AN In vitro ASSESSMENT OF SUPPLEMENTARY EFFECT OF CONCENTRATES CONTAINING GRADED LEVELS OF GROUND LINSEED (*Linum usitatissimum*) TO HOUSEHOLD WASTES ON ORGANIC MATTER DEGRADABILITY, SHORT CHAIN FATTY ACIDS, MICROBIAL PROTEIN, METABOLIZABLE ENERGY AND RELATIVE FEED VALUES

[EVALUACIÓN *in vitro* DE LA SUPLEMENTACIÓN CON CONCENTRADO CONTENIENDO NIVELES CRECIENTES DE LINAZA (*Linum usitatissimum*) EN RESIDUO DOMÉSTICO SOBRE LA DEGRADABILIDAD DE LA MATERIA ORGÁNICA, PROTEÍNA MICROBIAL, ENERGÍA METABOLIZABLE Y VALOR DE ALIMENTACIÓN RELATIVO]

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SUMMARY

With the objective to assess effect of level of linseed inclusion in feeds on nutritive value, an *in vitro* OM digestibility (IVOMD), total short chain fatty acids (SCFA:mMol l^{-1}), microbial crude protein (MCP:g kg^{-1} DM), metabolizable energy (MEruminants:MJ kg^{-1} DM) and relative feed values (RFV) of household wastes=HW (Areke-Atela=AA, carrot peels=CaP, cabbage leaf=Cle, cabbage leftover=Clef, onion leaf=OL, onion peels=OP, potato peels=PP, Tela-Atela=TA) supplemented with linseed (LS) containing concentrates=LSC1…LSC5 (39.32, 37.32, 35.32, 33.32, 31.32% noug cake + 58.99% wheat bran + 1.69% salt + 0, 2, 4, 6, 8% LS, as fed basis) were estimated via an *in vitro* gas trial. HWs were mixed with LSC (LSC-HW) at 3:1 ratio. Samples were incubated *in-vitro* with rumen fluid in duplicate and readings recorded at 0, 3, 6, 12, 24, 48, 72 and 96 h of incubation. LSC-HW mixtures had higher CP, IVDMD, IVOMD, ME and SCFA, MCP and RFV than those of HWs alone. IVDMD and IVOMD of the LSC-HW improved with increasing LS levels, mostly at 2 and 4%LSC; but at higher concentrations they declined. AA, TA had high IVOMD. However, IVDMD of AA (with the lowest IVDMD) was much more influenced than TA (with highest IVDMD) by LS levels. Clef had lowest and AA and TA highest ME. SCFA increased over incubation periods and with increasing levels of LS, in Clef improvement (from 0.25 to 0.61 mMol l^{-1}) was significant but in AA (from 0.69 to 0.72 mMol l^{-1}) moderate. Mixing HWs with LSC gave best results at 2%LSC.

Key words: concentrate; gas test; linseed; digestibility; household waste.

RESUMEN

Con el objetivo de evaluar el efecto del nivel de inclusión de linaza en los alimentos sobre el valor nutritivo, digestibilidad in vitro de la MO (DIVMO), ácidos grasos de cadena corta (AGCC: mmol l^{-1}), proteína cruda microbiana (MCP: g kg^{-1} DM), energía metabolizable (EMrumiantes: MJ kg^{-1} MS) y los valores de alimentación relativos (RFV) de residuos domésticos = HW (Areke-Atela = AA, cáscaras de zanahoria = CaP, hoja de col = Cle, repollo sobrante = Clef, hoja de cebolla = OL, cáscaras de cebolla = OP, cáscaras de papa = PP, Tela-Atela = TA), complementado con concentrados conteniendo semillas de lino (LS) = LSC1 ... LSC5 (39.32, 37.32, 35.32, 33.32, 31.32% de torta noug + 58.99% salvado de trigo + 1.69% sal + 0, 2, 4, 6, 8% LS, base fresca) se estimaron a través de análisis producción de gas in vitro. HWs se mezclaron con LSC (LSC-HW) en proporción de 3:1. Las muestras se incubaron in vitro con fluido ruminal por duplicado y lecturas registradas a 0, 3, 6, 12, 24, 48, 72 y 96 h de incubación. Las mezclas LSC-HW tuvieron mayor PC, DIVMS, DIVMO, EM y AGCC, MCP y RFV que los de HWs solo. La DIVMS y DIVMO de la LSC-HW mejoraron con el aumento de los niveles de

LS, en su mayoría a los niveles de 2 y 4% LSC; pero en concentraciones más altas fueron menores. AA y TA tuvieron alta DIVMO. Sin embargo, la DIVMS de AA (con la DIVMS más bajo) fue mucho más influenciada que TA (con mayor DIVMS) por los niveles de LS. El Clef tuvo el menor y AA y AT el mayor contenido de EM. Los AGCC aumentaron durante el período de incubación y con el aumento de los niveles de LS, la mejora de Clef (de 0.25 a 0.61 mmol l-1) fue significativo pero en AA (de 0.69 a 0.72 mMol l-1) fue moderada. La mezcla HWs con LSC dió mejores resultados en el 2% de LSC.

Palabras clave: concentrado; prueba de gas; linaza; digestibilidad; residuos domésticos.

INTRODUCTION

A major constraint to animal production in Ethiopia is inadequacy in the amount and quality of livestock feed. Animals are normally fed on natural pasture and crop residues of low feeding value. These characteristics hamper productivity of ruminants. Crop residues constitute more than 90% of the feed resources available for ruminant livestock in most developing countries (Tchinda *et al*., 1993).

