

NITROGEN BALANCE AND MORPHOMETRIC TRAITS OF WEANLING PIGS FED GRADED LEVELS OF WILD SUNFLOWER (*TITHONIA DIVERSIFOLIA*) LEAF MEAL

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ABSTRACT

Leaves of *Tithonia diversifolia* (wild sunflower) were harvested, processed and subsequently referred to as *Tithonia diversifolia* leaf meal (TDLM). Proximate composition and amino acid analyses revealed that TDLM is a relative rich protein source at 20.6% crude protein (CP) content. Lysine, leucine and isoleucine were particularly abundant in TDLM and compared favourably with conventional protein sources such as groundnut cake and hen's whole egg. Pre-feeding trial on standard grower pig diet for 10 days revealed uniform growth among the 24 Large White breed experimental pigs allotted into 4 treatments. Thereafter, a 63-day feeding trial was conducted using four diets containing 19.0% CP and digestible energy value of about 12.55MJ/kg. TDLM progressively replaced soybean at 0 (control), 10, 20 and 30% inclusion levels in diets 1, 2, 3 and 4, respectively. There was a significant reduction ($p \geq 0.05$) in daily feed consumption for pigs across the experimental diets with a range of 390.8 g/day in pigs on the control diet to 261.4 g/day in pigs on diet with 30% TDLM. Average daily weight gain (AWG) and feed conversion ratio (FCR) had a similar trend with AWG varying significantly ($p \geq 0.05$) from 114.3 g/day for pigs on the control diet to 11.7 g/day for pigs on diet with 30% TDLM. Nitrogen retention (NR) had the highest significant ($p \geq 0.05$) value of 2.87 g/N/pig/day for pigs on 10% TDLM followed by 2.26 g/N/pig/day for pigs on the control diet. There were negative net nitrogen retention values of -2.60 g/N/pig/day and -1.70 g/N/pig/day for pigs on 20% and 30% TDLM inclusion levels, respectively. Body length ranged from 55.3 to 61.8 cm; knee to floor ranged from 14.4 to 16.3 cm; and hock to floor ranged from 18.4 to 19.3 cm. Pigs on 10% TDLM based diet surpassed the control diet in body length. Other parameters (live weight, height at withers, chest depth and chest girth) had slightly varying ($p \geq 0.05$) values for pigs across different diets with pigs on the control diet and diet with 10% TDLM inclusion level having consistently higher values. The similarities in most parameters determined for pigs on control diet and 10% TDLM strongly suggests the suitability of TDLM at inclusion levels not exceeding 10%. We concluded that further processing techniques may be used to facilitate better utilization of TDLM in pig rations.

Key words: Morphometric trait, nitrogen balance, pigs

INTRODUCTION

Wild sunflower (*Tithonia diversifolia*) is a green plant that originated from Mexico and widely distributed throughout the humid and sub-humid tropics of Central and South America, Asia and Africa [1]. *Tithonia* was probably introduced into Africa as an ornamental plant and can be found on roadsides and as invader of field crops in the forest savanna transition zones in Nigeria [2].

Tithonia has been a subject of research interest because of the relatively high nutrient concentrations found in its biomass and because of its ability to extract relatively high amounts of nutrients from the soil [3]. The reported uses of *Tithonia* include fodder, poultry feed, pig feed, fuel-wood, compost, land demarcation, soil erosion control, building materials and shelter for poultry. It has also been reported that extracts from *Tithonia* plant parts protect crops from termites [4] and contain chemicals that inhibit plant growth and control insects [5]. Extracts of *Tithonia* also have medicinal value for treatment of hepatitis [6] and control of amoebic dysentery [7]. The use of *Tithonia* as feed stuff for livestock has been suggested and reported [8, 9, 10]. *Tithonia diversifolia* leaf meal has been reported by some of these researchers as a good alternative to maize in non-ruminant diets.

Since pigs performed well when fed with forage meals, the conventional protein required for pigs could be reduced by up to 40% when the ideal dietary protein has required balance of essential and non-essential amino acids. Therefore, an ideal protein can be complemented by the leaf meal [11], hence the choice of pig as the experimental animal in this study. In addition, it has been reported that pigs have the capacity to consume and digest fibre, and leaves from trees, shrubs and crop plants which are relatively high in dietary fibre [12].

The aim of this study, therefore, was to investigate the performance characteristics of growing pigs when fed varying levels of *Tithonia diversifolia* leaf meal as a replacement for conventional protein.

MATERIALS AND METHODS

Preparation of test ingredients

The test ingredients *Tithonia diversifolia* leaf meal (TDLM) was prepared by harvesting daily, the fresh and matured leaves of *Tithonia diversifolia* plants of different ages before flowering. The whole leaves were chopped manually using kitchen knives and then sun-dried. Sun-drying was done for 4 days, and the chopped leaves were manually turned using a rake so as to guarantee even-drying to 12-13% moisture content. Fresh and dried samples of *Tithonia diversifolia* leaves were taken to the laboratory for proximate and chemical analyses even before the inclusion of the dried samples into the diets.

Amino-acid analysis of *Tithonia diversifolia* leaf meal (TDLM)

The amino acid profile of dry *Tithonia diversifolia* leaf meal (TDLM) was determined using described methods [13]. This sample was dried to constant weight, defatted,

hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino acid Analyzer (TSM).

Experimental animals

A total of 24 male growing pigs (about 2¹/₂ months old) of commercially available crosses with a mean body weight of 13.3±0.5kg were used for this study. The experimental pigs were given intramuscular iron dextran injection (0.25ml/piglet) to prevent piglet anaemia and they were also de-wormed at the first week of their arrival.

Pre-feeding trial study

The 24 male growing pigs were all randomized into separate pens for this pre-feeding trial. They were all served a standard growing diet for pigs formulated with conventional feedstuffs as shown in Table 1. Daily feeding rate was 3.30% / live weight [14] and the pre-feeding trial lasted for 10 days. Water was given to the pigs *ad libitum* throughout the period of the trial. The daily feed consumption in g/day was calculated and divided by the average daily weight gain also in g/day to obtain the feed conversion ratio means for all the pigs on the standard pig diet.

Feeding trial

The feeding trial was carried out at the piggery unit of the Teaching and Research Farm (TRF) of the University of Ado-Ekiti for a period of 63 days. Four diets (D1, D2, D3 and D4) were formulated to contain about 19.0% crude protein and a digestible energy value of about 12.55MJ/kg. The control diet (D1) was a standard growing diet for pigs compounded with conventional feed stuffs e.g. maize, soybeans, palm kernel cake (PKC), oyster shell, bone meal, brewer's dried grains (BDG), wheat offals, fish meal, salt and grower premixes. The other three diets were compounded such that *Tithonia diversifolia* leaf meal (TDLM) progressively replaced soybeans at 10%, 20% and 30% inclusion levels in diets 2, 3 and 4, respectively. Daily feeding rate was 3.30% / live weight [14]. Water was given to the pigs *ad libitum* throughout the period of the experiment and FCR was calculated as done for the pre-trial period.

Nitrogen balance trial

For nitrogen retention trial, four pigs with similar weights and sex were selected from each treatment after 63 days of the experiments, and were transferred into metabolism cages locally constructed to facilitate easy feed intake, collection of urine and faeces. Total faeces voided during the last 5 days were collected, weighed, dried at 65-70⁰C in an air circulating oven for 72 hrs and preserved while the corresponding feed consumed was also recorded for nitrogen studies. The nitrogen contents of the samples were determined by an appropriate method [15]. Nitrogen retained was calculated as the algebraic difference between feed nitrogen and faecal nitrogen (on dry matter basis) for the period. Nitrogen digestibility was computed by expressing the nitrogen retained as a fraction of the nitrogen intake multiplied by 100.

Live morphometric traits

The following live morphometric traits were determined using a measuring tape [16]: height at withers, body length, chest girth, chest depth, chest width, hock/knee to floor and live weight.

Statistical analysis

The data collected in the completely randomized experimental design were subjected to statistical analysis using the Minitab Computer Software package (2005 version) [17].

RESULTS

Amino acid profile of TDLM

Amino acids profiles shown on Table 2 indicate that TDLM is rich in some essential amino acids particularly isoleucine, leucine and lysine; also rich in aromatic amino acids like phenylalanine and valine when compared with amino acid profiles of commonly used conventional protein sources like groundnut cake. The comparison with whole egg and FAO/WHO recommended pattern also indicated a fairly balanced amino acid profile (Table 1). However, there seemed to be a deficiency in the quantities of histidine, arginine, glycine and tyrosine when compared to that of groundnut.

The daily feed consumption, average daily weight gain and feed conversion ratio were statistically similar ($p \geq 0.05$) for all the experimental pigs assigned to the four dietary treatments. The daily feed consumption had a range of 350.2 to 360.0 g/day, while the daily weight gain ranged from 112.1 to 114.1 g/day (Table 3).

Feeding trial

The composition of experimental diets fed to the pigs after pre-feeding trial is shown in Table 4. Growth performance indices are shown in Table 5. The daily feed consumption (FC) value was significantly highest ($p \leq 0.05$) for the pigs on the control diet 1 at 390.8g/day and decreased across the other diets at 330.6, 296.4 and 261.4 g/day in diets 2, 3 and 4, respectively.

The average weight gain (AWG) values followed the same trend as above with the highest ($p \leq 0.05$) AWG value obtained for pigs on the control diet 1 at 114.3 g/day with subsequent statistically significant lower values ($p \leq 0.05$) of 60.5, 15.8 and 11.7 g/day for pigs on diets 2, 3 and 4, respectively. There were significant differences ($p \leq 0.05$) among all the FCR values with pigs on the control diet having the best FCR value of 3.4 compared to 5.5, 18.9 and 22.5 recorded for pigs on diets 2, 3 and 4, respectively.

Nitrogen balance trial

Nitrogen balance is shown in Table 6. Nitrogen retention (NR) values of pigs on all diets were significantly different ($p \leq 0.05$). However, those on diet 2 had the highest value of 2.87gN/pig/day, followed by the control diet 1 at 2.26gN/pig/day. Negative nitrogen retention values were recorded for pigs on diets 3 and 4 at -2.60 and -1.70 gN/pig/day, respectively.

Live morphometric traits

Live morphometric traits of the experimental pigs are shown in Table 7. Only live weight value of pigs on control diet 1 had a significantly higher ($p \leq 0.05$) value of 20.2kg. The height at withers of pigs on diets 1 and 2 were similar and higher ($p \leq 0.05$) at 45.6cm than for other diets 3 and 4 at 40.7 and 41.7 cm, respectively.

Body length values obtained for experimental pigs on all diets were similar ($p \geq 0.05$) at 57.8, 61.8, 58.9 and 55.3 cm for pigs on diets 1, 2, 3, and 4, respectively. Pigs on diet 3 had the highest chest width value of 15.9 cm. However, this value was similar ($p \geq 0.05$) to chest width values obtained for pigs on diets 1 and 4 at 15.3 and 15.4 cm, respectively. The significantly lowest ($p \leq 0.05$) value of 13.3 cm was obtained for pigs on diet 2.

The chest depth values of pigs placed on diets 1 and 2 were higher and similar ($p \geq 0.05$) at 28.6 and 25.6 cm, respectively. However, pigs on diets 2 and 3 also had similar ($p \geq 0.05$) chest depth values of 25.6 and 24.7 cm, respectively. Chest girth values of pigs on diets 1 and 2 (56.6 and 54.6 cm, respectively) were also similar ($p \geq 0.05$) and higher than other chest girth values. The knee- to- floor values were similar ($p \geq 0.05$) for all pigs on the experimental diets at 16.3, 16.3, 14.4 and 14.4 cm for diets 1, 2, 3 and 4, respectively. There were no significant differences ($p \geq 0.05$) in the hock to floor values of the experimental pigs placed on diets 1, 2, 3 and 4 at 19.3, 19.3, 19.7 and 18.4 cm, respectively.

DISCUSSIONS

Amino acid profile of TDLM

There is no doubt that TDLM is a potential source of protein with abundant essential amino acids. The abundant protein in some green leaves [18] could be attributed to the ability of green leaves to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as water, carbon dioxide and atmospheric nitrogen. Utilization may however be hindered by the presence of anti-nutritional factors [18, 19].

Pre-feeding trial study

The pre-feeding trial study confirmed the growth uniformity among all experimental pigs. This further confirmed the seeming uniformity in the genetic constitution of the pigs used for the feeding trials. The phenotypic manifestation in the growth performance could be attributed to the different dietary treatments during the experimental feeding trial with varying TDLM levels.

Growth Performance

The reduction in daily feed consumption and daily weight gain as the inclusion levels of TDLM increased from 10% to 30% could be attributed to the low palatability of wild sunflower (*Tithonia diversifolia*) in experimental diets 2, 3, and 4 [9, 20, 21]. Pigs respond to taste faster than human beings due to the fact that pigs have 15,000 taste buds, while human beings have 9,000 [22]. The increase in the bulkiness of the

feed and its inability to satisfy the pigs' energy and protein requirements may be another plausible factor [5, 23].

The alteration in the texture, colour and odour of the finished feed may have contributed to the reduction in feed consumption [9, 20]. According to the reports [5, 23], the presence of anti-nutrient factors in wild sunflower meal could be responsible for growth reduction in both pigs and chicks. It has been observed that depressed feed intake in broilers served TDLM led to reduced weight gain as inclusion level increased. The presence of anti-nutrients such as phytin, tannin, oxalate, alkaloids and flavonoids may have also contributed in part to the growth reduction among the experimental pigs. Phytate represents about 80% of the total concentration of anti-nutrients in both plants and fed grains [24]. A high value of 79.10mg/100g obtained for phytate during phytochemical screening of *Tithonia diversifolia* leaves [19] could have been responsible for the lowered bioavailability of minerals and inhibition of several proteolytic enzymes and amylases [25]. The anti-nutritional nature of phytin lies in its ability to chelate certain mineral elements especially Ca, Mg, Fe and Zn, thereby rendering them metabolically unavailable and leading to the subsequent development of osteomalacia when certain legumes and cereals are fed to growing animals [26]. The variation in FCR was as a result of reduced feed consumption and subsequent poor weight gained in this study. This result suggests further processing technique to improve the voluntary intake of diets containing TDLM.

Nitrogen balance trial

The nitrogen balance recorded in this study had a similar trend with previous report [27] in which pigs were fed with ensiled taro (*Colocasia esculenta*) leaves as replacement for fish meal. Similar report also had the same trend for diets of sugar cane juice and *Xanthosoma* leaves as replacement for soybean protein in pigs' diet [28]. TDLM at 10% inclusion in the experimental diet had the highest N retention (NR) value compared to pigs on other experimental diets, control diet inclusive. However, the N retention were lower at the range of -2.60 to 2.87g N/pig/day than those reported at range 2.9 to 5.4g N/pig/day [27] and at 6.1 to 9.7g N/pig/day [28]. The highest value of 2.87g N/pig/day for N retention for pigs on 10% TDLM inclusion level suggests that the amino acid profile may not be a limiting factor to pig performance. Tropical tree leaves and shrubs offer a variable amount (170 to 240g/kg) of proteins that are well balanced in essential amino acids, but not well digested by pigs [29].

Live morphometric traits

The decrease in live morphometric traits as the level of TDLM increased could be attributed to the decrease in feed consumption which was complicated by the increased bulkiness of the feed and poor palatability of the diets containing TDLM [23]. Since live morphometric traits are affiliated with body size and weight, poor feed intake would definitely lead to poor weight gain. Decreased feed intake, poor weight gain and high feed conversion ratio had been attributed to the decreased live morphometric traits due to the presence of anti-nutrients such as tannin which usually form insoluble complexes with proteins, thereby interfering with their bioavailability [5, 30].

The similarities in most live morphometric traits between pigs on 10% TDLM diet and the control diet suggest that 10% TDLM could be desirable since the values compared favourably with live morphometric trait values of pigs on conventional diets. It is noteworthy that pigs on 10% TDLM based diet even surpassed the control diet in body length.

CONCLUSIONS

Growing pigs could perform optimally with 10% inclusion level of TDLM in their diets. They could also tolerate the inclusion level of TDLM not more than 20% in their diet as there were no apparent setbacks in their live morphometric traits. Reduction in feed intake as TDLM inclusion increased could be attributed to poor palatability occasioned by the ample presence of anti-nutrients such as phytin, tannin, oxalate, alkaloids and flavonoids. A 30% inclusion level of TDLM adversely affected feed consumption, daily weight gain, feed conversion ratio. It is evident that improvement in the utilisation of TDLM can be engendered by further processing techniques with a view to reducing the content of anti-nutritional factors.

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Table 1: Standard diet fed for 10d before feeding trial study

Ingredients	Quantity
Maize	35.0
Soybean meal (45.0% CP)	15.0
Palm kernel cake (18.8% CP)	10.0
Brewers dried grains (25.3% CP)	12.0
Wheat offals (11.9%)	20.0
Fish meal (63.1% CP)	2.0
Bone meal	4.0
Oyster shell	1.0
*Premix	0.25
Salt (NaCl)	0.25
Total	100.0
Analysed Composition	
Crude protein	19.28
Crude fibre	6.51
Ether extracts	4.73
Ash	4.06
Moisture content	11.06
Carbohydrate	53.82

**Premix contained: vitamins A(10,000,000iu); D(2,000,000iu); E(35,000iu); K(1900mg); B12(19mg); Riboflavin(7,000mg); Pyridoxine(3800mg); Thiamine(2,200mg); D Pantothenic acid(11,000mg); Nicotinic acid(45,000mg); Folic acid(1400mg); Biotin(113mg); and Trace elements as Cu(8000mg); Mn(64,000mg); Zn(40,000mg); Fe(32,000mg); Se(160mg); I₂(800mg) and other items as Co(400mg); Choline(475,000mg); Methionine(50,000mg); BHT(5,000mg) and Spiramycine(5,000mg) per 2.5kg.*

Table 2: Proximate analysis and amino acid profile of TDLM compared with groundnut cake, whole egg and FAO/WHO recommended pattern (g/100g protein)

FAO/WHO	*TDLM	^a GNC	^b Hen's whole egg	
Dry matter, %	11.0			
Crude protein	20.6			
Crude fibre	18.9			
Ether extracts	4.0			
Carbohydrate	42.5			
Ash	14.0			
Amino acid				
Lysine	5.35	3.80	3.94	3.44
Histidine	2.25	2.40	1.50	-
Arginine	6.19	11.00	3.81	-
Aspartic acid	13.32	-	-	-
Threonine	4.26	2.80	3.19	2.50
Serine	5.13	-	-	-
Glutamic acid	12.19	-	-	-
Proline	3.91	-	-	-
Glycine	5.09	6.00	6.00	-
Alanine	6.08	-	-	-
Valine	5.29	5.20	4.75	3.13
Methionine	1.56	1.10	2.00	-
Cystine	1.04	1.60	1.13	-
Meth.+Cys.	2.60	2.70	3.13	-
Isoleucine	4.28	-	3.50	2.50
Leucine	7.55	6.50	5.19	-
Tyrosine	3.53	3.90	2.50	3.75
Phenylalanine	5.47	5.20	3.19	-

*TDLM, *Tithonia diversifolia* leaf meal, GNC is groundnut cake

Amino acid profile for TDLM was determined using methods described by Speckman et al (1958)

^aAmino acid profile for groundnut cake was adopted from Ravindran and Blair (1992).

^bAmino acid profile for hen's whole egg was cited by Robinson (1987).

FAO/WHO and whole egg amino acid profiles were converted to g/100g from initial unit of g 16 g⁻¹ N by Robinson (1987) before conversion to g kg⁻¹ and cited by Fasuyi (2006).

Table 3: Growth performances of pigs placed on pre-feeding trial standard diet

Parameters	Standard diet			
	T1	T2	T3	T4
Daily feed consumption (g/day)	350.2 ^a	352.1 ^a	360.0 ^a	358.2 ^a
Average daily weight gain (g/day)	112.1 ^a	112.4 ^a	114.1 ^a	114.0 ^a
Feed conversion ratio	3.1 ^a	3.1 ^a	3.2 ^a	3.1 ^a

T₁, T₂, T₃ and T₄ are experimental treatments each containing 6 pigs that are eventually used for the TDLM feeding trial.

No significant differences ($p > 0.05$) were found across means in the same row.

Table 4: Composition of experimental diets (g/100g)

Ingredients	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Maize (11.0% CP)	35.00	30.00	25.00	20.00
Soybean meal (45.0% CP)	15.00	10.00	10.00	5.00
Palm kernel cake (18.8% CP)	10.00	20.00	15.00	15.00
Brewers dried grain (25.3% CP)	12.50	12.50	12.00	12.00
Wheat offals (11.9% CP)	20.00	10.00	10.00	10.00
Fish meal (63.1% CP)	2.00	2.00	2.00	2.00
*TDLM (20.62% CP)	0.00	10.00	20.00	30.00
Bone meal	4.00	4.00	4.00	4.00
Oyster shell	1.00	1.00	1.00	1.00
**Premix	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25
Total %	100.00	100.00	100.00	100.00
Analysed composition				
Crude protein	19.28	19.43	19.68	18.94
Crude fibre	6.51	6.85	6.80	7.03
Ether extract	4.73	4.94	4.76	4.78
Ash	4.06	4.93	6.00	7.03
Moisture Content	11.60	11.05	11.51	11.86
Carbohydrate	53.82	52.80	51.25	50.36

*TDLM, *Tithonia diversifolia* leaf meal

**Premix contained: vitamins A(10,000,000iu);D(2,000,000iu); E(35,000iu); K(1900mg); B12(19mg); Riboflavin(7,000mg); Pyridoxine(3800mg); Thiamine(2,200mg); D Pantothenic acid(11,000mg); Nicotinic acid(45,000mg); Folic acid(1400mg); Biotin(113mg); and Trace elements as Cu(8000mg);Mn(64,000mg); Zn(40,000mg); Fe(32,000mg); Se(160mg); I₂(800mg) and other items as Co(400mg); Choline(475,000mg); Methionine(50,000mg); BHT(5,000mg) and Spiramycine(5,000mg) per 2.5kg.

Table 5: Growth performance of pigs fed varying dietary levels of TDLM

Parameters	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Daily feed consumption (g/day)	390.8 ^a	330.6 ^a	296.4 ^c	261.4 ^d
Average daily weight gain (g/day)	114.3 ^a	60.5 ^b	15.8 ^c	11.7 ^d
Feed conversion ratio	3.4 ^a	5.5 ^b	18.9 ^c	22.5 ^d

TDLM, Tithonia diversifolia leaf meal.

Means with the same superscripts in the same row are not significantly different (p>0.05)

Table 6: Nitrogen utilization of pigs fed varying dietary inclusion levels of TDLM based diets

Parameters (g/N/pig/day)	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Nitrogen Intake	7.49 ^a	7.02 ^b	2.04 ^c	2.04 ^c
Faecal Nitrogen	3.33 ^a	3.32 ^a	3.77 ^b	3.03 ^c
Urinary Nitrogen	1.90 ^a	0.85 ^b	0.96 ^c	0.66 ^d
Nitrogen retention	2.26 ^a	2.87 ^b	-2.60 ^c	-1.70 ^d

TDLM, Tithonia diversifolia leaf meal

Means with the same superscripts in the same row are not significantly different (p>0.05)

Table 7: Live morphometric traits of pigs fed varying dietary inclusion levels of TDLM based diets

Parameters	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Live weight (kg)	20.2 ^a	15.6 ^b	14.4 ^b	13.9 ^b
Height at Withers (cm)	45.6 ^a	45.6 ^a	40.7 ^b	41.7 ^b
Body Length (cm)	57.8 ^a	61.8 ^a	58.9 ^a	55.3 ^a
Chest width (cm)	15.3 ^a	13.3 ^b	15.9 ^a	15.4 ^a
Chest depth (cm)	28.6 ^a	25.6 ^{ab}	24.7 ^b	19.7 ^c
Chest girth (cm)	56.6 ^a	54.6 ^a	50.7 ^b	46.7 ^c
Knee to floor (cm)	16.3 ^a	16.3 ^a	14.4 ^a	14.4 ^a
Hock to floor (cm)	19.3 ^a	19.3 ^a	19.7 ^a	18.4 ^a

TDLM, Tithonia diversifolia leaf meal

Means with the same superscripts in the same row are not significantly different ($p > 0.05$)

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