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Reproductive Response of Rabbit Does to Diets Containing Varying Levels of Horseradish (Moringa oleifera) Leaf Meal

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Abstract
Twenty-four females and four male rabbits (New Zealand White and Chinchilla breed) aged between four to five months, with initial body weight of 1363-1460 g were used to evaluate the reproductive performance of rabbits fed diets containing varying levels of horseradish (*Moringa oleifera*) leaf meal (MOLM).The rabbits were assigned into four treatment groups consisting of six rabbits per group and replicated thrice in a complete randomised design and fed four diets designated T_1, T_2, T_3 and T_4 with 0, 10, 20 and 30 % MOLM. The study lasted for 16 weeks. The treatment diets were offered to the rabbits at 8:00 am and 5:00 pm daily. The rabbitry, faeces trays, feeders and drinkers were washed daily and clean water was provided *ad-libitum* throughout the experimental periods. The does (female rabbits) were mated at five months of age at a mating ratio of 1 buck to 6 does and at weight not less than 1.5 kg. Mating was carried out in the morning and evening and the does were taken to the bucks hutches for mating. twelve days after mating, the does were palpated for pregnancy and if no foetus were detected; the does were re-mated. The does were weighed prior to mating, weekly thereafter and following parturition. The birth traits evaluated were Gestation Length (GL), Gestation Gain (GG), Litter size at birth (LS), Litter birth weight (L B W), Kindling loss (KL), Neo-natal mortality (NNM) which is expressed in percentage. Also, co-efficient of milking capacity was computed. The weaning traits measured were Litter size at weaning (LSW), Litter weight at weaning (LW), Litter weight gain (LWG), Weaning sex ratio (WSR), Survival ratio to weaning (SRW) which is usually expressed in percentage. All data obtained were subjected to analysis of variance (ANOVA).The litter birth weight ranged from 32.10 – 38.07 g for T_1 –T_3 (0 - 20 % MOLM) while T_4 (30 % MOLM) and T_3 (20 % MOLM) recorded the highest values ($P<0.05$) of 55.20 and 59.10, respectively for gestation gain. The weaning traits were significantly affected by the dietary treatments, with T_3 having the highest ($P< 0.05$) litter size (3.33) at weaning and survival rate at weaning 66.60 %. T_2 (10 % MOLM) and T_3 (20 % MOLM) recorded excellent performance 442.30 g, 408.40 g and 462.52 g, 421.84 g for mean litter weight at weaning and litter weight gain, respectively. Consequently, it was concluded that up to 20 % MOLM can be included in the diet of rabbits without any deleterious effect.
Keywords: Reproductive Performance, Rabbit Does, Moringa

Introduction

Interest in consumption of Rabbit meat is growing because of its low sodium, cholesterol and fat content as compared with other meat sources. Also the rabbit meat is nearly white, fine grained, delicately flavoured, nutritious, appetizing, high in good quality protein, contains a high percent of minerals than other meats like, beef, poultry, mutton and chevon. It has a good meat – bone ratio and is acceptable to the general consumers in most countries of the world.(Amaefule *et al*., 2005).

The feeding habit of rabbit (*Oryctolagus cunniculus*) does not compete with humans, because it can survive on vegetable basal diets. (Omole and Ajayi, 2006). However, rabbit breeders are encountering feeding problems because of increase in the number of livestock industry in most African countries today who have solely depended on concentrates for their animals. (Omole and Ajayi, 2006). To make rabbit production more viable as a small scale business, there is need for the development of cheap sources of feed as an alternative to supplement or replace cereal or protein sources in diet of rabbit in order to make production of rabbit more profitable. The high cost of conventionally used plant protein sources mainly soyabean, groundnut and cotton seed cake pose a major problem.

It is evident that the conventional sources of feed can no longer adequately supply the needs of the fast growing livestock industry. (Abubakar , 2008). Despite the large number of legume grains in Nigeria, only soyabean, cotton seed cake and groundnut cake are mainly used in livestock feeding. Several other locally available species that exhibit remarkable adaptation to tropical conditions have been underutilized and under-exploited for livestock feeding (Apata and Ologhobo, 2004). Alternative or unconventional plant protein sources are therefore, been sought for, to be adapted in new feeding scheme. (Omole and Ajayi , 2006).

Horseradish *(Moringa oleifera)* leaf meal is a good natural source of protein and can be conveniently

used to replace soyabean or groundnut cake in livestock diet (Ozumba, 2000). The author stated that, *Moringa* leaves contain, four times the calcium in milk, seven times the vitamin C in orange, three times the potassium in banana, two times the protein in milk and four times the vitamin A in carrot, that *Moringa oleifera* is also a natural source of protein with great potential. The leaves contain magnesium, potassium, and all of the essential amino acids like, cysteine, histidine, arginine, methionine, lysine, phenylalanine, leucine, Isoleucine and valine. *Moringa* leaves are eaten by sheep, pigs, cattle, goats and rabbits, the leaves are also used to feed fishes. *Moringa* leaves are probably ranked as the best of all the vegetables in the tropical. They contain very strong concentration of vitamins A, C and B – complex vitamins, iron, protein, zinc, selenium which is unusual for a plant source, (F A O, 1995).

Church World Service, Dakka, (1994) recommended the addition of dried leaf powder of *Moringa* to improve the protein content of food since 6.7 g of protein is contained in 100 g of fresh leaves. The authors also recommended that during the months of pregnancy or breast feeding, a woman should consume 65 g of protein daily, so 38 % of her protein needs will be satisfied by 100 g of *Moringa* pod. 10.3 % of her protein needs for each day will be satisfy by 100 g fresh *Moringa* leaves. *Moringa l*eaves (fresh) contain more than twice the amount of protein (2.8 g/100 g) found in spinach.(Olugbemi *et al*., 2010).

The public are now conscious of high quality animal protein, like rabbit meat hence the need for the use of non-conventional feed ingredient like horseradish (*Moringa oleifera)*, which is draught resistant and unexploited (Ozumba, 2000).

Rabbit is prolific, easy and cheap to maintain, produces animal protein of public health interest, consequently rabbit can bridge the gap between the demands and supply of quality animal protein, hence the need for intervention to boost productivity.

MATERIALS AND METHOD

Location of study

The research work was conducted at the Federal Capital Territory (F C T) Rabbit Multiplication Centre, Zuba - Abuja. F C T is located in the Derived Savannah and Guinea Savannah Agro-ecological zone of Nigeria, within latitudes 09 ^{o} 40' N, and longitudes 07 ^{o} 29 $^{'}$ E with an altitude of 341.92 m above sea level (Federal Capital Territory Metrological Records, 2011). It is characterised by two seasons namely, rainy/wet season (April-October) and dry/harmathan season (November- March). The south-westerly wind is the predominant wind during the rainy season while north easterlies prevail during the dry season. Dust haze and heat waves are the dominant weather conditions during the dry season, while lightning, thunderstorm, rainstorm and rain shower occur in the rainy season (Federal Capital Territory Metrological Records, 2011).

The hottest and coldest periods in the F C T are in February and December respectively, Normal mean maximum temperatures range between 29 ^{0}C and 37 ^{0}C while minimum temperature ranges between 18 ^{0}C and 24 ^{0}C. The annual rainfall ranges between 1198 mm and 1940 mm. (Federal Capital Territory Metrological Records, 2011).

Source and preparation of *Moringa oleifera* leaf meal

Moringa leaves were harvested during the dry season from Kuje Area Council of Federal Capital Territory, Abuja. The leaves were washed, spread out on a concrete clean floor and dried for a period of 3-4 days under a shady condition to reduce loss of vitamins, especially Vitamin A as described by the Church World Service, Dakka, (1994). The dried leaves were ground, sieved and packaged for analysis.

Four experimental diets were formulated, with crude protein content of 18 % in line with Aduku and Olukosi, (1990). Diet T_1 was the control with 0 % horseradish (*Moringa oleifera*) leaf meal, while diet T_2, T_3 and T_4 contained 10 %, 20 % and 30 % horseradish (*Moringa oleifera*) leaf meal, respectively formulated on nutrient to nutrient basis (Table 1).

Twenty four (24) female and 4 male rabbits all of New Zealand White, and American Chinchilla breeds between the ages of 4–6 weeks were used for this research work. Two weeks before the arrival of the weaned rabbits, the rabbitry, hutches, feeders and drinkers were properly cleaned, washed and disinfected. The pens were illuminated with electric bulbs to provide warmth for the experimental animals. Each animal was housed in a wooden hutch with wire mesh and net at the base, top and side s of the hutches .The rabbits were administered with 0.5 ml of ivomec each, sub-cutenously for the control of both ecto and endo-parasites and they were allowed a two week period for acclimatization. After the acclimatization period, the rabbits were randomly allotted to four dietary treatments comprising of six rabbits per treatment group of three replicate with two rabbits per replicate using the complete randomized design (CRD). The treatment diets were offered to the rabbits at 8:00 am and 5:00 pm daily. The rabbitry, faeces trays, feeders and drinkers were washed daily and portable water provided *ad-libitum* throughout the experimental periods.

The does (female rabbits) were mated at five months of age and at weight not less than 1.5 kg. The does were weighed prior to mating, weekly thereafter and following parturition until the end of experiment. The mating ratio of 1 buck to 6 does as recommended by Aduku and Olukosi, (1990), was adopted. Mating was

carried out in the morning and evening and the does were taken to the bucks hutches for mating. Twelve days after mating, the does were palpated for pregnancy and if no foetus were detected; the does were re-mated.

The birth traits evaluated were Gestation Length (GL), Gestation Gain (GG), Litter size at birth (LS), Litter birth weight (LBW), Kindling loss (KL), Neo-natal mortality (NNM) which is expressed in percentage as described by Aduku and Olukosi ,(1990) and co-efficient of milking capacity while the weaning traits measured were Litter size at weaning (LSW), Litter weight at weaning (LW), Litter weight gain (LWG), Weaning sex ratio (WSR), Survival ratio to weaning (SRW).All data obtained were subjected to analysis of variance (ANOVA) according to the procedure of statistical package for social science, 16.00 (SPSS 16, 1980). Means were separated using the Duncan multiple range test.

RESULTS AND DISCUSSION

The proximate composition of horseradish (*Moringa oleifera*) leaf meal

The proximate composition of MOLM (Table 2) showed that, it contains, crude protein, 26.20 %, crude fibre 10.00 %, ether extract 13.00 %, ash 10.00 %, nitrogen free extract 33.00 % and metabolizable energy value of 3538.00 (kcal/kg). The crude protein, ether extract and energy content of *Moringa oleifera* as shown in Table 2, meets the nutritional needs of rabbits. This conforms to the findings of Gupta *et al*. (1999), Abubakar, (2008), Okereke *et al*. (2009) in rabbit feeding. The fibre content (10.00 %) is in line with the recommended range by Aduku and Olukosi, (1990). Ozumba (2000) confirmed that horseradish (*Moringa oleifera)* leaf meal is a good protein source that can be conveniently used to replace soyabean in rabbit diet, The dry matter and ether extract values are in close agreement with the findings of Onyekwere *et al*. (2010) who evaluated the effects of Bambara nut waste meal on rabbits.

The proximate composition of the experimental diet fed to rabbits.

It was observed (Table 3) that, the values of dry matter increased with increase in *Moringa oleifera* leaf meal inclusion (96.88-97.40 %) The crude fibre content also followed the same trend with the highest values in T_4 (12.43 %) and the least values in T_1 (10.30 %) while values for ether extract and nitrogen free extract declined with increasing levels of MOLM (5.00 and 52.03 % in T1 – 4.00 and 48.74 % in T4, respectively). Crude protein content fell within the same range (18.55-18.59 %) for control and treatment groups.

The dry matter values obtained in this study are in conformity with the values obtained by Lawal *et al*.(2010) who evaluated the effects of soyabean based meal diet on Albino Rats with or without mineral supplementation while the crude protein content (18.55-18.59 % for T_1 and T_4, respectively), fall within the recommended ranges by Johnson (2006), Aduku and Olukosi, (1990) and Cheeke *et al*. (2006). The value for ether extract obtained in this study were higher than the values obtained by Onyekwere *et al.* (2010) but the values obtained for ash content are in conformity with the values obtained by Onyekwere *et al*. (2010) but lower than that obtained by Odeyinka *et al*. (2008).

The anti nutritional factor analysis of horseradish (*Moringa oleifera*) leaf Meal

The result obtained on anti nutritional factor revealed that MOLM contains 0.45 mg of oxalate, phytate 2.57 mg, trypsin inhibitor 3.0 mg, tannin 21.19 mg and saponin 1.60 mg per 100 g, However, they were found to fall within the normal range (Table 4)

The anti-nutrient content of horseradish (*Moringa oleifera*) leaf meal is below lethal levels. This is in conformity with the findings of Enechi and Odunwodu, (2003), who stated that, phytochemical composition and anti-nutrients in *Moringa oleifera* leaves are low. This low level of anti- nutrient content as revealed on Table 4 makes the inclusion in animal feed non- toxic and will not adversely affect their health and growth as observed by Ozumba (2000), who also stated that *Moringa* is a good source of forage for livestock and fish. Olugbemi *et al*. (2010) in evaluating the suitability of MOLM confirmed that, it contains all essential amino acids, which makes it (*Moringa oleifera*) one of the most valuable sources of feed ingredients for monogastric animals.

Reproductive Performance

Effects of feeding diets containing varying levels of horseradish (*Moringa oleifera)* leaf meal on birth traits of does.

Of all the parameters studied in Table 5, only the Litter birth weight and gestation gain were significantly ($p<0.05$) affected by MOLM. Diet T_3 (20 %) MOLM inclusion level recorded the significantly highest values of birth weight compared to others. Also, It was observed from the study that 20 % MOLM inclusion level had the significantly highest gestation gain of 59.10 g with T_I and T_2 having the least values of 39.07 g. The co-efficient of milking capacity was significant ($P< 0.05$) across the treatment, with T_3 having the significantly highest value of 113.70 g while T_1 recorded the significantly lowest value of 101.20 g

The values obtained for litter birth weight from this study is in line with the reported values of Aduku and Olukosi (1990), Guptal *et al*. (1999) and Okereke *et al*. (2009) who investigated the factors influencing pre-weaning body weight of rabbits and effect of feeding graded levels of soaked acacia pods in rabbits feeding but contradicted that of Odeyinka *et al*. (2008) who recorded no significant effects on birth weight of litters from does fed varying levels of MOLM replacing Centrosema at 30 % inclusion level.

According to Dauda *et al*. (2009), the best way in assessing the suitability of a feeding material for rabbit nutrition is to include graded levels in the diets at the same time ensuring all that nutrients required by the animal are supplied and the measure of performance to know the optimum inclusion level. The authors noted that *Moringa* plant is of great scientific interest, since it possesses many valuable properties (Dauda *et al*., 2009), which include high protein content of the twigs, leaves and stem, high protein, oil, and sugar content in the seed which are all needed for proper growth , reproduction and overall performance of rabbits.

Effects of feeding varying levels of horseradish (*Moringa oleifera)* leaf meal on weaning traits of does

Feeding varying levels of *Moringa oleifera* leaf meal to does showed significant effect of MOLM on litter weaning size, litter body weight gain and survival rate (Table 6). The litter size at weaning was significantly higher in does on 20 % (3.33) MOLM. The litter weaning weight was significantly ($P<0.05$) different across the treatments. From this study it was observed that, the weaning weight increased from rabbits fed diets T_1 to T_3 and then decreased in those on diet T_4 [T_1(401.60), T_2 (442.30), T_3 (462.52) and T_4 (347.21)].

Dietary treatment significantly, affected the litter weight gain with values ranging from 309.81 in T_4 (30 %) - 424.45 in T_3 (20 %). The litter weight gain followed the same pattern as the litter weight at weaning with T_3 (20 %) and T_2 (10 %) dietary treatments groups recording the highest litter weight gain. T_3 (20 % MOLM inclusion level) had the highest survival rate at weaning (66.67 %), with T_4 recording (58.25 %), T_2 (55.88 %) and T_1 (50.00%). The weaning sex ratio revealed that more male rabbits survived to weaning with T_3 having the highest number of males to females at weaning (3:1), followed by T_2 (2:1) and T_4 (2:1) with T_1 recording the least value (1:1)

The values recorded agrees with the reported values of Isaac *et al*, (2010) who reported average litter size at weaning of 3, 2.3, 3 and 3.5 for four respective breeds of rabbits (New Zealand white, American Chinchilla, Giant white and Californian white). The values were lower than the findings of Odeyinka *et al.* (2007), who reported litter size at weaning of (5.2) in a study to evaluate the effects of feeding does with varying levels of *Moringa* leaf meal on. reproduction. This finding contradicts the report of Odubote and Somade (1992) who reported no significant ($p> 0.05$) difference for litter size at weaning when they investigated the effect of breeds on reproductive performance of rabbits. These findings were in line with those reported by Aduku and Olukosi, (1990), Odubote and Somade, (1992) and Ilori *et al*. (2003) in a study on post weaning performance of rabbits in rainforest ecological zone in Nigeria. The values reported by the authors mentioned above, were rather lower than those reported by Prayaga and Eadyu, (2002) who reported significant ($p<0.05$) effects in the weaning weights of litters among the following breeds of rabbits, Flemish Giant, Californian White and American Chinchilla. The authors recorded average weaning weights of 1050 g, 810 g, and 475 g for the breeds, respectively. The authors also indicated that the large litter sizes at birth will not likely result in a large weight at weaning and that parity could influence weaning weight of rabbits. The lowest value of litter weight at weaning obtained in T_4 may be an indication that the weaned rabbits may not have been able to digest the fibre content at that level (30 %) MOLM inclusion while high percentage survival rate at weaning is an indication of good nursing and mothering ability (Isaac *et al*. 2010).

Cost benefit analysis of feeding diets containing graded levels of horseradish (*Moringa oleifera*) leaf meal to weaned rabbits

Table 7 showed progressive decrease in the cost of feed with increase in horseradish (*Moringa oleifera*) leaf meal in the diet.The result showed significant ($P<0.05$) difference across the treatments, with T_1 (control) having the highest cost per kg weight gain (₦12. 85) and T_3 (20 %) recording the lowest cost of ₦ 3.05 per kg weight gain

Conclusion And Recommendations

It can be concluded based on the results of this study that the anti-nutritional factors in *(Moringa oleifera*) leaf meal are below lethal levels and that the animals fed 20 % inclusion level of *Moringa oleifera* leaf meal performed significantly better in term of litter size, litter birth weight, gestation gain and survival rate. Consequently it is concluded that, up to 20 % level of inclusion of MOLM in rabbits' diets did not have any deleterious effect on the performance of the rabbits. It is also recommended that further studies on the effects of feeding diets containing horseradish (*Moringa oleifera*) leaves on haematological indices and diseases management should be conducted to harness the role played by the horseradish (*Moringa oliefera*) tree also known and called the "miracle tree".

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Table 1. Composition of experimental diet

	Dietary treatments %			
Ingredients	T_1	T_2	T_3	T_4
Maize	46.69	45.13	43.39	41.53
Groundnuts cake	27.91	26.52	24.97	23.15
MOLM	0.00	2.95	6.24	9.92
Rice offal	20.00	20.00	20.00	20.00
Bone meal	3.00	3.00	3.00	3.00
Growers premix	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Salt	0.50	0.50	0.50	0.50
Palm oil	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Crude protein	18.00	18.00	18.00	18.00
Crude fibre	10.17	10.36	10.55	10 .78
Metabolizable energy (Kcal/kg)	2722.25	2712.25	2634.27	2521.57

KEY: MOLM- *Moringa oleifera* leaf meal

*** vitamine A=10,00011:D_3 =2,000IU :E51U : Nicotinic acid=20mg K=2mg: Riboflvine=4.2mg: =5mg:: B=0.01mg:pantothenic acid**

***Minerals: Se= 100mg: Cu=1.0mg:Fe=20mg:Iodine=0.8mg:Choline=3mg:Mg=56mg:Ca=1.25mg:Lysine,Mehtionine and TeTramycine(Broad –spectrum anti-biotics and promoters)**

Table 2. Proximate composition and energy value of horseradish (*Moringa oleifera)* leaf meal

Nutrients	Composition (%)
Dry matter	92.20
Moisture	7.80
Crude protein	26.20
Crude fibre	10.00
Ether extract	13.00
Ash	10.00
Nitrogen free extract	33.00
Metabolizable Energy (kcal/kg)	3538.00

Table 3. Proximate composition of experimental diet fed to rabbits

	Dietary treatments (%)			
Nutrients (%)	T_1	T_2	T_3	T_4
Dry matter	96.88	96.90	97.00	97.40
Moisture	3.12	3.10	3.00	2.60
Crude protein	18.55	18.56	18.57	18.59
Crude fibre	10.30	10.49	11.65	12.43
Ether extract	5.00	4.50	4.02	4.00
Ash	11.00	11.99	13.50	13.64
Nitrogen free extract	52.03	51.36	49.26	48.74
Total	100.00	100. 00	100.00	100.00

Table 4. Anti nutritional factors of horseradish (*Moringa oleifera*) leaf meal

Anti- nutritional factors	Composition(mg/100 g)	Recommended Safe Level *
Oxalate	0.45	0.54
Phytate	2.57	23.40
Trypsin (mg/10	3.0	16.90
Tannin (mg/10	21.19	31.20
Saponin	1.60	7.02

*Kumar and Amit (2010).

Table 5. Effects of feeding diets containing varying levels of horseradish *(Moringa oleifera)* leaf meal on birth traits.

	Dietary treatment					
Parameters	T_1	T_2	T_3	T_4	SEM	LS
Litter size at birth	4.00	4.17	5.00	4.00	0.18	NS
Litter birth weight (g)	32.10^{bc}	33.65^{b}	38.07^{a}	37.40^{ab}	1.33	*
Gestation length (days)	30.50	30.50	31.00	30.33	0.18	NS
Gestation gain (g)	39.07^{c}	39.07^{c}	59.10^{a}	55.20^{b}	180	*
Kindling loss (g)	45.25	4 4.86	45.83	44.50	1.07	NS
Co-efficient of milking Capacity (g)	101.20^{b}	114.50^{ab}	128.60^{a}	113.70^{ab}	0.14	*
Neo-natal mortality ((%)	1.33	0.33	0.33	0.50	0.15	NS

KEY: ^{abc} means with different superscript on the same horizontal row are significantly ($p<0.05$) different
S E M-Standard error of mean *-Significantly different ($p<0.05$)
L S- Level of significance N S-Not significantly different ($p>0.05$)

Table 6. Effects of feeding varying levels of *Moringa oleifera* leaf meal on weaning traits.

	Dietary treatment					
Components	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**LS**
Litter size at weaning (no)	2.00^{b}	2.33^{b}	3.33^{a}	2.33^{b}	0.17	*
Litterweight at weaning(g)	401.60^{b}	442.30^{a}	462.52^{a}	347.21^{c}	10.86	*
Litter weight gain (g)	369.50^{b}	408.25^{a}	424.45^{a}	309.81^{c}	10.85	*
Survival rate to weaning (%)	50.00	55.88^{b}	66.60^{a}	58.25^{b}	3.05	*
Weaning sex ratio (M:F)	1 :1	2:1	3:1	2:1		

KEY: ^{abc} means: with different superscript on the same horizontal row are significantly ($p<0.05$) different.
S E M-Standard error of means LS - Level of significance M:F-Male to female ratio

Table 7. Cost/ benefit analysis of feeding diets containing graded levels of horseradish (*Moringa oleifera*) leaf meal to weaned rabbits

	Dietary treatments					
Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**LS**
Total feed intake (g)	6888.00^{b}	6846.00^{b}	7007.28^{a}	6888.00^{b}	2.58	*
Cost of feed/kg (~~₦~~)	120.00	110.00	97.45	85.70		
Cost of daily feed intake / rabbit (~~₦~~)	10.00^{a}	9.00^{ab}	7.84^{b}	6.80^{bc}	0.40	*
Cost of total feed consumed/ Rabbit (~~₦~~)	826.60^{b}	821.52^{bc}	840.84^{a}	826.60^{b}	0.3	*
Ave. body weight gain (g)	9.35^{b}	10.00^{b}	31.90^{a}	8.00^{c}	5.52	*
Cost of feeding /kg weight gain	12.83^{a}	11.00^{b}	3.05^{d}	10.63^{c}	3.26	*

KEY ^{abcd} means with different superscripts on the same horizontal row are significantly ($p<0.05$) different
S E M- Standard error of means NS- Non-signifiant ($P>0.05$)
LS- Level of significance
*-Significant difference ($p<0.05$)

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