



Effect of Storage Period on Quality and Lipid Composition of Eggs Obtained From Hens Fed Restricted Diet.

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ABSTRACT: This study was carried out to investigate the effect of storage period on the quality and lipid composition of table eggs obtained from birds fed on restricted commercial feed. The study was carried out at the University of Port Harcourt Teaching and Research Farm. Seven-two (72) laying hens which were 74 weeks old were assigned to three (3) treatments which had three (3) replicate each. The treatment consisted of T1 (control, ad libitum feeding), T2 (four hours feed restriction from 7 am – 11 am) and T3 (eight hours feed restriction from 7 am – 3 pm). At the end of the 12 weeks feeding trial, 60 eggs were collected from each treatment, (20 eggs per replicate), stored at room temperature and used for analysis of the external (egg weight, egg width, egg length, shell weight, shell thickness and egg index), internal (albumen and yolk weight, yolk diameter and height, albumen height, yolk index and haugh unit, HU) and lipid composition (total cholesterol, triglyceride, high-density lipoprotein, HDL and the low-density lipoprotein, LDL) at week 1, 2, 3, 4 and 5. The result showed that the length of storage significantly affected ($P < 0.05$) the egg weight, egg width, egg length and egg index, some internal characteristics and the lipid composition of the eggs examined. The eggs analyzed at the end of week 4 and 5 gave lower HU of 53.3 – 59.2 in all the treatments which tallied with the classification of low quality eggs grade (HU of 31 – 59) compared to those analyzed at the end of the first to third week (68.1 – 76.4) which were within the AA grade (HU > 72) and A grade (HU of 60 – 70) termed high quality eggs. The result of the interactive effect of storage and restricted feeding regimen did not affect the egg quality and the lipid composition of the eggs. The egg HDL increased after 2 weeks of storage in T1 and T2 and the LDL decreased during same period in the two treatments. It was concluded that farmers could practice restricted feeding regime especially for four hours (7 am – 11 am) since similar egg quality in terms of HU was obtained throughout the storage duration as well as an increase in the good cholesterol, HDL, and a corresponding decrease in the bad cholesterol, LDL after two weeks of storage.

KEYWORDS: Egg lipids, External quality, Hens, Internal quality, Restricted feeding, Storage

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I. INTRODUCTION

Chicken rearing is one of the most suitable activities which improve the livelihood of the poor due to the advantages it has in terms of the amount of capital required and the relative ease to set-up such a production system [1,2]. Poultry has become a popular industry for the small-holders with tremendous contribution to Nigeria gross domestic product and creation of employment opportunities [3,4]. Eggs obtained from chicken have long been regarded as the store-house of important nutrients such as protein, lipids, enzymes and active biological substances which include growth promoting factors [5]. According to [6] egg is the complete food that is available to man, consisting of carbohydrates, lipids, proteins, essential fatty acids and minerals which satisfy human needs. However, changes in the content of the egg after lay had been the most challenging problem associated with the egg industry, especially in developing countries. High temperature [7,8], time [9] and storage condition, treatment method and

packaging [10] are some factors that cause deterioration in the internal content of the egg. According to [11] the level of loss of the egg quality depend on the duration of storage, the humidity, temperature, air movement and the handling methods. Egg quality is also affected by nutrition, the breed of the animal, post-lay handling practices and climatic factors [12].

Poultry production depends on the supply of feed that is ready-to-use [13] which accounts for 70% of the total cost of egg and broiler production [14], farmers target high profit by minimizing feed cost [15], better utilization of available feed resources [16] and restricting the amount of daily feed for a period of time which stimulate compensatory growth [17], maintaining correct body weight, prevented over-eating, and limit health risks and maintained high fertility for parent stock [18]. Such restricted feeding programmes have been found not to affect egg quality traits [19] and egg number, hen-day production, egg weight and egg quality [20]. However, feed restriction affected yolk index and Haugh unit between Hisex Brown and Bovan white strains of layers [21], yet the effect of feed restriction on the quality of stored eggs have not been well documented. This study was therefore aimed at evaluating the effect of storage period on the quality and lipid composition of eggs that were obtained from hens fed on restricted commercial feeds.

