

## **EFFECT OF GREEN TEA AND BIOTITE ON PERFORMANCE, MEAT QUALITY AND ORGAN DEVELOPMENT IN ROSS BROILER**

By

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**Abstract:** *A study was conducted with green tea (GT) and biotite to evaluate their effect on broiler growth, meat quality, and the development of internal organs. A total of 210 Ross day old chicks (DOC) were distributed to 6 dietary feed additive groups having 5 treatments with 7 chicks in each group. The groups were control (basal), antibiotic (basal + 30 ppm oxytetracycline), GT (0.5 and 1.0% with basal) and biotite (0.5 and 1.0% with basal). The birds were reared in wire cages following a completely randomized design for a period of 5 weeks under recommended starter and finisher diets. The results can be summarized as below:*

- 1. The final weight, weight gain, and feed intake of broiler were statistically lower in 1% green tea than the others ( $P < 0.05$ ).*
- 2. Green tea and biotite addition as feed additives showed a potential in livability like the antibiotic fed broilers compared to control group.*
- 3. The crude protein content of meat was significantly higher in biotite fed broiler than other groups ( $P < 0.05$ ) and 1% biotite reduced crude fat content in meat like the antibiotic. Addition of biotite in feed significantly increased crude ash in broiler meat compared to other groups.*
- 4. The TBA value of broiler meat in 1.0% biotite was significantly lower on the 1st week after preservation ( $P < 0.05$ ).*
- 5. The weight of small intestine was significantly smaller in the 0.5% green tea than other groups ( $P < 0.05$ ). In 1.0% biotite fed broiler had the shortest length of the small intestine ( $P < 0.05$ ) compared to antibiotic.*
- 6. There was no adverse effect observed in addition of lower level of green tea*

*(0.05%) and upto 1% biotite as feed additives in respect of growth performance, meat quality and internal organ development*

*In conclusion, addition of 0.5% green tea and upto 1.0% biotite may have the potential in replacing oxytetracycline for broiler production.*

## INTRODUCTION

Due to the outbreaks of resistant bacteria and residues of antibiotics in poultry products, using antibiotics is regulated by Korean Government (Yu et al., 2004). There are several kinds of antibiotics alternative developed and used currently, among which readily memorable natural substances for customers and medicinal plants with excellent physiologically activity are getting attention by researchers (Hernandez et al., 2004). Some examples of medicinal plants are green tea, artemisia, acanthopanax, and others (Yang et al., 2003; Kwon et al., 2005). Green tea (*Camellia sinensis*) has been used for centuries by Korean, Japanese, and Chinese people as an anti-aging herb. Green tea inclusion in broiler diets had positive effects on growth performance and lean meat production of the broilers (Kaneko et al., 2001). The tea plant is commonly used in Chinese herbalism, where it is considered to be one of the 50 fundamental herbs. Modern research has shown that there are many health benefits to drinking tea, including its ability to protect the drinker from certain heart diseases. It has also been shown that drinking tea can protect the teeth from decay, because of the fluoride naturally occurring in the tea. Green tea is made from the steamed and dried leaves and it contains polyphenols, these are antioxidants that help to protect the body against heart diseases, stroke and cancer. Catechins differ slightly in chemical structure from other flavonoids, but share their chemoprotective properties. The most common catechins are gallic esters, named epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). All are found in green tea, and are thought to be responsible for the protective benefits of this beverage.

Biotite is a common phyllosilicate mineral that contains potassium, magnesium, iron and aluminium. It is sometimes called "iron mica" and is found in granitic rocks, gneisses, and schists. It is an antibiotics substitute which is made from natural substances is feed supplement for poultry and livestock. It is in powder form that makes it possible to easily mix in feed. It increases feed efficiency, weight gain and improves meat quality. Biotite increases immunity, the antibodies immunoglobulin IgG cell increases 67.8 - 86.6%. as protection against infection (Kim, 2003). It is an environmental friendly feed additive for livestock because it reduces phosphorus, nitrogen and ammonia gas concentration in feces.

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat products through adverse changes in flavor, color, texture and nutritive value (Ura et al., 2008). Chicken meat enriched with PUFA contains longer fatty acids (FA) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). One such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among many methods proposed for assessing MDA the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. In practice, meat is stored and cooked for consumption.