During dry seasons in the tropics and also in Ethiopia there are setbacks in the growth of ruminant domestic livestock due to shortage of feed and thus food processing byproducts and crop residues become the main sources of energy for use by ruminants (Ahn *et al*., 1989; Kibon and Ørskov, 1993, Okojie, 1999), therefore household waste (HW) could add to the feed base and contribute nutrients to livestock. Most HWs are nutritionally poor and deficient in protein and mineral and, therefore, unable to provide proper nourishment for ruminants (Preston and Leng, 1984). Efficient utilization of HWs for animal feeding could improve nutritional status of livestock on poor quality roughages (Getachew *et al*., 2001). High levels of nutritious feeds such as concentrates with linseed supplementation is important to improve the nutritive value of HWs by supplying different nutrients such as protein, energy and essential fatty acids (omega-3 fatty acids, Lardy and Anderson, 1999). Linseed depresses methane production in the rumen, and thus reduces the energy loss and improves energy utilization of ruminant livestock (Machmuller *et al*., 2000).

Although highly relevant to Ethiopian condition, limited information on the feeding value of ground linseed are available when given with HWs (1. kitchen waste: potato, onion and carrot peels, onion leaves and cabbage leftovers; 2. traditional brewery and distillery byproducts: Tela-Atela and Areke-Atela, respectively).

Optimization of HW utilization in ruminant production becomes important because of their poor nutritional qualities due to the presence of refractory and inhibitory substances. Determination of feed factors affecting microbial biomass synthesis *in vivo* is too expensive. Evaluations using *in vitro* techniques are considered relatively simple and economical as it allows assessment of feed energy, rumen fermentation characteristics and microbial biomass synthesis (Krishnamoorthy *et al*., 2005).

This trial was thus conducted with the objectives to evaluate the effect of supplementing HWs with a concentrate containing graded levels of ground linseed on *in vitro* dry matter and organic matter digestibility, total short chain fatty acids (SCFA) production, microbial protein production and metabolizable energy (ME) values of the HWs and the concentrate-HW mixtures.

MATERIALS AND METHODS

Experimental site

The laboratory analyses were conducted in Animal Nutrition laboratory of School of Animal and Range Sciences of the College of Agriculture of Hawassa University, southern Ethiopia.

Sample collection and processing

Linseed, wheat bran, noug cake, brewery/distillery by-products, coffee byproducts and crop residues were purchased from local markets of Hawassa. Household wastes were collected from the cafeteria of the College of Agriculture located at Hawassa and its vicinity and dried according to Negesse *et al*. (2009). The HWs (Table 1) and concentrate (Table 2) were dried in an oven at 65ºC for 72 hours. Samples were then milled using Wiley Mill to pass through a 1.0 mm screen for the *in vitro* gas test and proximate analysis.

Experimental Design and Treatments

The experimental design is shown in Table 2. The 8 HWs were mixed with each of the concentrates, containing five levels of ground linseed, giving rise to 40 combinations. The roughage to concentrate ratio used for *in vitro* analysis was 3:1. The *in vitro* gas tests were done in duplicate.

Table 1. The household wastes used for the *in-vitro* gas test

No	Feed stuff	Abbreviation	Species name	No	Feed stuff	Abbreviation	Species name
1	Areke-Atela	AA	Not applicable	5	Onion leaf	OL	*Allium cepa*
2	Carrot peels	CaP	*Daucus careta*	6	Onion peels	OP	*Allium cepa*
3	Cabbage leaf	Cle	*Brassica oleracea*	7	Potato peels	PP	*Solanum tuberosum*
4	Cabbage left over	Clef	*Brassica oleracea*	8	Tela atela	TA	Not applicable

Table 2. Experimental design and composition of concentrates (% as fed)

Components	Treatment diets (HW-LSC combinations)				
	T1-T8[a]	T9-T16[b]	T17-T24[c]	T25-T32[d]	T33-T40[e]
Household wastes	1-8	1-8	1-8	1-8	1-8
Concentrate-LS mix	LSC1	LSC2	LSC3	lSC4	LC5
Ingredients	Linseed containing concentrate (%, as fed)				
	LSC1	LSC2	LSC3	lSC4	LC5
Wheat bran	58.99	58.99	58.99	58.99	58.99
Linseed	0.00	2.00	4.00	6.00	8.00
Noug cake	39.32	37.32	35.32	33.32	31.32
Salt	1.69	1.69	1.69	1.69	1.69

[a, b, c, d, e] all 8 household wastes mixed with each of 0%, 2%, 4%, 6% and 8% linseed-concentrates, respectively

In vitro gas test

Rumen liquor was obtained from three sheep fed on Rhode grass *(Chloris gayana Kunths*) hay and concentrate in the morning before feeding by using a vacuum pump connected to a glass vacuum container in turn connected to a semi-flexible oro-ruminal probe, flushed with CO_2, filtered through three layers of cheese-cloth and mixed (1:2, v/v) with an anaerobic mineral buffer solution as described by Makkar *et al.* (1995) and revised by Makkar (2000). About 200 mg of each of the air dried and ground (1.00 mm) sample was incubated *in vitro* with rumen fluid in to a calibrated glass syringe of 100 ml following the procedure of Menke and Steingass (1988). *In vitro* analysis for each sample was done in duplicate. The piston was then lubricated with pure oil to ease movement and to prevent escape of gas. The syringes were pre-warmed (39°C) for 1 h, before the addition of 30 ml of rumen-buffer mixture into each syringe. The syringes were incubated in a water bath maintained at 39°C, and were gently shaken every hour during the first 8 h of incubation. Readings were recorded before incubation (0 h) and 3, 6, 12, 24, 48, 72 and 96 h after incubation. The mean gas volume readings (a, b and c) were fitted to the exponential equation: $P= a + b (1 - e^{-ct})$ according to Orskov and Mcdonald (1979) and Blummel and Orskov (1993) using the Neway computer program (X.B Chen, Rowet Research Institute, Aberden) where P=volume of gas produced at time‘t’ (ml); a= gas produced from soluble fractions (ml); b= gas produced from insoluble but with time fermentable fraction (ml); a+b= potential gas production, c= rate of gas production (ml/hr); t= time at measurement (h).