II. MATERIALS AND METHODS

This research was conducted at the Poultry Unit of University of Port Harcourt Teaching and Research Farm, Choba. Port Harcourt Rivers State.

Seven-two (72) ISA Brown laying birds of 74 weeks old were used for this research. The hens were properly housed in battery cage system in an open-sided building. The hens were assigned randomly to 3 treatments where the feeding time served as the treatments consisting of T1 (control, *ad libitum* feeding), T2 (four hours feed restriction from 7 am – 11 am) and T3 (eight hours feed restriction from 7 am – 3 pm). Each treatment had three replicates with 8 birds each. Routine management practices and good hygienic conditions were maintained throughout the experimental period. The experimental design was the Completely Randomized Design.

Commercial layers feed which contained 2700 Kcal ME/kg, 15.0 % crude protein (CP), 10.0 % crude fibre (CF), 5.0 % fat, 3.5 % Ca, and 0.4 % P was purchased from a reliable source and given to the birds. The duration of the experiment was 12 weeks (April - July).

On completion of the study, 60 eggs, (20 eggs per replicate) were collected within 96 hours, stored at room temperature and used for analysis of the external, internal and lipid composition at week 1, 2, 3, 4 and 5. Thus, four eggs from each replicate were weighed weekly using an electronic weighing balance. The length and the width of the eggs were obtained by measuring the long and widest axes with a Vernier caliper. The eggs were carefully broken in the middle to keep the yolk intact and emptied into a petri-dish. The individual yolk was separated from the albumen and weighed. Yolk height was recorded and the diameter was taken as the maximum cross-sectional diameter with a pair of Vernier caliper. Albumen heights were also obtained by measuring the widest expanse of the thick albumen between the yolk and the yolk edge and the eternal edge of the thick albumen. Individual eggshell was carefully washed, air-dried for 24 hours and weighed. The shell thickness was measured with a micrometer screw gauge. The albumen weight was estimated by deducting the yolk and the shell weight from the egg weight. Egg index, yolk index and yolk to albumen ratio were computed from quality measurements. The weekly Haugh unit (HU) was calculated using a mathematical expression from the values obtained from egg weight and albumin height with the formula:

Haugh unit (HU) = $100\log(H+7.5-7w^{0.37})$, where H= Albumin height in cm, w = egg weight in grams. The egg shape index was obtained using the formula: width of egg ÷ length of egg x100%). The yolk ratio was obtained using the mathematical expression: yolk weight ÷ egg weight x 100). The eggs were also analyzed weekly for yolk lipid composition such as total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL). The eggs were weighed, hard boiled and allowed to cool. They were cracked and the yolk and albumen were separated. One gramme of the yolk was extracted, homogenized and filtered. The method for analyzing cholesterol using the Randox test kit was used, after which the lipids that were extracted were incubated at 37 °C for 5min and the absorbance of sample obtained using the spectrophotometer.

All data collected were analyzed statistically using the variance procedures of SAS Inc. Duncan's procedure of the same software was used to separate the differences between the means where they existed.

III. RESULTS

The effect of storage on the external qualities of eggs collected is presented in Table 1. There were significant differences ($P < 0.05$) in the weekly weight of the eggs and the egg index. The egg weight was significantly lower in week 4 and 5 in all the treatments. There were no significant differences ($P < 0.05$) in egg width, egg length, shell weight and shell thickness of the stored eggs.

The effect of storage on the internal quality of egg is presented in Table 2. Significant differences ($P < 0.05$) were obtained in all parameters measured except in yolk diameter and yolk height. It was noticed that while the albumen weight decreased in all the treatments in week 4 and 5, the yolk weight was higher in week 4 and 5. The albumen height was observed to decrease after the first 3 weeks of storage in T1 and T3 and after the first 2 weeks in T2. The Haugh unit (HU) followed similar pattern like the albumen weight, resulting in significant decrease ($P < 0.05$) after the first three weeks.

Statistical analysis of the data showed that the interactive effect of storage and treatment on the external and the internal qualities of the eggs showed no differences ($P < 0.05$).

