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Nutrient digestibility of pregnant WAD ewe fed Mexican sunflower leaf meal (MSLM) based diets

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ABSTRACT
Fifteen days prior to parturition, studies was conducted using sixteen West African dwarf (WAD) ewes weighing between 22.80 and 26.03kg on a basal diet of *Panicum maximum* were allotted into 4 treatment groups A, B, C and D of 4 replicates each. The MSL replaced Wheat bran (WB) gravimetrically at 0, 15, 30 and 45%. Treatment A served as control, while animals in treatments B, C and D received Mexican sunflower leaf (MSL) at 15, 30 and 45% respectively. The experiment lasted for one week. Digestibility was determined using a 6-d total fecal collection. Ewes were given *ad-libitum* access to feed and water and routine vaccination and medication followed standard procedures. The 16 ewes were previously brought to heat (Oestrus) by synchronization and served by two rams (1:8). Parameters measured were voluntary dry matter intake (VDMI), which comprised concentrate dry matter intake (CDMI) and grass dry matter intake (GDMI), apparent digestibility coefficients of DM, CP, EE, NDF, ADF, ADL, organic matter (OM) and energy. Data were analyzed using descriptive statistics and ANOVA. The VDMI (g/d) varied from 392.30 - 695.00 for ewes during digestibility. CDMI (g/d) varied from 191.35 – 565.01 and GDMI (g/d) varied from 137.52 – 227.32 for ewes and this was significant ($p < 0.05$) with animals on treatment B having the highest CDMI while animals on treatment C having the highest GDMI respectively. Apparent digestibility coefficients of EE, NDF, ADF, ADL, OM, and energy were similar. Approximately 75.5 ±1.1% of the VDMI came from the supplement. Diets containing 15% MSLM was superior to others for CDMI (191.35 – 565.01 g/day), C P absorbed (42.05 – 99.45 g/day), CP apparent digestibility (86.49 – 91.47%), Digestible DMI (52.34 – 85.20 g/day/kg$W^{0.75}$) and Digestible CPI (14.31 – 28.35 g/day/kg$W^{0.75}$) while GDMI (137.52 – 227.32 g/day) for diets containing 30% and 45% MSLM were significant ($p < 0.05$). Therefore Mexican Sunflower Leaf Meal could suitably replace Wheat bran in the diets of pregnant ewe up to 30% level of inclusion without eliciting any adverse effect.

Keywords*: *Mexican Sunflower, Nutrient digestibility, Pregnant West African dwarf ewe

INTRODUCTION
In Nigeria, *Tithonia diversifolia "*Mexican sunflower" grows in the wild and is available all year round in fadama areas. It is a perennial shrub that grows to a height of 1.5 to 3m. The leaves are ovate to triangular from 15 to 30cm long. The flowers resemble the single dahlia; and are 5 to 8cm

across with bright yellow colour. It reproduces from seeds and vegetative re-growth of the basal stem when the plant is slashed. *Tithonia diversifolia* produces higher number of capitula and seeds per plant (Muoghalu and Chuba, 2005). Sorn Suheang (2003) reported that when peeling the bottom 3-4cm of cuttings, it increased the rate of appearance of green shoots compared with non-peeling. *Tithonia* is drought tolerant and needs well drained soil, which is moist to dry. According to Rios, (2002) it is easy to grow by planting cuttings derived from the lower, more fibrous part of the stem. It can also be established from seed which is said to have a germination of about 16% when the seeds are collected immediately but that after four months storage the germination may reach 90%. Under practical conditions, over 75% of seeds germinated when the seeds were planted in the field during the rainy season (Moughalu and Chuba, 2005). In addition, extracts from *Tithonia* plant parts reportedly protect crops from termites (Adoyo *et al.*, 1997) and contain chemicals that inhibit plant growth (Baruah *et al.*, 1994; Tongma *et al* (1997) and control insects (Carino and Rejestes, 1982; Dutta *et al.*, 1993). Extracts of *Tithonia* are said to have medicinal value for treatment of hepatitis (Lin *et al.*, 1993; Kuo and Chen, 1997) and control of amoebic dysentery (Tona *et al.*, 1998). Among the various uses of *Tithonia*, its value is one that farmers in western Kenya frequently report (Jama *et al.*, 2000).

There is however, paucity of information on its value as a fodder for sheep. The performance of pregnant West African Dwarf ewes fed Mexican Sunflower Leaf (MSL) was investigated.