Gas volume from 24 h was used to estimate metabolisable energy (ME), organic matter digestibility (OMD) and total short chain fatty acids (SCFAs) (Menke *et al*. 1979, Menke and Steingass 1988, Blümmel *et al*. 1999).

1) IVOMD (g/kgDM) = 8.89 Gv(ml/200 mg DM per 24 h) +0.448 CP(g/kgDM) + 0.651 crude ash(g/kgDM) + 149
2) ME ruminants (kJ/kg DM) = 146 Gv (ml/200 mg DM per 24 h) + 7 CP (g/kg DM) + 22.4 EE (g/kg DM) + 1242
3) SCFA (m mol L^{-1}) = 0.0239 x Gv – 0.601

Where: Gv, CP, EE and CF are gas volume (ml 200 mg^{-1} DM), crude protein, ether extract and crude fiber (g kg^{-1} DM) contents of the incubated samples, respectively. In vitro dry matter digestibility (%, IVDMD) and organic matter digestibility (%, IVOMD) Digestible organic matter (DOM) and microbial crude protein (MCP) productions were then calculated using the following equations:

4) Digestible organic matter (g/kg DM) = OM (g/kg DM) x IVOMD (%)
5) Microbial crude protein (g/kg DM) = DOM x 0.032 (AFRC, 1993)

Relative feed value

The relative feed value (RFV) is an index used to rank feeds relative to the typical nutritive value of full bloom alfalfa hay, containing 41% ADF and 53% NDF on a DM basis, and having an RFV of 100, which is considered to be a standard score.

6) RFV = DDM (%DM) x DMI (%BW)/1.29 (Uttam *et al.*, 2010);

Where: DDM (digestible dry matter) and DMI (dry matter intake potential as % of body weight) were calculated from ADF and NDF, respectively as:

7) DDM (% DM) = 88.9 - 0.78 x ADF (% DM)
8) DMI (%BW) = 120/ NDF (% DM),
9) RFV = (88.9- 0.78 x ADF (%DM)) x 120/NDF (%DM) / 1.29.

Chemical analyses

The representative samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and ash contents using the method of AOAC (2005). Nitrogen was determined according to the procedure of Kjeldhal and protein was calculated as N x 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al* (1991) using the Ankom Fiber Analyzer (Ankom Technology, 2005). Hemicellulose was computed as a difference between NDF and ADF, and cellulose as difference between ADF and ADL contents.

Statistical analysis

The data were analyzed using SPSS v 19 for Windows. The HW means were calculated using descriptive statistics while they were compared using Duncans Multiple range test. The values were considered significant at P<0.05. The means for the *in sacco* analysis of the data were fitted to the equation of Ørskov and McDonald (1979) using SAS 9.0 Institute (2004) software.

RESULTS AND DISCUSSION

Nutrient content of the feed ingredients, household wastes and concentrates

The nutrient content of the feed ingredients used for concentrate making, household wastes and concentrates are indicated in Table 3.

As presented in Table 3 the CP content of the HWs varied between 8.39 (Cle) and 19.68%DM (OL). Except those (AA, Cle and clef) that are close to the lower limit in CP requirement (8%) of rumen microbes (NRC, 2000 and Elliot 2006) the HWs could provide reasonably good amount of CP. The crude protein (CP) content of the concentrates was reasonably high and complimented the CP value of the HWs when three parts of HWs were mixed with one part of the concentrates (containing graded levels of linseed).

Neutral detergent fiber differed among HWs ranging from 11.01 to 70.75%DM (Cle to PP, respectively). Acid detergent fiber (ADF) constituted about two third of the portion of NDF in the HWs (7.62 and 43.91%DM in Clef and PP, respectively). Nearly half of the ADF of the HWs was found as ADL, possibly because the samples collected were obtained from matured vegetables or roots.

As can be seen from the Table, as expected the concentrate-HW mixtures had higher CP values than those of the corresponding HWs alones but lower than the concentrates. The CP content in OL was in excess of recommended minimum requirements for lactation (120 g/kg DM) and growth (113 g/kg DM) in ruminants (ARC, 1984) (where early and late lactation of dairy cows require 19 and 13%CP: NRC 1989) (optimum growth of growing cattle: 15%CP, Parish and Rhinehart, 2008) but OP, PP and TA were close to lactation and growth CP requirements, and AA, Cle and Clef had slightly lower values as compared to the requirements.

Metabolizable energy

As shown in Table 4, the metabolizable energy (ME) content of the first two treatment diets (6.92 and 6.92 MJ/kg DM) were only slightly different from the last two (7.36 and 7.53 MJ/kg DM).

Clef had the lowest (3.25 MJ ME/kg DM), TA (5.59) and AA (5.87 MJ ME/kg DM) the highest while the rest of the HWs had intermediate ME contents (p<0.05) of which PP had higher (5.01 MJ ME/kg DM) than those of OP, CaP and Cle (4.43, 4.43 and 4.16, respectively).

Table 3. The nutrient content of the household wastes and concentrates containing graded levels of linseed that are used for the *in vitro* trial