The effect of storage on the lipid composition of the egg is presented in Table 3. There were significant differences ($P < 0.05$) in all the parameters measured. The level of cholesterol increased as the duration of storage increased. The triglyceride followed similar trend except that in week 4 for T1 and T2, the level was not increased. The good cholesterol, high-density lipoprotein (HDL) was significantly higher in week 3 and 5 in T1 and T2 and only in week 5 in T3 while the bad cholesterol, low-density lipoprotein (LDL) was lower in week 2 to 5 in T1 and T2 and higher during same weeks in T3.

IV. DISCUSSION

The egg weight which was lower in week 4 and 5 across the treatment groups indicated that the storage duration had an effect on the egg weight. This could possibly be due to the longer duration of storage (June - July) in which water loss from the egg may have caused the difference in weight. This finding agreed with the report of [22,23] who stated that the eggs that were collected from the market, shops, and farms reduced in weight after storing for 3 weeks at room temperature. The decreasing weight of eggs during storage at room temperature had been attributed to the loss of carbon-dioxide, nitrogen, ammonia, hydrogen sulfide and water from the eggs [24-27] while [28] reported that such weight loss in eggs was due to the loss of humidity through evaporation from the inside of the egg. This finding however confirmed that eggs stored in the humid tropics retain good quality for up to 3 weeks since [29] recorded faster egg deterioration (after 14 days) when eggs were stored at room temperature of 37 °C and [30] stated that eggs that were stored for 2 weeks in Bauchi State (northern Nigeria with hot environment) lost more weight compared to those stored for a day and those stored for one week respectively. Recent report by [31] showed that eggs that were collected from hens fed commercial layers feed (FT₃) which had a declared crude fibre (CF) content as 6.5 %, although proximate analysis showed 8.01%, had greater ($P < 0.05$) egg weight at room temperature till week 5, which declined at the end of week 6 (day 42) compared to the eggs collected from the hens that were fed the feeds FT₁ and FT₂ whose declared CF content was 6 and 7 %, although the analyzed values gave 11.6 and 12.2% respectively and gave higher egg values up to day 28, the end of the 4th week. The absence of differences ($P < 0.05$) in egg width, egg length, shell weight and shell thickness of the stored eggs was similar to [24] but differed from the report of [32] who found differences in these parameters possibly because of the two strains of birds that were studied. The egg shape index which was lower in week 5 for only T₁ did not tally with [30] who found no difference in the shape index of the eggs studied. The albumen weight which decreased in all the treatments in week 4 and 5 while the yolk weight increased in week 4 and 5 across the treatments implied that some physical and chemical modifications are associated with increased storage period of eggs. This could be as a result of the storage time and temperature [33] and the breakdown of the protein structure of the thick albumen and vitelline membrane [34]. The result tallied with the report of [23,35] who found that the yolk weight of eggs increased sharply as the storage period increased. According to [36] the increased yolk weight was caused by the movement of water from the albumen to the yolk as a result of the difference in osmotic pressure during the storage period which makes the yolk to acquire a flabby shape (flattened shape) instead of the normal spherical shape of freshly laid egg while the albumen gets thinner reducing in weight as the length of storage increases. The yolk diameter and yolk height which did not differ despite the duration of storage was at variance with the report of [37] who stated that the yolk height decreased significantly with increase in duration of storage when stored eggs that were obtained from birds fed five commercial vitamin-mineral premixes under two rearing systems were studied. The decreasing

pattern of the yolk index agreed with [37]. The values obtained from the Haugh unit (HU) which showed that the eggs were of good quality for 3 weeks and deteriorated in weeks 4 and week 5 agreed with earlier findings by [23,37,38] who reported that as the week of egg storage increased the HU decreased. However, according to [31], eggs collected from hens that were fed normally (not restrictively) with three commercial feeds and stored were observed to have good HU up to week 5 (day 35) for eggs collected from FT₁ and FT₂ and week 6 (day 42) for FT₃, with the eggs having the AA grade (HU of 72 and above) which later decreased to the A grade (HU of 60 – 70) till week 5 in FT₁ and FT₂ and week 6 in FT₃. The result obtained in this study, therefore, showed that while best egg quality can be obtained when eggs are freshly laid which usually decrease with prolonged storage [39], restricted feeding can also lead to fast deterioration of the egg quality when compared to adequate feeding regime. The level of cholesterol which increased as the duration of storage increased tallied with the finding of [40] who stated that the cholesterol content of the egg yolk increased from 52.83 mg/g yolk in the first week to 95.00 mg/g yolk in the 5th week. However, eggs collected from birds that were not fed restrictively on three commercial feeds were found to have significantly decreased cholesterol across the treatments as the length of storage of the eggs were prolonged [41]. Although previous researchers have shown that the cholesterol content of the egg yolk depended on the breed, species, the line and the age of the hen whose egg was used and the yolk weight [12,42], the similarity in the trend of the result obtained in this study supports the finding by [43] who stated that the cholesterol content of eggs is most times the same except when the content of the feed nutrient altered. The increasing trend of the triglyceride as the length of storage increased except in week 4 for T₁ and T₂ showed that storage duration could alter the level of the lipid in eggs. The good cholesterol, high-density lipoprotein (HDL) which was higher in week 3 and 5 in T₁ and T₂ and only in week 5 in T₃ while the bad cholesterol, low-density lipoprotein (LDL) was lower in week 3 to 5 in T₁ and T₂ and higher during same weeks in T₃ suggest that egg stored for 2 weeks and above may favour the better cholesterol level except when eggs were collected from hen that were starved for up to 8 hours. The lower level of the HDL at the end of the first two weeks in T₁ and T₂ with a corresponding high LDL implied that the eggs stored for two weeks from hens fed normally and restrictively for 4 hours will not favour reduction in heart disease, stroke and health problems, since [44,45] stated that only higher levels of HDL with a corresponding lower level of LDL can lead to lower risk of heart disease, stroke and health problems.

V. CONCLUSION

From this study, restrictive feeding of hens up to 8 hours a day can be practiced by farmers based on the HU of 53 – 59 which was obtained from eggs at the end of the 4th and 5th week of storage since it was above the HU of 40, termed poor quality eggs. The higher level of HDL after two weeks of egg storage, for eggs obtained from T₁ and T₂ will be of interest to egg consumers and need to be studied more closely to ascertain if the restriction of feed up to 4 hours coupled with more than 2 weeks egg storage will continuously favour higher levels of the good cholesterol, HDL in eggs.

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Effect Of Storage Period On Quality And Lipid Composition Of Eggs Obtained From Hens Fed

Table 1: Effect of storage on external qualities of the eggs

Parameters	T ₁					T ₂					T ₃				
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
Egg weight (g)	60.1 ± 4.0 ^a	60.0 ± 4.0 ^a	61.5 ± 0.5 ^a	56.0 ± 1.0 ^b	53.0 ± 4.0 ^b	61.6 ± 1.5 ^a	61.8 ± 1.5 ^a	65.0 ± 1.0 ^a	60.0 ± 0.5 ^b	60.0 ± 0.0 ^b	64.5 ± 4.0 ^a	63.0 ± 4.0 ^a	68.5 ± 0.5 ^a	60.1 ± 0.5 ^b	60.0 ± 0.0 ^b
Egg width (cm)	3.20 ± 0.0	3.00 ± 0.0	3.10 ± 0.0	2.90 ± 0.1	2.45 ± 0.4	3.00 ± 0.1	2.95 ± 0.0	3.10 ± 0.1	3.05 ± 0.1	3.05 ± 0.1	3.05 ± 0.1	3.05 ± 0.1	3.20 ± 0.14	3.20 ± 0.01	3.05 ± 0.1
Egg length (cm)	4.27 ± 0.2	4.25 ± 0.2	4.20 ± 0.2	4.15 ± 0.1	4.00 ± 0.0	4.48 ± 0.1	4.35 ± 0.1	4.50 ± 0.0	4.35 ± 0.2	4.50 ± 0.1	4.45 ± 0.1	4.35 ± 0.1	4.50 ± 0.2	4.45 ± 0.1	4.50 ± 0.0
Shell weight (g)	6.50 ± 0.5	6.50 ± 0.5	7.00 ± 0.0	7.00 ± 0.0	6.00 ± 1.0	6.5 ± 0.5	6.50 ± 0.5	7.50 ± 0.5	7.00 ± 0.0	7.00 ± 1.0	6.50 ± 0.5	6.50 ± 0.5	7.00 ± 0.0	7.00 ± 0.0	6.00 ± 0.1
Shell thickness (mm)	1.32 ± 0.3	1.31 ± 0.3	1.19 ± 0.1	1.13 ± 0.1	1.08 ± 0.01	1.45 ± 0.5	1.43 ± 0.4	1.08 ± 0.02	1.03 ± 0.1	1.04 ± 0.0	1.14 ± 0.02	1.12 ± 0.03	0.83 ± 0.2	0.97 ± 1.1	1.05 ± 0.02
Egg index	74.9 ± 2.5 ^a	70.6 ± 2.5 ^a	73.8 ± 3.5 ^a	69.9 ± 1.6 ^a	61.3 ± 3.8 ^b	67.91 ± 0.5 ^a	67.0 ± 0.4 ^a	68.9 ± 0.0 ^a	70.1 ± 1.3 ^a	67.8 ± 0.6 ^a	70.2 ± 0.3 ^a	70.1 ± 0.3 ^a	71.1 ± 5.4 ^a	71.9 ± 0.8 ^a	67.8 ± 1.1 ^a

^{ab} = means within each row that bear different superscripts differ significantly ($P < 0.05$)

Table 2: Effect of storage on internal qualities of the eggs

Parameters	T ₁					T ₂					T ₃				
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
Alb. Wt. (g)	35.5 ± 2.5 ^a	34.5 ± 2.5 ^a	36.5 ± 1.5 ^a	31.0 ± 2.0 ^b	28.5 ± 3.5 ^b	34.0 ± 0.0 ^b	33.0 ± 0.0 ^b	38.5 ± 0.5 ^a	30.0 ± 2.0 ^b	30.1 ± 1.0 ^b	39.5 ± 2.4 ^a	36.5 ± 3.5 ^a	39.5 ± 0.5 ^a	35.5 ± 1.5 ^b	34.0 ± 1.0 ^b
Yolk wt (g)	14.0 ± 1.0 ^b	15.0 ± 1.0 ^b	14.2 ± 0.0 ^b	16.8 ± 0.0 ^a	16.5 ± 0.5 ^a	15.8 ± 0.0 ^{1b}	16.0 ± 0.0 ^b	17.5 ± 2.5 ^{ab}	20.5 ± 1.0 ^{ab}	18.5 ± 0.5 ^a	14.0 ± 4.0 ^b	14.0 ± 4.0 ^b	15.0 ± 0.0 ^b	18.0 ± 0.0 ^a	16.5 ± 0.5 ^{ab}
Yolk diameter (cm)	3.05 ± 0.1	3.05 ± 0.1	2.70 ± 0.0	2.95 ± 0.2	3.00 ± 0.0	3.21 ± 0.2	3.10 ± 0.1	3.05 ± 0.1	3.40 ± 0.0	3.10 ± 0.1	2.60 ± 0.2	2.55 ± 0.2	3.05 ± 0.2	2.90 ± 0.1	2.85 ± 0.2
Yolk ht. (cm)	0.81 ± 0.1	0.75 ± 0.1	0.65 ± 0.1	0.60 ± 0.1	0.65 ± 0.1	0.80 ± 0.0	0.70 ± 0.0	0.75 ± 0.1	0.75 ± 0.1	0.60 ± 0.0	0.70 ± 0.1	0.70 ± 0.1	0.55 ± 0.1	0.70 ± 0.1	0.75 ± 0.1
Alb. Ht. (cm)	3.85 ± 1.4 ^a	3.82 ± 1.9 ^a	3.90 ± 0.2 ^a	1.49 ± 0.2 ^c	1.7 ± 0.1 ^b	3.66 ± 0.2 ^{1a}	3.51 ± 0.2 ^{5a}	3.00 ± 0.0 ^{0b}	1.47 ± 0.5 ^{5c}	1.6 ± 0.0 ^c	3.51 ± 0.2 ^{0a}	3.48 ± 0.3 ^{0a}	3.30 ± 0.2 ^{0a}	1.45 ± 0.5 ^{0c}	1.8 ± 0.05 ^b
Yolk index	26.6 ± 0.1 ^a	24.6 ± 0.1 ^a	24.1 ± 0.3 ^a	20.3 ± 0.5 ^b	21.7 ± 3.0 ^b	24.9 ± 0.8 ^{6a}	22.6 ± 0.7 ^{6ab}	24.6 ± 3.4 ^a	22.1 ± 0.5 ^{6ab}	19.4 ± 0.7 ^{8b}	26.9 ± 4.4 ^{7a}	27.1 ± 5.1 ^{4a}	18.0 ± 0.1 ^{9b}	24.1 ± 0.1 ^{8ab}	26.3 ± 0.7 ^{9a}
Yolk: Alb. ratio	0.40 ± 0.0 ^{2b}	0.43 ± 0.0 ^{1b}	0.39 ± 0.0 ^{3b}	0.54 ± 0.0 ^{4a}	0.57 ± 0.0 ^{8a}	0.46 ± 0.0 ^b	0.48 ± 0.0 ^b	0.53 ± 0.0 ^{6b}	0.68 ± 0.0 ^{0a}	0.61 ± 0.0 ^{3a}	0.35 ± 0.0 ^{5b}	0.38 ± 0.1 ^b	0.38 ± 0.0 ^{1b}	0.51 ± 0.0 ^a	0.48 ± 0.0 ^a
HU	76.4 ± 1.2 ^a	76.2 ± 1.2 ^a	76.4 ± 1.1 ^a	55.0 ± 2.2 ^b	59.2 ± 1.9 ^b	74.6 ± 2.6 ^a	74.8 ± 2.1 ^a	68.1 ± 1.8 ^a	53.5 ± 10.0 ^{0b}	55.1 ± 0.0 ^b	72.6 ± 2.3 ^a	72.8 ± 2.7 ^a	69.8 ± 4.3 ^a	53.3 ± 10.8 ^b	57.5 ± 1.2 ^b

^{ab} = means within each row that bear different superscripts differ significantly ($P < 0.05$)

Effect Of Storage Period On Quality And Lipid Composition Of Eggs Obtained From Hens Fed

Alb. Wt. = Albumen weight, Wt. = Weight, ht. = height,

Table 3: Effect of storage on lipid composition of the eggs

Parameters (mmol/L)	T ₁					T ₂					T ₃				
	Wee k1	Wee k2	Wee k3	Wee k4	Wee k5	Wee k1	Wee k2	Wee k3	Wee k4	Wee k5	Wee k1	Wee k2	Wee k3	Wee k4	Wee k5
TC	10.7 ±0.9 b	10.9 ±0.9 b	20.2 ±0.1 a	18.7 ±0.6 a	22.1 ±0.1 a	11.2 ±0.1 b	11.3 ±0.1 b	19.9 ±0.5 a	19.9 ±0.5 a	22.6 ±0.1 a	12.8 ±0.1 ^b	13.9 ±0.2 b	20.7 ±0.7 a	20.8 ±0.2 a	22.5 ±0.5 a
TG	2.80 ±0.1 b	2.90 ±0.1 b	4.60 ±0.2 a	3.10 ±0.1 b	5.00 ±0.1 a	3.04 ±0.2 b	3.05 ±0.2 b	4.70 ±0.1 a	3.30 ±0.1 b	5.25 ±0.1 a	3.03 ±0.6 ^b	3.05 ±0.6 b	4.80 ±0.1 a	4.10 ±0.1 a	5.10 ±0.1 a
HDL	2.20 ±1.0 b	2.30 ±1.1 b	4.10 ±0.1 a	1.80 ±0.3 b	3.10 ±0.1 a	2.24 ±0.1 b	2.30 ±0.1 b	3.70 ±0.2 a	2.50 ±0.3 b	3.60 ±0.1 a	2.70 ±0.2 4 ^b	2.80 ±0.3 b	2.40 ±0.1 b	2.70 ±0.1 b	3.10 ±0.1 a
LDL	3.80 ±2.9 a	3.90 ±2.9 a	1.30 ±0.1 b	1.40 ±0.4 b	1.30 ±0.2 b	2.70 ±0.6 a	2.80 ±0.7 a	1.50 ±0.1 b	1.20 ±0.5 b	1.50 ±0.5 b	3.90 ±2.3 ^b	3.90 ±2.4 b	6.90 ±0.2 a	7.80 ±0.3 a	4.30 ±0.4 b

^{a,b} = means within each row that bear different superscripts differ significantly ($P < 0.05$)

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