MATERIAL AND METHODS

Plant materials (Mexican sunflower)

Mexican sunflower "*Tithonia diversifolia*" leaf obtained at the Teaching and research farm, University of Ibadan was harvested at approximately 6 weeks by slashing and carrying after the onset of rains. The stems were cut 50cm above the ground and sorted into leaves (Tarawali *et al.*, 1995). The stems were sun-dried on a clean cemented platform until crisp. The leaves were partially ground and packed into sacks, weighed and stored in a silo. The samples were bulked together and manually mixed to obtain as uniform a product as possible. A representative sample was collected from it for proximate analysis. The MSL sample was oven dried at 105^0C for 24 hours (to constant weight), milled and stored in air tight, sealed polythene bags prior to chemical analysis.

Pen Management

The pen and metabolic cages were swept and dusted. They were later fumigated with Izal (Saponated cresol) at the ratio of 1: 200 water (1: 200 liters of water) and also with diazintol (diazinon) at the rate of 2ml/litre of water (diazintol a strong and broad spectrum insecticide, acaricide and larvicide). Wood shavings were later spread on the floor of individual pens including the adaptation and spare pens; the wood shaving was changed fortnightly till the end of the trial.

Experimental Design and treatments

Sixteen WAD sheep were divided into four groups of four animals each based on dentition. Each group was randomly assigned to one of 4 treatments and individual animals were completely randomized within the experimental pens in the unit.

The statistical model was: $\gamma_{ij} = \mu + \alpha_i + e_{ij}$

where γ_{ij} = individual observation

μ = general mean of population

α_i = treatment effect due to diets

e_{ij} = error effect

Animal Feeding

The WAD sheep were fed Mexican sunflower wheat bran blended ration (Table 1). *Panicum maximum* leaves were harvested from pasture and range management unit of Animal Science Department at the Teaching and Research farm of the University of Ibadan. Leaves were allowed to wilt over-night before feeding and this was chopped manually with cutlass into 3-5cm pieces just before feeding.

Panicum maximum was given to all the treatments as basal diets. Feeding was done daily at 08:00 and 16:00 hrs (GMT). Fresh water was provided for each animal *ad libitum* daily. The animals were fed at 5% of body weight calculated from the diets on dry matter basis.

Table 1: Ingredient composition of experimental ration.

Ingredients %	Rations			
	A	B	C	D
MSLM[1]	0.00	15.00	30.00	45.00
Wheat bran	45.00	30.00	15.00	0.00
Cassava peel	33.20	33.20	33.20	33.20
Palm kernel meal	10.00	10.00	10.00	10.00
Ground nut cake	10.00	10.00	10.00	10.00
Oyster shell	0.50	0.50	0.50	0.50
Bone meal	0.50	0.50	0.50	0.50
Mineral/Vit. Premix	0.30	0.30	0.30	0.30
Common salt	0.50	0.50	0.50	0.50

MSLM = Mexican Sunflower Leaf Meal, A = 0% MSLM, B = 15% MSLM, C = 30% MSLM, D = 45% MSLM

Digestibility Study

Digestibility was carried out by the total faecal and urine collection method (McDonald *et al*., 1995). Animals were weighed and each animal was penned in an individual cage for 14 days, with a 7 day adjustment and another 7days collection period. Faeces and urine voided were collected. Individual total urine was collected and a 10% aliquot were kept in a refrigerator (0-4 ^{0}C) for analysis. Faecal samples were dried at 65 ^{0}C for 48hrs to a constant weight wrapped in aluminium foil, milled and stored in air-tight bottles until analyzed.

Apparent Digestibilities (AD) of dry matter (DM), organic matter (OM), energy, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of experimental diets (Treatments) were calculated using the formula:

$$\frac{\text{Nutrient consumed (as feed)} - \text{Nutrient in feaces}}{\text{Nutrient consume}} \times 100$$

OR

$$\frac{\text{Nutrient [Input} - \text{Output]}}{\text{Input}} \times 100$$

Laboratory Analysis

Samples of dried MSLM and *Panicum maximum* were oven dried at 105 ^{0}C to constant weight, milled and stored in air tight, sealed polythene bags prior to chemical analysis. The nutrient composition of dried milled sample of MSLM, *Panicum maximum* leaves or branchlets and faeces were determined according to the procedure of AOAC (1990). Nitrogen content of feeds, faeces and urine were determined by the micro-kjeldahl technique using the Markham's distillation apparatus.

Results obtained were used for the calculation of DM, nutrient digestibility by the WAD sheep.

Neutral Detergent Fiber (NDF): NDF was determined by the Van Soest and McQueen (1973) methods as the residue after the reflux with 0.5m H_2SO_4 (TetraoxosulphateVI acid) and acety/trimethy/ammonium bromide.

Acid Detergent Fiber (ADF): ADF was determined by the Van Soest (1963) method as the residue after extraction with boiling neutral solutions of sodium lauryl sulphate and EDTA.

The percentage hemicellulose was obtained by deducting the ADF from the NDF. The percentage cellulose was obtained by deducting the percentage lignin from the ADF. ADF residue is primarily lignocellulose. The cellulose is dissolved by using 72% H_2SO_4 (TetraoxosulphateVI acid) solution. The remaining residue consists of lignin and acid soluble ash.

Statistical Analysis

The experimental design was completely randomized and the Data obtained were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of SAS software (SAS, 1999). Treatment means were compared by Duncan test of the software.

RESULTS AND DISCUSSION

Nutrient Digestibility during Pregnancy

Dry Matter Intake (DMI)

Dry Matter Intake (DMI) at the pregnancy stage is presented in Table 5. Ewe on diet B recorded the highest Concentrate Dry Matter Intake (CDMI) (565.01g/d) and this was significant *(P<0.05)* while ewe on diet D recorded the least CDMI. An increase in the inclusion level of Mexican sunflower leaves in the concentrate diet brought about a reduction in CDMI of ewes on diet D, suggesting a substitution effect. On the contrary, ewe on diets C (227.32g/d) and D (221.57g/d) had the highest Grass Dry Matter Intake (GDMI) and these were significantly *(P<0.05)* higher than GDMI consumption for ewes on diets A (137.52g/day) and B (166.59g/d). Dietary effects on digestible DMI were significant *(P<0.05)* with optimum values (85.20 g/day /kg$W^{0.75}$) being observed with ewes on treatment B (15%MSLM) in the diet dry matter. The improvement in DM digestibility was probably the direct result of increased dietary density of fermentable carbohydrate as the organic matter in the mixture that is Wheat bran / Mexican sunflower leaves was highly digestible. Effects of feeding level and its implication on DMI digestibility have been stressed (Araba et al., 2002).

Crude Protein

As shown in Table 5, Treatment effects on observed values of crude protein (CP) was not significant (*P>0.05*) for CP digestibility. However, treatment effect on observed values of CP were significant (*P<0.05*) for CP intake and absorbed. Ewes on treatment B (33.67 g/d /kg$W^{0.75}$) had the highest CP intake and this was numerically higher than ewes on treatments A (23.64 g/d /kg$W^{0.75}$) and C (23.49 g/d /kg$W^{0.75}$) but significantly (*P<0.05*) higher than ewes on treatment D (18.41 g/d /kg$W^{0.75}$). Similarly, ewes on treatment B (28.35 g/d /kg$W^{0.75}$) had the highest CP absorbed and this was numerically higher than observed values for ewes on treatments A (19.81 g/d /kg$W^{0.75}$)and C (19.13 g/d /kg$W^{0.75}$) but significantly (*P<0.05*) higher than ewes on treatment D (14.31 g/d /kg$W^{0.75}$). The trend in CP intake, absorbed as obtained in this experiment is in consonance with earlier reports (Reid *et al.,* 1987; Robertson *et al.*, 1996) that higher feed intake leads to higher faecal excretion and absorbed. This report is also in line with digestibility values obtained although no significant difference (*P>0.05*) were observed among treatments.

Energy

Values obtained for energy intake, digestible and digestible coefficient for diets A, B, C and D are shown in Table 5.

Treatment effects on observed values of energy profile were not significant. Ewes on diet B had the highest energy intake (383.94Mcal/kgW$^{0.75}$) and the highest digestible energy (226.91Mcal/kgW$^{0.75}$) but these values were not statistically significant from the rest diets. Ewes on diet D had the least energy profile across the diets

Acid Detergent Fiber (ADF)

As shown in table 6, treatment effect on observed value of acid detergent fiber (ADF) was significant (*P<0.05*) for faecal ADF. Ewes on treatment B (52.34g) had the highest faecal ADF and this was significantly (*P<0.05*) higher than ewes on treatments C (37.78g), A (36.24g) and D (35.38g).

There were no statistical significance (*P>0.05*) among ewes on treatments C, A and D respectively. Animals on treatment C which had the second highest ADF intake (189.08g/d) among the four treatments recorded the highest ADF digestibility (80.02%). This could be due to efficient use of relative amount of the fiber fraction and high absorptive rate in the intestine in order to meet its physiological needs (Jarrige, 1999). Also relative to wheat bran, sunflower digestion or legumes generally is characterized by a high quantity of readily fermentable substrate for microbial use after mastication.

Neutral Detergent Fiber (NDF)

Treatment effect on observed values of neutral detergent fiber (NDF) was significant (*P<0.05*) for faecal NDF as shown in table 6. Ewes on treatment B (69.36g) had the highest faecal NDF and this was numerically higher than observed values for ewes on treatment C (52.65g/d) but significantly (*P<0.05*) higher than ewes on treatments A (49.39g) and D (48.51g).

Dietary characteristics that promote relatively high numbers of protozoa may indirectly yield high ruminal fibrolytic activity by causing opposite bacterial shift (Mackie *et al.*, 1978) through a process known as defaunation as envisaged in Mexican sunflower leaf meal. Sunflower being a legume has some inherent leguminous factors which enable its better utilization by the rumen microbes amongst which are high propionate production, greater particle breakdown which lead to high ruminal outflow, low rumen fill, and more time is spent in grazing and ruminating grass than legume (Galyean and Goetsch, 1993).

Acid Detergent Lignin (ADL)

Treatment effect on observed value of acid detergent lignin (ADL) was significant (*P<0.05*) for faecal ADL as shown in table 6. Ewes on treatment B (52.68g) had the highest faecal ADL and this was significantly (*P<0.05*) higher than observed values for ewes on treatments A (42.78g) and D (41.76g) but numerically higher than ewes on treatment C (46.52g)

There were no statistical significance (*P>0.05*) among ewes on treatments A and D respectively. Ewes on treatment D which had the second least ADL intake among the four treatments recorded the highest ADL digestibility. This could be due to efficient use of relative amount of the fiber fraction due to high absorptive rate in intestine in order to meet its physiological requirements (Jarrige, 1999). However, the improvement for in digestibility of the cell wall fraction is more probably the result of an improved rumen environment for cellulolysis caused by an increase in rumen pH when Wheat bran is replaced with Mexican sunflower. Araba et al., 2002 also shares this view. The resultant reduction in the rate of starch fermentation would be expected to have a positive outcome for digestion of cell wall constituents.

Table 2 Proximate composition of experimental diet

	Rations			
Constituents %	A	B	C	D
Dry matter	92.00	91.00	90.00	89.00
Crude protein	17.10	16.90	16.50	16.20
Crude fiber	15.70	16.40	17.00	17.50
Ether extract	3.47	3.63	3.70	3.75
Ash	8.60	9.40	10.10	11.30
NFE[1]	55.13	53.67	52.70	51.25
ADF[2]	22.54	26.70	30.85	35.01
NDF[3]	43.15	44.50	45.85	47.20
ADLs[4]	7.92	8.41	9.03	9.85
Gross energy(kcal/kg)	3829.5	3805.5	3781.0	3735.5

NFE= Nitrogen free extract
ADF= Acid detergent fiber
NDF= Neutral detergent fiber
ADL= Acid detergent lignin

Table 3: Chemical Composition of Dried Mexican Sunflower Leaf Meal (MSLM), Wheat bran and *Panicum maximum*

Components	MSLM	Wheat bran	*Panicum maximum*
Dry matter	89.00	89.00	26.00
Crude protein (CP)	16.33	17.00	7.95
Crude fiber (CF)	21.80	8.50	31.00
Ether extract (EE)	2.81	3.50	4.00
Ash	14.68	13.01	8.90
NFE[1]	44.38	57.99	48.15
ADF[2]	42.63	25.00	42.70
NDF[3]	60.00	51.00	74.30
Hemi cellulose	17.37	26.00	31.60
ADL[4]	9.96	8.60	13.87

1. NFE= Nitrogen free extract
2. ADF= Acid detergent fiber
3. NDF= Neutral detergent fiber
4. ADL=Acid detergent lignin

Table 4: Anti Nutritional Factors in MSLM

Component	Quantity (mg/100g)
Total Alkaloid	6.32
Saponin	1.05
Oxalate	5.25
Phytate	8.81
Tannin	5.19
Glycosides	0.42
Phenol	0.53