		Nutrient content (% DM)						
	DM (%)	Ash	OM	EE	CP	NDF	ADF	ADL
Household Wastes								
AA	28.78^{a}	6.33^{a}	93.68^{d}	0.36^{c}	8.76^{a}	26.69^{bc}	20.86^{c}	10.55^{c}
Cle	29.82^{a}	14.33^{c}	85.68^{b}	026b^{c}	9.64^{b}	11.01^{a}	7.63^{a}	3.53^{a}
Clef	30.79^{a}	17.50^{d}	82.50^{a}	0.17^{ab}	17.39^{e}	22.11^{b}	15.68^{b}	7.80^{b}
CaP	56.21^{e}	18.07^{d}	81.94^{a}	0.27^{bc}	8.40^{a}	23.17^{b}	15.24^{b}	5.24^{a}
OL	36.62^{b}	12.63^{c}	87.38^{b}	0.24^{b}	19.68^{e}	24.63^{bc}	18.99^{c}	8.99^{bc}
OP	48.81^{d}	14.31^{c}	85.69^{b}	0.13^{a}	12.46^{d}	28.39^{c}	23.58^{d}	13.58^{d}
PP	42.49^{c}	9.01^{b}	90.99^{c}	0.26^{bc}	10.81^{c}	70.76^{e}	26.36^{e}	28.91^{f}
TA	36.55^{b}	7.61^{ab}	92.39^{cd}	0.12^{a}	10.43^{c}	53.80^{d}	38.91^{f}	16.36^{e}
Concentrates								
LSC1	92.31^{bc}	7.19	92.82	0.15^{a}	17.00^{ab}	51.51	20.13	5.95
LSC2	91.15^{a}	8.04	91.96	0.21^{b}	18.69^{a}	50.76	19.70	6.11
LSC3	92.38^{bc}	7.49	92.52	0.23^{b}	20.24^{c}	46.18	19.13	5.40
LSC4	92.93^{c}	7.32	92.69	0.24^{bc}	19.71^{bc}	48.18	20.12	5.39
ISC5	91.39^{ab}	7.02	92.99	0.27^{c}	20.65^{c}	44.52	19.14	5.71

Means for household wastes and concentrates within columns with different superscript letters were significantly different ($p<0.05$).

AA= Areke Atela, ADF=acid detergent fiber, ADL=acid detergent lignin, CaP=carrot peels, Cle=cabbage leaf, Clef=cabbage leftover, CP=crude protein, DM=dry matter, EE=ether extract, LSC=linseed containing concentrate, NDF=neutral detergent fiber, OL=onion leaf, OM=organic matter, OP=onion peels, PP=potato peels, TA=Tela Atela

Table 4. *In vitro* DM and OM digestibility (%), gas volume (ml/200 mg DM), ME (MJ/kg DM), short chain fatty acids (mMol/l), digestible organic matter (g/kg DM), microbial crude protein production (g/kg DM) and relative feed value (%) of the household wastes and concentrates.

Household	Parameters							
wastes	IVDMD	IVOMD	GV	ME	SCFA	DOM	MCP	RFV
AA	43.48^{a}	86.85^{d}	31.0^{e}	5.87^{d}	0.69^{e}	808^{d}	26.0^{d}	31.89^{ab}
Cle	63.01^{cd}	68.76^{a}	19.5^{b}	4.16^{b}	0.41^{b}	605^{bc}	19.5^{bc}	38.79^{bc}
Clef	57.66^{bc}	66.62^{a}	13.0^{a}	3.25^{a}	0.25^{a}	501^{a}	16.0^{a}	37.47^{bc}
CaP	64.64^{cd}	71.73^{ab}	21.0^{b}	4.43^{b}	0.45^{bc}	568^{ab}	18.0^{b}	41.21^{c}
OL	74.87^{d}	73.10^{ab}	22.5^{bc}	4.64^{bc}	0.48^{bc}	633^{bc}	20.5^{c}	32.99^{ab}
OP	44.64^{a}	71.49^{ab}	21.0^{b}	4.43^{b}	0.45^{bc}	612^{bc}	19.5^{bc}	28.68^{a}
PP	48.19^{ab}	77.71^{bc}	25.5^{cd}	5.01^{c}	0.55^{cd}	657^{c}	21.0^{c}	48.96^{d}
TA	67.84^{cd}	83.87^{cd}	29.0^{d}	5.59d	0.64^{de}	770^{d}	24.5^{d}	53.29^{d}

Concentrates	GV	ME	SCFA	DOM	MCP
LSC1	30.5^{a}	6.92^{a}	0.67	797	25.5
LSC2	30.5^{a}	6.92^{a}	0.69	797	25.5
LSC3	31.0^{ab}	7.16^{ab}	0.68	794	25.5
LSC4	32.0^{ab}	7.36^{bc}	0.71	818	26.0
ISC5	33.0^{c}	7.53^{c}	0.72	815	26.0

Means for household wastes and concentrates within columns with different superscript letters were significantly different ($p<0.05$).

AA= Areke Atela, CaP=carrot peels, Cle=cabbage leaf, Clef=cabbage leftover, DOM=digestible organic matter, GV=gas volume, IVDMD= in vitro dry matter degradability, IVOMD= in vitro organic matter degradability, LSC=linseed containing concentrate, MCP=microbial crude protein, ME=metabolizable energy, OL=onion leaf, OP=onion peels, PP=potato peels, RFV=relative feed value, SCFA=short chain fatty acid, TA=Tela Atela

The ME content of the HWs was improved by mixing them with all the concentrates, although the improvement was relatively low at 2%LSC and those of Cle, OP and PP was in fact reduced by 2%LSC inclusion (Table 5). At most levels of LSC supplementation, the ME content of CaP, Cle and Clef were much more improved than the rest of HWs.

The HWs with low ME values (Cap, Cle, Clef, PP) have taken advantage of the 6 and 8% inclusion level of linseed in the concentrate. A general trend of reduction was noted in the calculated ME content of the mixtures especially at 2% inclusion level.

The higher levels of linseed reduced ME values because they must have reduced volume of gas production. Concentrate without linseed (0%LSC) and with 6%LSC have produced the highest ME values of most of the HWs.

Short chain fatty acids

Calculated total short chain fatty acids (SCFA) production (mMol/l) of the household wastes (HW) and concentrate-HW mixtures (LSC-HW) from the *in vitro* gas test is presented in Table 4 and 5.

The highest SCFA ($p<0.05$) was from AA and TA (0.69 and 0.64mMo/l) but lowest from Cle (0.25mMol/l), among those that are found in between, PP was still better than the rest (Table 5). Clef (0.25mMol/l) had the lowest and AA the highest SCFA values (0.69mMol/l) while the rest were found in between ($p<0.05$) of which TA and PP had higher SCFA value (0.64 and 0.55mmol/l, respectively).

The concentration of *in vitro* SCFA production of HWs was raised by mixing most of the HWs with the concentrates except with 2%LSC. The highest SCFA production of most of the HWs was produced with 0%LSC and 6%LSC supplementation, whereas Cle, OP and PP had reduced SCFA production, moreover the increments in SCFA production with increasing level of LS in most of the mixtures was not noticeable.

Among household wastes Clef and OL and OP produced lower amount of SCFA, which could be associated with their high amount of CP content (17.38, 19.68 and 12.45% of DM). Similar results were reported by Grings, Blümmel and Südekum (2005) where N-rich incubations produced less SCFA (0.64 to 0.75 mMol/250 mg) than N-low incubations (0.74 to 0.78 mMol/250 mg).

***In vitro* organic matter digestibility and calculated digestible organic matter (g/kg DM)**

Clef had lowest and AA and TA the highest ($p<0.05$) DOM contents; and from among those having DOM values in between, PP had higher DOM than the rest (Table 4).

The *in vitro* organic matter digestibility (IVOMD, %); and calculated digestible organic matter (DOM, g/kg DM) of the concentrates were only slightly higher than those of most of the HWs.

Microbial crude protein

The microbial crude protein production calculated using the formula MCP (g/kg DM) = DOM (g/kg DM) x 0.032 (AFRC, 1993) of the household wastes (HW) and concentrate-HW mixtures is presented in Tables 4 and 5.

Microbial crude protein (MCP) production of the concentrates calculated from DOM vary between 25.5±0.3 (0%LSC) and 26.2±0.4 g/kg DM (6%LSC). Clef produced the smallest (16 g/kg DM) and AA and TA the highest (26.0 and 24.5 g/kg DM) amount of MCP, the rest were found in between, of which PP produced significantly higher than CaP ($p<0.05$). The CP:ME ratio in Clef was very high (54 g CP/MJ ME) which indicates that there was comparatively less energy for the microbes to convert the CP to MCP and thus gave lower MCP yield which was much more improved by supplementing it with the concentrates than the rest of the HWs.

Household wastes (HW) with low MCP potential (Clef) was much more improved when mixed with the concentrates as compared to those with high MCP potential. In most cases 2 and 8%LSC have reduced but 0, 4 and 6%LSC improved MCP production. The trend is that MCP production increased linearly from 2%LSC up to 6%LSC and it started to decline with 8%LSC.

The actual MCP yield, although not the efficiency (4-5 g MCP/MJ ME), was improved by supplementing roughages with the concentrate, however, the level of LS had variable influence over this parameter. Earlier reports on microbial efficiency was approximately 10 g MP/MJ ME (AFRC, 1993) and 7 to 14 g MP/MJ ME (Lebzien, 1996) which are higher than the results in this study.

Microbial efficiency of HWs and LSC-HW mixtures was about 32 g MCP/kg DOM. The CP content of the HWs was ranging between 86 and 152 g/kg DOM and was complimented by the concentrates with higher range of CP (201 to 258 g/kg DOM) which must have improved efficiency of microbial CP production. Variable microbial efficiencies have been reported by different authors; some are not far from the results obtained for the HWs and LSC-HW mixtures in this study while others are different. Stern *et al*. (1994) reported 30 g microbial N/kg of truly degraded organic matter; and ARC (1984) reported the supply of microbial protein to the animal per unit of feed ingested to vary from 14 to 60 g N/kg digestible OM fermented in the rumen. Microbial efficiency expressed as g microbial nitrogen per kg dry matter and organic matter ranged from 21 to 27; and 35 to 44, respectively (Sniffen *et al*., 2006).

Table 5. Results of the *in vitro* gas test conducted on concentrate-household waste mixtures

Concentrate-HWs mixture	Parameters							
	IVDMD	IVOMD	GV	ME	SCFA	DOM	MCP	RFV
0%LSC-AA	72.86^{s}	88.9^{r}	32.50^{mn}	6.09^{fghij}	0.72^{n}	827^{rs}	26.5^{mn}	65.80^{ghij}
0%LSC-Cle	75.04^{v}	85.06^{opqr}	27.50^{ghi}	5.46^{ijkl}	0.60^{hi}	691^{ij}	22.0^{fg}	48.77^{abcd}
0%LSC-Clef	73.28^{stu}	89.00^{r}	27.50^{ghi}	5.34^{hijk}	0.61^{hij}	702^{ij}	22.5^{g}	49.57^{abcde}
0%LSC-CaP	62.88^{l}	82.91^{klmno}	27.00^{fghi}	5.20^{fghij}	0.58^{ghi}	715^{ijklm}	23.0^{gh}	54.77^{bcdef}
0%LSC-OL	78.62^{w}	86.42^{qrs}	30.00^{ijk}	5.73^{fghij}	0.65^{jkl}	752^{mno}	24.0^{hi}	50.10^{abcde}
0%LSC-OP	63.22^{l}	79.87^{hi}	27.00^{fghi}	5.20^{fghij}	0.58^{ghi}	681^{hi}	22.0^{fg}	45.00^{ab}
0%LSC-PP	75.09^{v}	84.37^{nopq}	28.00^{ghij}	5.34^{hijk}	0.60^{hi}	746^{lmno}	24.0^{hi}	70.53^{ij}
0%LSC-TA	74.24^{uv}	81.31^{ijkl}	33.00^{mn}	6.12^{fghij}	0.72^{n}	841^{s}	27.0^{n}	59.80^{efgh}
2%LSC-AA	63.00^{l}	83.15^{klmno}	34.00^{n}	6.23^{fghij}	0.63^{ijk}	742^{klmn}	24.0^{hi}	56.36^{defg}
2%LSC-Cle	73.05st	81.05^{ikj}	18.00^{b}	3.99^{b}	0.37^{b}	557^{b}	17.5^{b}	72.38^{ij}
2%LSC-Clef	80.31^{x}	66.54^{b}	23.00^{cd}	4.71^{cde}	0.49^{d}	602de	19.0^{cd}	50.10^{abcde}
2%LSC-CaP	62.25^{l}	81.31^{ijkl}	30.00^{ijk}	5.65^{fghij}	0.65^{jkl}	782^{opq}	25.0^{ijk}	54.49^{bcdef}
2%LSC-OL	68.61^{p}	74.90^{def}	25.00^{defg}	4.91^{defg}	0.53^{ef}	652^{gh}	21.0^{ef}	50.10^{abcde}
2%LSC-OP	50.90^{h}	80.22^{i}	12.50^{a}	3.15^{a}	0.24^{a}	476^{a}	15.0^{a}	46.12^{abcd}
2%LSC-PP	57.29^{k}	75.86^{ef}	17.50^{b}	3.81^{b}	0.35^{b}	571^{bcd}	18.5^{bc}	71.44^{ij}
2%LSC-TA	74.00^{tuv}	81.92^{ijklm}	31.50^{lmn}	5.92^{fghij}	0.68^{lmn}	802^{pqr}	25.5^{klm}	55.32^{bcdef}
4%LSC-AA	69.83^{q}	74.18^{de}	30.00^{ijl}	5.72^{fghij}	0.66^{klm}	764no	24.5^{ij}	63.08^{fghi}
4%LSC-Cle	69.26^{pq}	81.90^{ijklm}	23.50^{cde}	4.74^{cde}	0.50^{de}	614^{ef}	20.0^{de}	47.66^{abcd}
4%LSC-Clef	68.37^{p}	80.37^{ij}	22.50^{cd}	4.56^{cd}	0.48^{cd}	594^{cde}	19.0^{cd}	46.77^{abcd}
4%LSC-CaP	64.93^{mn}	73.08^{cd}	27.50^{ghi}	5.33^{hijk}	0.60^{hi}	724^{jklm}	23.0^{gh}	45.06^{ab}
4%LSC-OL	41.05^{d}	85.66^{pqrs}	24.00^{cdef}	4.82^{cdef}	0.51^{def}	629^{fg}	20.5^{e}	47.35^{abcd}
4%LSC-OP	46.10^{f}	86.51qrs	21.00^{c}	4.42^{c}	0.44^{c}	561^{bc}	18.0^{bc}	45.30^{abc}
4%LSC-PP	47.93^{g}	81.48^{ijkl}	28.00^{ghij}	5.35^{hijk}	0.61^{hij}	712^{ijkl}	22.5^{g}	68.62^{hij}
4%LSC-TA	41.07^{d}	83.90^{mnop}	31.50^{lmn}	5.88^{fghij}	0.69^{lmn}	807^{qrs}	26.0^{lmn}	48.78^{abcd}
6%LSC-AA	44.38^{e}	84.79^{nopqr}	32.50^{mn}	6.10^{ghij}	0.72^{n}	827^{rs}	26.5^{mn}	63.50^{fghi}
6%LSC-Cle	65.86^{no}	76.87^{fg}	27.00^{fghi}	5.25^{ghijk}	0.59^{ghi}	694^{ij}	22.0^{fg}	47.82^{abcd}
6%LSC-Clef	66.82^{o}	75.73^{ef}	28.00^{ghij}	5.46^{ijkl}	0.61^{hij}	708^{ijk}	22.5^{g}	45.61^{abc}
6%LSC-CaP	64.69^{m}	84.68^{nopq}	29.00^{hijk}	5.65^{ijklmn}	0.63^{ijk}	769^{nop}	24.5^{ij}	52.76^{abcde}
6%LSC-OL	71.41^{r}	85.11^{opqr}	30.00^{ijk}	5.69^{fghij}	0.66^{klm}	765no	24.5^{ij}	45.20^{abc}
6%LSC-OP	68.29^{p}	78.02^{gh}	26.50^{efgh}	5.19^{fghij}	0.58^{gh}	708^{ijk}	22.5^{g}	47.08^{abcd}
6%LSC-PP	64.39^{m}	80.21^{i}	31.50^{lmn}	5.95^{fghij}	0.70^{mn}	802^{pqr}	25.5^{klm}	52.72^{abcde}
6%LSC-TA	52.22^{i}	58.00^{a}	31.50^{lmn}	5.99^{fghij}	0.69^{lmn}	823^{rs}	26.5^{mn}	50.66^{abcde}
8%LSC-AA	66.39^{o}	71.14^{c}	31.00^{klmn}	5.90^{fghij}	0.66^{klm}	784^{opq}	25.0^{ijk}	55.79^{cdef}
8%LSC-Cle	55.69^{j}	74.57^{de}	24.00^{cdef}	4.90^{defg}	0.52^{def}	645^{fg}	21.0^{ef}	50.35^{abcde}
8%LSC-Clef	47.86^{g}	87.85^{rs}	27.00^{fghi}	5.31^{hikj}	0.59^{ghi}	683^{hi}	22.0^{fg}	49.75^{abcde}
8%LSC-CaP	55.95^{j}	79.68^{hi}	27.50^{ghi}	5.35^{hijk}	0.60^{hi}	741^{klmn}	24.0^{hi}	53.51^{abcdef}
8%LSC-OL	39.26^{c}	86.55^{qrs}	25.00^{defg}	5.06^{efghi}	0.55^{fg}	644^{fg}	21.0^{ef}	51.80^{abcde}
8%LSC-OP	37.97^{b}	83.53^{lmnop}	23.00^{cd}	4.76^{cde}	0.50^{de}	614^{efg}	20.0^{de}	43.64^{a}
8%LSC-PP	39.02^{c}	87.08^{qrs}	28.50^{hijk}	5.51^{ijklm}	0.63^{ijk}	746^{lmno}	24.0^{hi}	73.66^{j}
8%LSC-TA	34.70^{a}	82.66^{jklm}	31.50^{lmn}	5.96^{fghij}	0.69^{lmn}	825^{rs}	26.5^{mn}	53.66^{abcdef}

Means within columns with different superscript letters are significantly different ($p<0.05$). LSC=linseed containing concentrate, AA= Areke Atela, Cle=cabbage leaf, Clef=cabbage leftover, CaP=carrot peels, OL=onion leaf, OP=onion peels, PP=potato peels, TA=Tela Atela, IVDMD= in vitro dry matter degradability, IVOMD= in vitro organic matter degradability(%), GV=gas volume (ml/200mg DM), ME=metabolizable energy(MJ/kg DM), SCFA=short chain fatty acid (mMol/l), DOM=digestible organic matter (g/kg DM), MCP=microbial crude protein(g/kg DM), RFV=relative feed value(%)

Microbial biomass yield in concentrates and forages was 211 and 303 g/kg TDOM, respectively (Thirumalesh and Krishnamoorthy, 2013). On an average 22g of MCP per 100g organic matter apparently degraded in the rumen (OMDR) was observed in lactating cows when proportion of roughage was reduced from 40 to 32 %. High proportion of roughages in the ration (extremely low energy) yielded only 15 to 20 g MCP/100g OMDR, whereas with high levels of concentrates in the ration yielded 14 to 18 g MCP/ 100g OMDR (Hagemeister *et al*., 1981), which are very close results to the values found in this study.

Efficiency of microbial production in the rumen of steers fed spear grass hay alone or when supplemented with Dunaliella, cotton seed meal, Spirulina and Chlorella were 52, 57, 91, 91 and 106 g MCP/kg DOMI, respectively (Denis and Simon, 2013). Although this efficiency is related to intake, the figures, when converted to per unit of DOM, are not far from the MCP values obtained in this study.

Leng (1999) has shown that the breakdown of carbohydrate in the presence of adequate ammonia results in more microbial protein being produced. It is therefore clear that with readily soluble and fermentable protein the protein to energy ratio in the nutrients arising from the rumen may be decreased. This was what was observed in the roughages when supplemented with the energy rich concentrate.

Among the factors that affect the synthesis of microbial protein, the availability and synchronization between energy and nitrogenous compounds (N) in the rumen have been recognized as the most important factor (Russell et al., 1992).

Some of the results reported by Stokes *et al*. (1991) and Sinclair *et al*. (1995) might be taken as supporting explanations to the improvements achieved by adding concentrates to the HW. Microbial biomass yield from purified diets are highly variable which is probably due to quality of the diets. Diets contained 31 or 39% NSC and 11.8 and 13.7% RDP in diet DM supported greater MCP synthesis than a diet contained 25% NSC and 9% RDP (Stokes *et al*. 1991). The efficiency of MCP synthesis was 11 to 20% greater in sheep given diets with CHO source (barley) synchronous with rapeseed meal than in asynchronous with urea (Sinclair *et al*. 1995).

The NRC (1996) system assumes that the amount of protein required by the microorganisms is equivalent to the amount of MCP produced in the rumen. Not all of the MCP is available to the animal as metabolizable protein (MP). The NRC (1996) calculates true protein content in rumen microorganisms as 80% times crude protein, the rest 20% is nucleic acid. The NRC (1996) estimate is that only 80% of the true protein in microorganisms is actually absorbed by the animal. Thus even though enough protein must be supplied to meet the total CP requirements of the microorganism, this amount of CP only supplies 0.64 times (0.80 x 0.80 = 0.64) its weight as MP (Agriculture and Rural Development, 2014).

Relative feed value

Calculated relative feed value (RFV) of the roughages and concentrate-roughage mixtures are presented in Tables 4 and 5.

The lowest ($p<0.05$) RFV was obtained from OP, AA and OL and highest ($p<0.05$) from PP and TA while Clef, Cle and CaP had values in between.

The RFV of the HWs was improved by the addition of concentrate. When concentrate was supplemented, PP had highest improvement and OP the lowest (except at 6%LSC) and the rest of them were moderately improved when compared to standard, good quality alfalfa hay which is considered to have 100% RFV.

The concentrate without linseed (0%LSC) has extremely improved the RFV but addition of linseed did not bring any further improvement over and above the concentrate alone in fact in some of the HWs (TA and OL) it has reduced RFV. In general no visible trend in RFV was noticed with the increasing levels of linseed in the concentrate.

CONCLUSION

The *in vitro* gas tests conducted on eight household wastes (HWs) supplemented with concentrates containing five levels (0, 2, 4, 6 and 8%) of ground linseed (LSC) revealed significant improvements of the HW-LSC mix in the **CP** (smallest CP of 8.40% and 8.76% in AA and Clef was raised to over 12% CP) and ME (from 5.87 and 5.59 in AA and TA was slightly raised to over 5.90 MJ/kg DM at 0, 2 and 6%LSC) contents and IVOMD (from 87 and 67 in AA and Clef was raised to over 89% at 0%LSC); production of SCFA (from 0.7 and 0.64 in AA and TA it was very slightly raised to over 0.7mMol/l at 0%LSC) and MCP (from26.0 and 24.5 in AA and TA it was slightly improved to over 26 g/kg DM at 0,, 4 and 6%LSC) and RFV (from 32, 39, 49 in AA, Cle and PP it was raised to 65% at all linseed inclusion levels).

The SCFA production of HWs were increased over the incubation periods when the roughages were mixed with the concentrates and the increment was moderately and consistently influenced by the higher levels of linseed in the concentrates.

MCP production and RFV of the HWs were improved by mixing them with LSCs; the highest MCP was obtained with 0%LSC which corresponds with the highest DOM content of the mixtures. Improved utilization of the protein is only possible through increased degradability in the rumen and conversion to microbial protein.

It can thus be concluded that mixing three parts of the HWs with one part of concentrates improved CP, DOM, ME, total SCFA, MCP production and RFV. Up to 4 % LS in the concentrate has supported the positive effect and could be recommended.

REFERENCES

AFRC. 1993. Energy and Protein Requirements of Ruminants. CAB International Wallington, UK.

Agriculture and Rural Development. 2014. Nutrition and Management: Protein Requirements of Feedlot Cattle. Alberta.

Ahn, J.H., Robertson, B.H., Elliott, R., Gutteridge, R.C. and Ford, C.W. 1989. Quality assessment of tropical browse legumes: Tannin content and protein degradation. Animal Feed Science and Technology, 27(1-2) 147-156 DOI: 10.1016/0377-8401(89)90139-9.

Ankom Technology, 2005. Method for determining neutral detergent fiber (aNDF). Ankom Technology, Macedon, NY, USA

AOAC, 2005. Official Methods of Analysis of the Official Analytical Chemists, 18th Edn. (Horwitz, W., Eds.), Association of Official Analytical Chemists, Washington DC

ARC, 1984. The Nutrient requirements of Ruminant Livestock, Supplement No. 1. Report of the Protein Group of the Agricultural Research Council Working Party on Nutrient Requirements of Ruminants. CAB, Farnham Royal, UK.

Blümmel M, Aiple K, Steingas H, and Becker K. 1999. A note on the stoichiometrrical relationship of short chain fatty acid production and gas evolution *in-vitro* in feedstuffs of widely differing quality. Journal of Animal Physiology and Animal Nutrition, 81:157-167.

Blümmel, M. and Orskov, E.R. 1993. Comparison of an *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Animal Feed Science and Technology, 40:109-119.

Dennis Poppi and Simon Quigley, 2013. Increased efficiency of microbial protein production in the rumen through manipulation of nutrients and rumen microbial populations. University of Queensland. ISBN 9781925045659. Published by Meat and Livesstock Australia

Elliot Block. 2006. Rumen Microbial Protein Production: Are We Missing an Opportunity to Improve Dietary and Economic Efficiencies in Protein Nutrition of the High Producing Dairy Cow? 2006 High Plains Dairy Conference Industry Presentation Princeton, NJ

Getachew, G., H.P.S. Makkar and K. Becker, 2001. Method of polyethylene glycol application to tannin containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. Animal Feed Science and Technology, 92: 51-57.

Grings E.E., M. Blümmel, K.-H. Südekum. 2005. Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. Animal Feed Science and Technology, 123–124: 527–545

Hagemeister H, Lupping W and Kaufmann W. 1981. Microbial protein synthesis and digestion in the high yielding dairy cow. In: Recent developments in ruminant nutrition (Ed). Haresingh W and Cole DJA). Pp. 31. Butterworks, London. http://www.cipav.org.co/irrd/irrd3/3sarec2.htm.

Kibon, A. and E. R. Ørskov. 1993. The use of degradation characteristics of browse plants to predict intake and digestibility by goats. Animal Production 57: 247-251.

Krishnamoorthy U, Chandrapal Singh K and Kailas MM. 2005. Evaluation of roughages for rumen microbial biomass synthesis. Indian Veterinary Journal. 82:453-454.

Lardy, G. P. and V. L. Anderson. 1999. Alternative feeds for ruminants. North Dakota State University Extension Service Bulletin AS-1182.

Lebzien P. 1996. The German protein evaluation system for ruminants under discussion. Kraftfutter. 10:452-455.

Leng R. Chapter eleven. 1999. Feeding strategies for improving milk production (pp 207-224). In the Book: Smallholder Dairying in the Tropics. Edited by Flavey L and Chantalakhana, ILRI, Nairobi, Kenya.

Machmuller, A.; Ossowski, D.A.; Kreuzer, M. 2000. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Animal Feed Science and Technology, 85(1-2):41-60.

Makkar, H.P.S., Blümmel, M. and Becker, K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyathylene glycols and tannins, and their implication in gas production and true digestibility in *in-vitro* techniques. British Journal of Nutrition 73, pp. 897-913.

Makkar, H.P.S. 2000. Quantification of tannin in tree foliages. FAO/IAEA.

Menke K., Raab L., Salewiski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in-vitro*. Journal of Agricultural Science (Cambridge), 93:217-222.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research Development 28:7-55.

Negesse, T., Makkar, H.P.S., Becker, K. 2009. Nutritive value of some non-conventional feed sources of Ethiopia determined by chemical analyses and an in-vitro gas method. Animal feed Science and Technology, 154:204–217.

NRC, 1996. National Research Council, Nutrient Requirements of Beef Cattle, seventh ed. National Academy Press, Washington, DC, USA.

NRC. 1989. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.

NRC. 2000. Nutrient requirements of beef cattle, 2000 Update. National Academy Press, Washington, D C.

Okojie, J.A., 1999. The role of government and universities of Agriculture in improving animal production and consumption in Nigeria. Tropical Journal of Animal Science, 2: 1-7.

Ørskov, E.R.and McDonald, I., 1979 The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science 92: 499-503.

Parish, J. A. and J. D. Rhinehart. 2008. Protein in beef cattle diets. The Beef Site: The Sciennces of Heal Their Animal. 5m Publishing, Bench Mark House, 8 Smithy Wood Drive, Sheffield, 535, QN, England.

Preston, T.R. and Leng, R.A., 1984. Supplementation of diets based on fibrous residues and byproducts. In: F. Sundstol and E. Owen (editors), straw and other fibrous By-products as feed. Elsevier, Amsterdam, pp.373-413.

Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J., Sniffen, C.J. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science, 70: 3551–3561.

SAS Institute, 2004. The SAS System for Windows, version 10. SAS Institute Inc., Cary, NC, USA.

Sinclair, L.A., Garnsworthy, P.C., Newbold, J.R., