QUALITY EVALUATION OF EGGS FROM ISA BROWN AS INFLUENCED BY NATURAL ANTIOXIDANTS AND STORAGE TIME

ABSTRACT

This study aimed to assess the effect of natural antioxidants on storage time, physical and chemical properties of egg. A total of 200laying ISA brown birdswere distributed into eight dietary groups, each dietary group with 25birds (5 birds per replicate). They were fed with roselle, black pepper, green tea,combine (roselle + black pepper + green tea) at 0.5g/kg and 1.0g/kg in basal diet respectively and a control feed. At the end of eight (8) weeks of feeding trial, twelve eggs were collected from each dietary (six eggs per dietary group were analyzed for the internal and external properties while the remaining six were stored). Data collected include; Egg shape index, egg weight, shell thickness, membrane weight, yolk index, haugh unit, meat and blood spot, yolk color, lipid profile, lipid peroxidation and proximate analysis. Data generated were subjected to Analysis of variance using the General Linear Model for factorial within a completely randomized design.The natural antioxidants significantly (P<0.05) improves the proximate composition of the poultry eggs. Both Green tea and Black pepper have significant effect (P<0.05) on the yolk percentage. Black pepper increases (P<0.05) the Haugh unit while it shows to be lower (P>0.05) in the combination of the antioxidants. The inclusion levels of the natural antioxidants on the internal and external quality of egg reveals that there were no significant (P>0.05) differences in the two inclusion level of 0.5g/kg and 1.0g/kg of feed but they have numerically higher values in the external parameters and internal parameters. Eggs stored for 4weeks had the lowest value (P>0.05) for the proximate and lipid profiles, though there are no significant (P>0.05) differences in their values. The fresh eggs show high moisture content (P<0.05) but the value for nitrogen free extract is low (P>0.05) in the fresh eggs. The natural antioxidants improved significantly (P<0.05) the proximate composition of the poultry eggs, with the green tea at 1.0g having the highest value (P<0.05) for, black pepper 0.5g having the highest (P<0.05) for CP and combine 1.0g having the highest value (P<0.05) for E.E. Based on the result obtained from this study, the natural antioxidants (Black pepper, green tea and roselle) in layers' diet shows significant effects on the physical qualities of egg and the yolk color was also preferred with the inclusion of the natural antioxidants compared to the control. The chemical properties also deteriorate with the storage time. Natural antioxidants are hereby recommended for better and improved chemical qualities of eggs.

Key words: Egg quality, physical properties, chemical properties, antioxidants

Introduction

Poultry is by far the largest group of livestock species (FAO. 2000) contributing about 30% of all animal protein consumed in the world (Permin and Pedersen, 2000). Poultry refers to all birds of economic value to man as source of meat, egg and fiber. This industry in Nigeria occupies a prominent position as a major source of animal protein supply to the citizens. The persistence short supply of protein for populace is the main problem which was compounded by the accelerated increase in human population especially in Nigeria and thus created pressure on every form of food supply (FAOSTAT, 2011). According to Orji and Chukwuma (2007) the poultry eggs goes a long way in providing animal protein for the populace because it yields quickest returns and provides for egg in a very short time. Eggs are major sources of animal

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protein in human diets. Eggs constituted an important part of human diets for centuries because of its high quality protein (Forson*et al*., 2011).

Antioxidants can be defined as any substance that, when present in low concentrations compared to that of an oxidisable substrate, delays or inhibits the oxidation of that substrate (Murthy, 2001). The important commercially available natural antioxidants are tocopherols (Vitamin E) ascorbic acid (Vitamin C) and Rosemary extracts (Trombino*et al*., 2004). Natural Anti-oxidant includes Green Tea,Roselle and black pepper. The most abundant constituent of green tea extracts (GTE) is catechins which has antibacterial activities (Cao *et al*., 2005; Hara-Kudo, 2005), as well as anti-tumorigenic, anti-inflammatory, anti-proliferative, antiviral, anti-parasitic and anti-oxidative properties (Crespy and Williamson, 2004; Fujiki, 2005; Jang *et al*., 2007). Black pepper (*Piper nigrum*) is known as spices due to its pungent quality (Hassan, 2007). It is a flowering vine in the family *Piperaceae*, genus *Piper* and species *Piper nigrum*. Efficiency compounds of pepper consist: cupsaeesin, cupsisin and cupsantine that some of them allay rheumatic aches. Roselle (*Hibiscus Sabdarifa*) is leave use to make a drink locally known as Zobo. The chemical constituents of the Roselle include the flavonoids, gossypetine, and sabdaretine (Pietta, 2000). In addition to their antioxidant capacity they have the possibility to be used as feed colorants (Bottcher*et al*., 2015).

However, this present study is to determine the effects of different natural antioxidants (green tea, black pepper, and roselle) on performance and egg quality of Isa brown laying pullets.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry unit of the Teaching and Research Farm, LadokeAkintola University of Technology, Ogbomosho, Oyo – State, Nigeria. Ogbomoso is situated in derived savannah zone of Nigeria and lies on longitude 4^0 15^1 East of Greenwich meridian and 8^0 15^1 North of equator. The altitude is between 300 and 610mm above sea level, wuth the mean temperature and annual mean rainfall are 26.1^0C and 1217mm respectively. (BATC, 2012).

Procurement of Test Ingredients

Dried Roselle (*Hibiscus sabdariffa*), Green tea (*Camellia sinensis*) and Black pepper (*Piper nigrum*L.) were purchased from Ojajaguna local market in Ogbomoso, Oyo state. They were grounded into powdered form and stored for usage.

Experimental Animal and Management

Two hundred (200)layingbirdswere purchased from Amo farmSieberer Hatchery Limited, Awe, Oyo state and used for the experiment. They were stabilized for two weeks before the commencement of the feeding trial. The birds were randomly allotted to eight(8) dietary treatments of 25birds each in a Completely Randomized Design. On arrival, birds were fed diets and water mixed with vitamins and glucose to reduce transportation stress. Routine medication and vaccination programs were strictly followed as required for the birds.

They were fed with roselle, black pepper, green tea,combine (roselle + black pepper + green tea) at 0.5g/kg and 1.0g/kg in basal diet (%CP and kcal/kg)respectively and a control feed the layout is as indicated in Table 1. The feeding period lasted for eight(8) weeks.

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Experimental Layout

Table 1: Layout of the experiment

	Control	Black Pepper		Green Tea		Roselle		Combined
	T_1	T_2 (0.5g/kg)	T_3 (1.0g/kg)	T_4 (0.5g/kg)	T_5 (1.0g/kg)	T_6 (0.5g/kg)	T_7 (1.0g/kg)	T_8 (1.0g/kg)
Total of Birds	25	25	25	25	25	25	25	25

Data Collection

At the end of eight (8) weeks of feeding trial, 30 eggs were collected from each dietary treatment with a total of 240 eggs in all. Six eggs per dietary group were analyzed for the egg quality as fresh while the remaining were stored and analyzed at 1,2,3 and 4 week for internal, external and chemical qualities. Data collected were as follows:

External quality parameter:

Egg shape index-The egg shape index is defined as the ratio of width to length of the egg (Duman*et al*., 2016).

Egg Shape Index= $\frac{Egg\ Width}{Egg\ Lenght}$

The egg width and the egg length are both measured using the vernier caliper.

Eggshell thickness-this can be measured by various methods, which may include destruction of the egg (Roberts, 2004). The thickness was measured with a micrometer screw gauge.

Egg weight- The eggs are placed on the sensitive scale to measure the weight of the eggs.

Internal:

Yolk index- this is the ratio of yolk height to yolk length

Yolk index = $\frac{yolk\ height}{yolk\ length}$

Haugh unit-The use of haugh unit scores has been generally accepted as a measure of albumen quality in egg quality studies in recent years. The formula given by Haugh (1937) for haugh unit is HU=100*log(h-1.7w''0.37 + 7.6)

Where HU is haugh unit, h is observed albumen height, W= weight egg

Yolk color.-this was evaluated visually by means of the usual La Roche Scale also known as DSM Yolk Color fan (Bovskova*et al*., 2014).

Yolk weight-the yolk was separated and weighed using a sensitive scale

Blood and Meat spot-the total number of stain in the inner part of the eggs are been noted.

CHEMICAL ANALYSIS

Proximate Analysis

The proximate analysis of eggs was carried out according to the method of AOAC (2005).

Lipid Profile

Lipid profile of egg samples was determined as published by Richmond (1973) to estimate the total cholesterol, low-density lipoprotein, high-density lipoprotein and triacylglyceride and fatty acid profile.

Lipid Peroxidation

This is the oxidative degradation of lipids. Samples of yolk were analyzed for peroxide value (AOAC, 2000).

Statistical Analysis

The data collected was analyzed using a completely randomized design (SAS, 2000). Significant differences among treatments were separated by Duncan's multiple range test.

Results and Discussion

Results

Effects of Natural Antioxidants and Storage time on Physical Properties of Eggs

Table 2 shows the main and interaction effects of treatment of different natural antioxidants on both the internal and external egg quality. Result shows that Green Tea (*Camellia sinesis*) improves (P<0.05) the yolk color of the egg than the other. Both Green tea and Black pepper have significant effect (P<0.05) on the yolk percentage. Black pepper increases (P<0.05) the Haugh unit while it shows to be lowered (P>0.05) in the combine. The inclusion levels of the natural antioxidants on the internal and external quality of egg reveals that there were no significant (P>0.05) differences in the two inclusion level of 0.5g/kg and 1.0g/kg of feed but they have numerically higher values in the external parameters and internal parameters. Inclusion level 1.0g/kg has high effect (P<0.05) on egg shape index, haugh unit, yolk index, yolk%, yolk color, membrane weight, membrane % and blood spot compare to 0.5g/kg inclusion level. There were significant differences (P<0.05) in all the treatment stored at different weeks. Egg stored for 4 weeks has the height (P<0.05) effect on the egg weight as it tends to reduce the weight. The egg weight and membrane weight at the fourth week of storage is higher (P<0.05). Yolk colour was improved at week 2 of storage. At fourth week of storage membrane weight has the highest values.

Effects of Natural Antioxidants and storage time on Chemical Properties of Egg

Table 3 shows the effect of natural antioxidants on chemical properties of egg. The natural antioxidants significantly (P<0.05) improves the proximate composition of the poultry eggs. Green tea shows minimal (P>0.05) influence on crude protein, though the combination of the natural antioxidants (Green tea, Roselle, Black pepper) have a significant effect (P<0.05) on the ether extract. The different inclusion levels of the antioxidants have no effect (P>0.05) for both proximate and lipid profile except in the Lipid peroxidation where 1.0g gave higher values (26.87) than 0.5g (24.66). Eggs stored for 4weeks had the lowest value (P>0.05) for the proximate and lipid profiles, though there are no significant (P>0.05) differences in their values. The fresh eggs show high moisture content (P<0.05) but the value for nitrogen free extract is low (P>0.05) in the fresh eggs. The natural antioxidants improve significantly (P<0.05) the proximate composition of the poultry eggs, with the green tea at 1.0g having the highest value (P<0.05) for Moisture contents, black pepper 0.5g having the highest (P<0.05) for CP and combine 1.0g having the highest value (P<0.05) for E.E. The lipid profile was also improved significantly (P<0.05) by the inclusion levels of the natural antioxidants. The storage periods had a significant (P<0.05) effect on the M and CP value of the eggs. The Lipid profile was not significantly

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(P>0.05) affected either. The chemical analysis result shows that the storage periods of the 1.0g inclusion eggs does not have significant (P>0.05) effect on the proximate composition of the poultry egg. The lipid profile of the 1.0 inclusion was however affected significantly (P<0.05).

Discussion

Results of this study showed that all the egg quality parameters, such as egg weight, egg shape index, yolk color, e.t.c were significantly (P<0.05) affected by the inclusion of the natural antioxidants (roselle, green tea, black pepper) in the layer's diet. Our data showed that the egg weight was affected by dietary inclusion of green tea and black pepper (0.5 g/kg of diet) during the whole experimental period beyond the two other treatment group. This is in contrary to the research carried out by Melo*et al.* (2016), who reported no significant differences in egg weight between control and black pepper supplemented groups. Conversely, inclusion of black pepper in laying birds diet in the current study increased egg weights beyond the control.

Haugh unit is a measure of the egg quality inside the shell. Haugh unit value obtained from the relationship between height of albumen and egg weight (albumen quality). Inclusion of roselle in laying birds diet had the highest Haugh unit percentage compared with control and other treatment groups. However, Saki *et al.* (2014) observed no significant effect on Haugh unit from roselle supplementation. Hilmi*et al.* (2015) reported that supplementation of piperine in quail diets at a level of 30 mg/kg body weight increased Haugh unit. In contrary with the current findings, Lokaewmanee*et al.* (2013) found that Haugh unit was not influenced by dietary supplementation of 0.5% black pepper in laying birds diets. According Mohammed Al-harti (2014) Green tea had a beneficial effect on haugh unit value of stored eggs and yolk cholesterol of fresh eggs when compared to control which is in accordance with the result of this study. The use of phytogenic additives with antioxidant properties, such as black pepper and roselle, may improve albumen quality, as previously reported by Bozkurt *et al.* (2012). In addition, the bio-active ingredients of herbal plants were shown to protect magnum and uterus, and encourage the albumen secretion in laying birds (Nadia *et al.*, 2008).

The egg yolk colour score was significantly increased by the addition of green tea in laying hen diets compared with the other treatment groups. The egg yolk colour was stated to be influenced by the consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids (Hammershøj*et al.*, 2010). Santos-Bocanegra*et al.* (2004) and Lokaewmanee*et al.* (2013) reported that capsanthin improved egg yolk color and was responsible for the deep red color of the egg yolk. The yolk colour score (yellowness of egg yolk) was increased in the layers fed the 2.0% green tea diet compared with that of the control diet. Later, Uuganbaya*et al.* (2006) reported that the eggshell thickness and shape index were significantly reduced in layers fed 1.0% or 2.0% Japanese green tea diets compared to that of the control.

The percentage of composition for moisture, crude protein, ether extract and ash were affected by the antioxidants as there were significant difference between the antioxidants and the control. This correspond with Tounkara*et al.* (2011) which also stated that there were significant differences between Roselle seeds with respect to total ash. The result shows that the inclusion of green tea at 1.0 had the highest value for moisture content, showing a significant (P<0.05) effect on the moisture content of the egg. This result is close to agreement with a previous result from a research by Kojima and Yoshida (2008); Wei *et al.* (2012) who suggested that green tea treatment could affect the egg quality and egg production performance (Uuganbaya*et al.* 2005;

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Panja 2007), but the results were inconsistent and even very different from each other. The moisture content of the eggs without antioxidants (control) increase as the storage period increases, however the moisture contents of the stored egg containing antioxidants did not increase as the storage period increases, but remain almost stable with slight reduction as the storage period increases. The total ash was higher in the natural antioxidants compared to the control thou there were no significant differences in the inclusion level and the storage time. There was significant difference between the storage periods on nitrogen free extract (NFE)

Lipid oxidation is one of the main reasons for foods deteriorating and causes a significant reduction in their nutritional value and taste (Xi *et al.*, 2012). Triacylglyceride are the main constituents of body fats which have to be kept in low levels; addition of antioxidants increased the triacylglyceride levels in black pepper while there was a decrease in the levels of triglycerides in the combination of the antioxidants (black pepper, green tea and Roselle). The result of this study were in accordance to Melo*et al.* (2016) who also observed that Triacyglycerides level increased significantly (P< 0.05) with increase inclusion level of black pepper in the diet. Chen *et al*. (2020) result also shows that triacylglycerides decreases in green tea. The levels of high density lipoprotein (HDL), the good type of cholesterol in roselle compared to the combination of Roselle, green tea and black pepper while the levels of low density lipoprotein (LDL) were significantly increased in green tea. Though green tea significantly increased high-density lipoprotein and no significant difference was observed in the low-density lipoprotein (LDL) according to Chen *et al*. (2020). Malondialdehyde is an indicator of oxidative stress. Its level was significantly increased in green tea and low in Roselle. In the study of Chen *et al*., (2020) MDA in stored eggs numerically decreased, but not significantly, by dietary antioxidant supplementation, this is because storage period was relatively short. The combined inclusion at 1.0 had the lowest value for Cholesterol with significant difference (P>0.05). Green tea supplementation of layer diets reduced the cholesterol content of the egg yolk (Biswass and Wakita, 2000). Ariana *et al*. (2011) also observed that feeding green tea extract or powder improved FCR and decreased feed intake, low density lipoprotein (LDL) and blood cholesterol and reduced triacylglycerides and yolk cholesterol and increased HDL to cholesterol or LDL ratio. The beneficial effects of Green tea could be attributed to its contents of flavonoids, phenolic extracts, catechins and beta-carotene (Mohammed Al-Harthi, 2014).

Conclusion

Conclusively, no particular trend was observed with the influence of the various natural antioxidants on both external and internal characteristics of eggs from ISA brown. However, the yolk color was preferred with the inclusion of the natural antioxidant compared to the control.

Green tea improves the yolk color better than others. Black pepper greatly improves the CP, E.E and the lipid profile of the eggs. The inclusion levels have no influence on the proximate and the lipid profile. The chemical properties deteriorate with the storage time.

Recommendations

Natural antioxidants are hereby recommended for better and improved chemical qualities of eggs. Green tea as a natural antioxidant is hereby recommended to improve the physical properties of egg. There were no significant differences in the inclusion level on both physical and chemical properties, the two inclusion level (0.5g/kg and 1.0g/kg) are hereby recommended. Though the egg quality deteriorates with time, eggs should be stored at maximum of two weeks

Table 2: Effect of natural antioxidants on external, internal qualities and period of storage of layer's egg

Treatment	External qualities					Internal qualities							
	Egg Weight	Egg Shape Index	Shell Weight	Shell %	Shell Thickness	Albumen Height	Haugh Unit	Yolk Color	Yolk Index	Yolk Weight	Yolk %	Membrane Weight	Membrane %
Natural antioxidants													
Control	49.92^{b}	0.83^{c}	4.24^{c}	8.60^{c}	0.52^{b}	7.83^{b}	95.99^{b}	3.23^{b}	0.66^{a}	13.09^{c}	26.55^{bc}	0.22^{ab}	0.45^{ab}
Green Tea	49.39^{b}	0.89^{a}	4.39^{b}	8.97^{b}	0.45^{c}	8.10^{ab}	96.01^{b}	3.93^{a}	0.56^{b}	13.40^{ab}	27.44^{a}	0.22^{ab}	0.44^{ab}
Black Pepper	50.23^{ab}	0.87^{b}	4.47^{b}	8.95^{b}	0.50^{b}	8.24^{a}	96.68^{ab}	3.42^{b}	0.58^{b}	13.66^{a}	27.42^{a}	0.21^{b}	0.42^{b}
Roselle	50.12^{ab}	0.89^{a}	4.25^{c}	8.56^{c}	0.45^{c}	8.08^{a}	96.09^{a}	3.17^{b}	0.64^{a}	13.39^{abc}	27.05^{ab}	0.18^{c}	0.35^{c}
Combine	51.04^{a}	0.87^{b}	4.70^{a}	9.23^{a}	0.55^{a}	6.88^{c}	89.15^{c}	3.24^{b}	0.50^{c}	13.27^{bc}	26.24^{c}	0.23	0.46^{a}
SEM	0.25	0.01	0.04	0.07	0.01	0.08	0.29	0.07	0.01	0.09	0.17	0.01	0.01
P.Value	*	*	*	*	*	*	*	*	*	*	*	*	*
Inclusion Level													
0.5	50.27	0.87	4.47	8.96	0.49	7.68	93.95	3.36	0.56	13.36	26.86	0.20	0.41
1.0	49.88	0.88	4.33	8.75	0.49	8.16	96.48	3.51	0.62	13.44	27.25	0.21	0.43
SEM	0.17	0.00	0.03	0.05	0.01	0.05	0.20	0.05	0.01	0.06	0.12	0.00	0.01
P. Value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Period Of Storage													
Fresh	53.08^{ab}	0.78^{b}	4.74^{a}	8.96^{b}	0.40^{c}	11.67^{b}	95.74^{a}	2.19^{c}	0.38^{a}	13.30^{c}	25.08^{c}	0.14^{d}	0.26^{d}
1 Week	53.36^{a}	0.78^{b}	4.44^{b}	9.80	0.37^{d}	5.25^{d}	89.01^{b}	1.77^{d}	0.16^{c}	14.22^{ba}	31.26^{a}	0.18^{c}	0.39^{c}
2 Week	45.55^{c}	0.78^{b}	4.13^{c}	9.11^{b}	0.38^{d}	8.75^{c}	73.05^{c}	5.42	0.24^{b}	13.92^{b}	30.63^{b}	0.21^{b}	0.47^{b}
3 week	52.46^{b}	1.27^{a}	4.18^{c}	8.00^{d}	0.40^{b}	13.66^{a}	69.20d	5.38	2.17	12.48^{d}	23.89^{d}	0.25	0.47^{b}
4 Week	45.23^{c}	0.78^{b}	4.50^{b}	8.47^{c}	0.66^{a}	1.09^{e}	52.92^{e}	2.52^{b}	0.07^{d}	13.12^{c}	24.70^{c}	0.27	0.57^{a}
SEM	0.27	0.01	0.04	0.08	0.01	0.08	0.03	0.08	0.01	0.09	0.18	0.01	0.01
P.Value	*	*	*	*	*	*	*	*	*	*	*	*	*
Interactions													
Treatment* Inclusion	*	*	*	*	*	*	*	*	*	*	*	*	*
Treatment* Week	*	*	*	*	*	*	*	*	*	*	*	*	*
Inclusion level* Week	*	*	NS	NS	*	*	*	*	*	*	*	*	*
Treatment* inclusion*week	*	*	*	*	NS	*	*	*	*	*	*	*	*

NOTE: SEM: Group standard error of mean; ^{a,b,c,d}: Means with different superscripts on the same row are significantly different (P<0.05).

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Table 3: Effect of natural antioxidants on chemical properties of egg

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Treatment	Proximate					Lipid Profile				Lipid Peroxidation
	M%	C.P%	E.E%	T.A%	NFE%	CHO	TAG	HDL	LDL	MDA
Natural antioxidants										
Control	67.87^{b}	12.29^{b}	17.09^{a}	1.19^{c}	3.90^{a}	6.07^{a}	6.33^{a}	30.80^{b}	524.18^{a}	22.39^{d}
Green tea	68.61^{a}	11.59^{c}	15.77^{a}	1.24^{b}	2.79^{b}	647.26^{a}	490.07^{b}	25.07^{d}	450.34^{b}	31.79^{a}
Black pepper	68.14^{b}	13.31^{a}	14.41^{d}	1.29^{a}	2.09^{c}	521.33^{b}	521.48^{b}	28.67^{c}	388.36^{c}	25.21^{c}
Roselle	67.73^{b}	12.48^{b}	15.17^{b}	1.33^{a}	4.05^{a}	638.81^{a}	441.76^{c}	31.10^{a}	519.36^{a}	21.01^{d}
Combine	66.97^{c}	12.68^{b}	14.73^{c}	1.30^{a}	1.96^{c}	4.71^{b}	4.89^{b}	19.33^{e}	354.43^{c}	27.31^{b}
SEM	0.08	0.08	0.08	0.01	0.08	15.22	3.10	0.09	14.69	0.48
P.Value	*	*	*	*	*	*	*	*	*	*
Inclusion level										
0.5	67.72	12.56	15.14	1.27	3.41	6.23	5.23	27.97	4.89	24.66^{b}
1.0	68.24	12.48	15.49	1.28	2.53	5.51	4.82	26.98	4.27	26.87^{a}
SEM	0.06	0.06	0.06	0.01	0.06	11.09	2.24	0.06	10.7	0.36
P. Value	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Storage Period										
0 week (Fresh)	68.63^{a}	12.60^{a}	15.17^{b}	1.27	2.34^{b}	5.39^{c}	4.47^{b}	24.68^{c}	4.25^{b}	26.99^{b}
2 week	67.32^{C}	12.62^{a}	15.58^{b}	1.28	3.19^{a}	6.41^{a}	6.01^{a}	27.05^{b}	4.94^{a}	31.72^{a}
4 week	67.98^{b}	12.18^{b}	15.19^{a}	1.28	3.37^{a}	5.80^{b}	4.63^{b}	30.70^{a}	4.57^{b}	18.59^{c}
SEM	0.07	0.07	0.07	0.01	0.07	12.88	2.7	0.08	12.43	0.44^{a}
P. VALUE	*	*	*	NS	*	*	*	*	*	*
Interactions										
Treatment* Inclusion	*	*	*	*	*	*	*	*	*	*
Treatment* Week	*	*	*	*	*	*	*	*	*	*
Inclusion level* Week	*	*	NS	NS	*	*	*	*	*	*
Treatment* inclusion*week	*	*	*	*	NS	*	*	*	*	*

NOTE: SEM: Group standard error of mean; ^{a,b,c,d}: Means with different superscripts on the same row are significantly different (P<0.05). M= Moisture content, C.P= Crude protein, E.E- ether extract, T.A= Total Ash, N.F.E= Nitrogen Free Extract, CHO= Cholesterol, TAG= Triacylglycerides, HDL= High density lipoprotein, LDL= Low Density lipoprotein, MDA= Malondialdehyde

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