Source: Ekeocha (2009)

Table 5: Apparent Nutrient Digestibility of WAD sheep fed MSLM based diets during pregnancy

Parameters	Treatment				
	A	B	C	D	SEM
Concentrate Dry Matter Intake g/day	332.84^{ab}	565.01^{a}	298.29^{ab}	191.35^{b}	199.82
Grass Dry Matter Intake g/day	137.52^{b}	166.59^{b}	227.32^{a}	221.57^{a}	33.42
Total Dry Matter Intake g/day	470.36	731.60	525.61	412.92	211.37
Total Dry Matter Intake g/day/kg$W^{0.75}$	101.00	140.67	109.77	91.60	31.61
Organic Matter Intake g/day /$W^{0.75}$	94.34	130.76	101.79	84.63	29.10
Crude Protein Intake g/day /kg$W^{0.75}$	23.64^{ab}	33.67^{a}	23.49^{ab}	18.41^{b}	8.36
Energy Intake Mcal/kg $W^{0.75}$	273.14	383.94	299.25	249.00	85.82
<u>Digestible Nutrient g/day/kg$W^{0.75}$</u>					
Digestible dry matter intake	60.65^{ab}	85.20^{a}	68.11^{ab}	52.34^{b}	25.89
Digestible organic matter intake	55.97	77.91	62.02	47.35	23.71
Digestible crude protein intake	19.81^{ab}	28.35^{a}	19.13^{ab}	14.31^{b}	7.82
Digestible energy intake	159.20	226.91	181.42	138.51	68.53
<u>Digestibility coefficients</u>					
Dry matter	0.60	0.61	0.62	0.57	0.06
Organic matter	0.59	0.60	0.61	0.56	0.06
Crude protein	0.84	0.84	0.81	0.78	0.04
Energy	0.58	0.59	0.61	0.56	0.06

ab: means on the same row with different superscripts differ significantly *($P<0.05$)* S.E.M: Standard error of mean, A = 0% MSLM, B = 15% MSLM, C = 30% MSLM, D = 45% MSLM

Table 6: Cell Wall Fraction Digestibility of WAD sheep fed MSLM based diets during pregnancy

Parameters	Treatment				
	A	B	C	D	SEM
ADF Intake (g/day)	133.75	221.99	189.08	161.60	64.48
ADF Output (g/day)	36.24^{b}	52.34^{a}	37.78^{b}	35.38^{b}	8.82
ADF Absorbed (g/day)	97.51	169.65	151.30	126.22	59.51
ADF Apparent Digestibility (%)	72.90	76.42	80.02	78.11	4.94
NDF Intake (g/day)	245.80	375.20	305.66	254.94	100.38
NDF Output (g/day)	49.39^{b}	69.36^{a}	52.65^{ab}	48.51^{b}	11.95
NDF Absorbed (g/day)	196.41	305.84	253.01	206.43	91.08
NDF Apparent Digestibility (%)	79.91	81.51	82.77	80.97	3.07
ADL Intake (g/day)	45.44	70.62	58.47	49.58	19.48
ADL Output (g/day)	42.78^{b}	65.37^{a}	46.52^{ab}	41.76^{b}	12.79
ADL Absorbed (g/day)	2.66	5.25	11.94	7.82	13.33
ADL Apparent Digestibility (%)	6.74	7.97	9.32	15.26	16.85

ab: means on the same row with different superscripts differ significantly ($P<0.05$) . S.E.M: Standard error of mean, A = 0% MSLM, B = 15% MSLM, C = 30% MSLM, D = 45% MSLM

CONCLUSION

From the results of this study, it appears that replacing wheat bran with MSLM up to 30% level of diet dry matter had a favorable effect on the rumen ecosystem leading to increases in the microbial breakdown of the cell wall constituents in the diet. Replacing Wheat bran with Mexican sunflower leaf meal for pregnant ewes led to increases in apparent digestibility of dry matter and cell wall constituents with the optimum response being achieved with 30% MSLM in the diet. However, these improvements in digestibility of the overall diet and the fibrous fraction of the diet were not reflected in animal performance at above 30%, which deteriorated as the level of MSLM in the diet was increased. Inclusion of up to 30% MSLM in the diet of pregnant ewe appeared most beneficial to ewes as it will not adversely affect growth performance, nutrient intake but improved digestibility.

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