The purpose of this study was to assess possibility of green tea and biotite to be used as broiler feed additives as antibiotic alternatives and their effect on productivity, meat quality and organ development.

## MATERIALS AND METHODS

### Birds and Experimental Design

A total of 210 "Ross" day old broiler chicks were housed in a close ventilated caged-broiler house. From 1 to 14 days of age, supplemental heat was provided by electric heater which placed inside the chicken house, thereafter the room temperature was kept at  $22\pm 2^{\circ}\text{C}$  through a supplemental heating system. The birds were assigned to 6 treatments with 5 replications having 7 chicks in each using completely randomized design (CRD). There were six dietary treatments, namely control, antibiotics (basal+30 ppm oxytetracycline), green tea (basal+0.5% and basal+1.0%) and biotite (basal+0.5% and basal+1.0%). The feed and drinking water were provided *ad libitum*.

### Experimental Diets and Feeding

Experimental diets were formulated to meet the nutrient requirement of broiler suggested by NRC (1998). The starter diet used for this study was ME 3,000 kcal/kg and CP 21.50%, and the finisher diet was ME 3,070 kcal/kg and CP 19% (Table 1). The broiler chicks were housed individually in 3-storied cages and they were provided starter diet from day old to 3 weeks and then up to 5 weeks finisher diet. These additives were used by mixing with the control diets.

## **Record Keeping**

### ***Body weight gain, Feed intake and Feed conversion ratio***

Body weights were measured on weekly basis from initial day to the final day of the experiment. Feed intake had been determined by measuring feed residue on weekly basis since the beginning of the experiment. Feed conversion ratio was obtained by dividing the feed intake to body weight gain.

### ***Carcass rancidity***

For determination carcass rancidity for broiler meat the method described by Vernon *et al.* (1970) was used with small modifications. For this analysis, 10 g of thigh and breast meat mixture was blended at full speed for 1.5 min in chilled stainless Watering Blender cup with 25 ml of extracting solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid. The resulting sediment was transferred quantitatively to 50 ml volumetric flask with 20 ml distilled water and homogenized by shaking. A 25 ml portion was filtrated through Whatman No.6 filter paper. Then 5 ml filtrate was transferred to a test tube and added 5 ml of 2-thiobarbituric acid (0.005 M in DW). The solution was shaking at water bath 80°C for 30 min. After cooling, the color development was measured at 530 nm in a spectrophotometer, biochrom, Libra S22 (Biochrom Ltd., Cambridge, England). Thiobarbituric acid (TBA) reactive substance values were expressed as micromole of malondialdehyde (MDA) per hundred gram of meat.

### ***Body composition and Organ development***

At the end of the experiment, 24 chickens were slaughtered and samples were collected from breast and thigh muscle. The chemical composition of the carcass was determined according to common method of AOAC (1995). The organs development was measured by taking weight and length of the broilers after slaughtering.

### ***Statistical Analysis***

The data obtained from this study were analyzed by general linear models (GLM) of SAS Package Program (1990) to estimate variance components for a completely randomized design and the differences were statistically assessed at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Growth performance and Feed intake

Table 2 shows significantly lowest weight gain was observed in 1.0% green tea fed broilers at starter period though there was no statistical difference with other groups except antibiotic fed broilers. Significantly highest weight gain (1210.61 g/bird) was observed in finishing period in 0.5% green tea fed broilers compared to 1.0% (1033.36 g/bird). This trend was even the whole 5 weeks total weight gain. Highest weight gain. This amount of increasing green tea had effect in lowering body weight. These results were similar with Kaneko et al. (2001) who reported that 1.0, 2.5 and 5.0% of green tea in broiler diets linearly reduced body weight gain of the chicks and Uganbayar (2004) reported that 1.0 to 1.5% green tea supplement in broiler diet had effect to reduce body weight gain of the chicks. The death rate in relation to addition of green tea and biotite. This is in agreement with the opinion of Vohar and Kratzer (1964), they reported that the high crude fiber content of green tea may reduce body weight gain of the broilers. There was no significant difference in feed conversion ratio at both starter and finisher stage among the feed additives group and the addition of biotite (0.5 and 1.0%) possessed almost similar feed conversion ration like antibiotic. The suitability of 1.0% biotite use as feed additives in current broiler study is agreed with the findings of Lee et al. (2003a,b) layer chicken and pig. Uganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet did not affect crude protein and crude fat content of broiler meat. This result is similar to the green tea treatment group of our study. Biotite has also positive effect in carcass composition especially reducing crude fat content compared to antibiotic which indicate it's potentiality as an antibiotic alternative. This findings are in agreement with the findings of Uganbayar, 2004.

### Livability

Green tea and biotite has the potential in immune response like the antibiotics which can be revealed by our study. It was observed that no morbidity and mortality occurred in both green tea and biotite added group like the antibiotic compared to control fed birds. In control 2 birds were died so the mortality was 5.71% compared to all the feed additives groups. This findings reveals that the antioxidant effect of green tea catechin and the natural complex mineral substance Biotite, possibly have potentiality like the antibiotic. The potentiality of biotite in our study also supported by an experienced poultry farmer in Korea (Kim, 2003). According to his experience Biotite increases the feed conversion and livability in broilers (Kim, 2003). This findings in green tea addition in broiler diet is also supported by our previous studies (Uganbayar, 2004, Yang et al., 2003).

### **Lipid oxidation of meat**

Table 3 shows that water content of the green tea and biotite added groups were significantly lower ( $P < 0.05$ ) than the control group. Addition of biotite possessed the significantly highest ( $P < 0.05$ ) protein content compared to antibiotic and 1.0% green tea added diet. Table 4 shows the result of TBA analysis in relation with addition of green tea and biotite. The eventual amount of breast and thigh meat of all groups were analyzed to get TBA value. The average value of the control group was 7.28  $\mu\text{mol}/100\text{g}$  which was the highest than those of the green tea and biotite added groups. Addition of 1.0% biotite showed lowest TBA value, 5.94  $\mu\text{mol}/100\text{g}$ , followed by 0.5% green tea and other groups. Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life. The storage time also had an effect on these values, and the highest TBA occurred at the end of the storage period. This result is agreed with the findings of Muhammet *et al.*, 2005. In this study reducing of TBA value in 0.5% green tea and 1.0% biotite showed a positive trend because lipid oxidation. It has been reported to be a primary cause of off-odor in refrigerated raw beef and significant correlations between the TBA values and sensory scores of poultry meat have been reported Jae *et al.*, 2004. The findings in lowering malondialdehyde (MDA) value in lipid oxidation in fresh and preserved broiler meat by addition of green tea is also similar with our another study (Sarker *et al.*, 2009). They found at 3 weeks of storage, the TBA value of broiler meat in 0.5% green tea showed the lowest value among the groups, although no significant difference ( $P > 0.05$ ) from other dietary groups was observed.

### **Body composition and Organ development**

The results showed in table 5 that the ceum weight was decreased significantly in broilers fed diets containing 0.5% green tea supplement ( $p < 0.05$ ) compared to 1.0% though no significant difference were observed with other groups. The biotite-added group showed decrease in large intestine weight compared to the control and antibiotic group though there was no statistical difference ( $P < 0.05\%$ ). Addition of 1.0% both green tea and biotite groups showed lower abdominal fat content than the control compared to other groups but there was no statistical difference ( $P > 0.05\%$ ). Gizzard weight in 1.0% biotite showed significantly ( $P < 0.05$ ) highest compared to other groups showing similarity with antibiotic fed broiler chicks. The crop weight, heart weight, liver weight, pancreas weight, kidney weight and proventriculus weights among the groups didn't show statistical differences among the

treatment groups ( $p < 0.05$ ). Table 6, shows the length of small and large intestines of the broilers. The addition of 1.0% biotite showed significantly lower small intestine length (151.83 cm) followed by 0.5% green tea (160.83 cm) and other treatment groups. So it can be assumed that green tea and biotite has potential in reducing intestinal length. Uganbayar (2004) reported diets containing 0.5% green tea showed a significant weight loss of the small intestine compared to the control diet, which is similar to our study. Kim *et al.* (2006) also expressed same opinion. Although there was no statistical significant difference in abdominal fat observed among the treatments but the lowest abdominal fat content was recorded in 1.0% biotite supplemental broiler chicks. This finding is agreed with Leestra *et al* (1986) that fat accumulation in visceral cavity has a proportional relation with weight. Considering all the factors and parameters it may be concluded that addition of 0.5% green tea and 1.0% biotite had the potential in replacing oxytetracycline in broiler production.

### CONCLUSION

In conclusion, addition of green tea at lower level (0.5%) and the natural mineral substance, biotite (to some extend higher level, i.e. 1.0%) may be suitable for improving growth performance, meat quality and internal organ development of broilers instead of antibiotic (oxytetracycline) use. More research studies are needed to conduct for drawing a rigid conclusion especially on Biotite because of limited works are done in this potential supplement.

**Table (1).** Feed formula and chemical composition of the experiment diets

Ingredient	Starter	Finisher
Corn grain	57.81	60.00
Soybean meal	26.50	25.40
Corn gluten	5.00	4.20
Soybean oil	2.20	1.50
Animal fats	4.50	5.00
Salt	0.25	0.25
Tricalcium Phosphate	2.14	2.00
Limestone	0.92	0.88
Vit-Min. premix <sup>1)</sup>	0.30	0.30
Choline	0.08	0.07
Enzyme	0.10	0.30
Yeast	0.20	0.10
Total	100.00	100.00
Chemical composition <sup>2)</sup>		
ME (kcal/kg)	3.000	3.070
Crude protein (%)	21.50	19.00
Crude fat (%)	4.00	4.50
Crude ash (%)	8.00	8.00
Crude fiber (%)	6.00	6.00

<sup>1)</sup>Vit-min. premix. provided nutrients per kg of diet : Vitamin A. 9,000,000 IU; Vitamin D<sub>3</sub>. 2,100,000 IU; Vitamin E. 15,000 IU; Vitamin K. 2,000 mg; Vitamin B<sub>1</sub>. 1,500 mg ;Vitamin B<sub>2</sub>. 4,000 mg; Vitamin B<sub>6</sub>. 3,000 mg; Vitamin B<sub>12</sub>. 15 mg.; Pan-Acid-Ca. 8500 mg; Niacin. 20,000 mg; Biotin. 110 mg; Folic-Acid. 600 mg; Co. 300 mg; Cu. 3,500 mg; Mn. 55,000 mg; Zn. 40,000 mg; I. 600 mg; Se. 130 mg.

<sup>2)</sup> Calculated values



Green Tea, Biotite, Meat Quality, Organ Development, Broiler.

**Table (2).** Effect of dietary green tea and biotite on growth performance and feed intake of broiler (g)

Items	Control	Antibiotics	Green Tea		Biotite	
			0.5%	1.0%	0.5%	1.0%
0-3 weeks						
Initial weight	44.82±0.15	44.86±0.23	44.72±0.21	44.79±0.10	44.86±0.33	44.75±0.11
Final weight	903.75 <sup>a</sup> ±14.98	922.11 <sup>a</sup> ±18.77	877.25 <sup>ab</sup> ±25.74	850.22 <sup>b</sup> ±11.88	899.75 <sup>ab</sup> ±27.12	907.64 <sup>ab</sup> ±7.15
Weight gain	858.93 <sup>ab</sup> ±15.07	877.25 <sup>a</sup> ±18.90	832.54 <sup>ab</sup> ±25.67	805.43 <sup>b</sup> ±11.81	854.89 <sup>ab</sup> ±26.87	862.89 <sup>ab</sup> ±7.08
Feed intake	1269.53 <sup>a</sup> ±18.31	1293.32 <sup>a</sup> ±32.18	1251.61 <sup>ab</sup> ±19.55	1186.43 <sup>b</sup> ±9.99	1215.00 <sup>ab</sup> ±19.32	1267.14 <sup>a</sup> ±38.55
FCR	1.48±0.02	1.48±0.02	1.51±0.04	1.47±0.02	1.42±0.05	1.47±0.06
4-5 weeks						
Initial weight	903.75 <sup>a</sup> ±14.98	922.11 <sup>a</sup> ±18.77	877.25 <sup>ab</sup> ±25.74	850.22 <sup>b</sup> ±11.88	899.75 <sup>ab</sup> ±27.12	907.64 <sup>ab</sup> ±7.15
Final weight	2079.29 <sup>a</sup> ±51.67	2073.57 <sup>a</sup> ±58.38	2087.86 <sup>a</sup> ±52.35	1883.57 <sup>b</sup> ±63.10	2064.29 <sup>a</sup> ±61.20	2088.57 <sup>a</sup> ±27.65
Weight gain	1175.54 <sup>a</sup> ±37.45	1151.47 <sup>ab</sup> ±41.03	1210.61 <sup>a</sup> ±31.14	1033.36 <sup>b</sup> ±68.02	1164.54 <sup>ab</sup> ±46.54	1180.93 <sup>a</sup> ±23.86
Feed intake	2184.47 <sup>a</sup> ±59.27	2130.11 <sup>a</sup> ±39.79	2210.68 <sup>a</sup> ±36.65	1986.04 <sup>b</sup> ±71.64	2178.79 <sup>a</sup> ±31.11	2174.36 <sup>a</sup> ±38.67
FCR	1.86±0.04	1.86±0.04	1.83±0.03	1.93±0.07	1.88±0.06	1.84±0.03
0-5 weeks						
Initial weight	44.82±0.15	44.86±0.23	44.72±0.21	44.79±0.09	44.86±0.34	44.75±0.11
Final weight	2079.29 <sup>a</sup> ±51.67	2073.57 <sup>a</sup> ±58.38	2087.86 <sup>a</sup> ±52.35	1883.57 <sup>b</sup> ±63.10	2064.29 <sup>a</sup> ±61.20	2088.57 <sup>a</sup> ±27.65
Weight gain	2034.46 <sup>a</sup> ±51.79	2028.72 <sup>a</sup> ±58.55	2043.14 <sup>a</sup> ±52.24	1838.78 <sup>b</sup> ±63.08	2019.43 <sup>a</sup> ±60.91	2043.82 <sup>a</sup> ±27.55
Feed intake	3453.99 <sup>a</sup> ±71.78	3423.43 <sup>a</sup> ±59.48	3462.29 <sup>a</sup> ±37.28	3172.47 <sup>b</sup> ±77.42	3393.79 <sup>a</sup> ±46.24	3441.50 <sup>a</sup> ±4.26
FCR	1.70±0.02	1.69±0.02	1.70±0.03	1.73±0.02	1.68±0.05	1.69±0.02

<sup>a,b</sup> Mean with different superscripts within the same row are significantly different (P<0.05).  
Data = mean ± Standard error

**Table (3).** Effects of dietary green tea and biotite on the carcass composition of broiler (%)

Meat composition	Control	Antibiotics	Green Tea		Biotite	
			0.5%	1.0%	0.5%	1.0%
Moisture	74.92 <sup>a</sup> ±0.55	73.74 <sup>a</sup> ±0.13	73.69 <sup>a</sup> ±0.63	73.07 <sup>b</sup> ±0.63	72.47 <sup>a</sup> ±0.26	72.83 <sup>a</sup> ±0.62
Crude protein	23.61 <sup>a</sup> ±0.30	22.11 <sup>b</sup> ±0.68	23.55 <sup>ab</sup> ±0.79	22.06 <sup>b</sup> ±0.29	24.70 <sup>a</sup> ±0.41	24.63 <sup>a</sup> ±0.42
Crude fat	0.71±0.08	0.85±0.07	1.25±0.18	1.53±0.52	1.19±0.12	0.88±0.22
Crude ash	1.10 <sup>a</sup> ±0.01	1.10 <sup>a</sup> ±0.03	1.13 <sup>a</sup> ±0.02	1.13 <sup>a</sup> ±0.03	1.19 <sup>a</sup> ±0.01	1.22 <sup>a</sup> ±0.01

<sup>a,b</sup> Mean with different superscripts within the same row are significantly different (P<0.05).  
Data = mean ± Standard error

**Table (4).** Effects of dietary green tea and biotite on meat TBA in broiler ( $\mu\text{mol}/100\text{g}$ )

Items	Control	Antibiotics	Green Tea		Biotite	
			0.5%	1.0%	0.5%	1.0%
Fresh	2.31±0.09	2.06±0.22	1.69±0.13	2.22±0.19	2.22±0.32	1.85±0.00
1week	4.52 <sup>a</sup> ±0.05	2.77 <sup>c</sup> ±0.45	2.55 <sup>dc</sup> ±0.21	3.54 <sup>b</sup> ±0.22	2.34 <sup>dc</sup> ±0.24	1.91 <sup>d</sup> ±0.08
2week	7.28±0.74	6.55±0.45	6.43±0.74	6.49±0.41	7.35±0.85	5.75±0.35
3week	15.02±2.14	14.62±3.26	13.88±1.85	13.57±0.96	14.71±1.20	14.25±1.60
Average	7.28±0.69	6.50±0.70	6.14±0.66	6.45±0.17	6.65±0.56	5.94±0.32

<sup>abc,d</sup> Mean with different superscripts within the same raw are significantly different (P<0.05).

Data = mean ± Standard error

**Table (5).** Effects of green tea and biotite on development of intestinal organs in broiler chickens (%)

Organ wt.*	Control	Antibiotics	Green Tea		Biotite	
			0.5%	1.0%	0.5%	1.0%
Crop wt.	0.29±0.05	0.30±0.03	0.26±0.04	0.27±0.03	0.26±0.02	0.26±0.02
Heart wt.	0.60±0.04	0.60±0.04	0.65±0.01	0.64±0.09	0.63±0.03	0.67±0.03
Liver wt.	1.87±0.15	1.86±0.20	1.91±0.14	2.20±0.11	1.87±0.11	1.92±0.24
Gizzard wt.	0.89 <sup>b</sup> ±0.05	1.01 <sup>ab</sup> ±0.06	0.94 <sup>b</sup> ±0.11	0.96 <sup>b</sup> ±0.03	0.90 <sup>b</sup> ±0.11	1.24 <sup>a</sup> ±0.10
Pancreas wt.	0.16±0.02	0.17±0.03	0.16±0.02	0.14±0.01	0.19±0.02	0.19±0.03
Cecum wt.	0.49 <sup>ab</sup> ±0.04	0.55 <sup>ab</sup> ±0.08	0.37 <sup>b</sup> ±0.04	0.61 <sup>a</sup> ±0.12	0.38 <sup>b</sup> ±0.02	0.47 <sup>ab</sup> ±0.03
Kidney wt.	0.75±0.03	0.70±0.05	0.74±0.09	0.79±0.01	0.71±0.08	0.78±0.09
Small intestine wt.	2.59 <sup>b</sup> ±0.26	2.33 <sup>ab</sup> ±0.09	1.98 <sup>ab</sup> ±0.14	2.60 <sup>b</sup> ±0.22	2.16 <sup>b</sup> ±0.11	2.22 <sup>ab</sup> ±0.08
Large intestine wt.	0.16±0.02	0.18±0.02	0.15±0.01	0.16±0.02	0.13±0.02	0.15±0.01
Abdominal fat wt.	1.85±0.27	1.91±0.17	2.12±0.36	1.39±0.38	2.05±0.57	1.51±0.39
Proventriculus wt.	0.38±0.06	0.40±0.07	0.40±0.04	0.33±0.03	0.30±0.01	0.42±0.09

<sup>ab</sup> Mean with different superscripts within the same raw are significantly different (P<0.05).

\*All the organ weights (%) are in respect of live weight.

Data = mean ± Standard error

**Table (6).** Effects of dietary green tea and biotite on the length of the intestines (cm)

Intestine	Control	Antibiotics	Green Tea		Biotite	
			0.5%	1.0%	0.5%	1.0%
Small	171.83 <sup>b</sup> ±7.83	182.50 <sup>a</sup> ±9.25	160.83 <sup>ab</sup> ±5.36	166.67 <sup>ab</sup> ±5.24	165.33 <sup>ab</sup> ±4.91	151.83 <sup>c</sup> ±8.19
Large	8.17 <sup>a</sup> ±0.44	10.83 <sup>b</sup> ±0.73	10.33 <sup>b</sup> ±0.44	9.30 <sup>abc</sup> ±0.67	10.00 <sup>ab</sup> ±0.29	9.00 <sup>bc</sup> ±0.00

<sup>abc</sup> Mean with different superscripts within the same raw are significantly different (P<0.05).

Data = mean ± Standard error

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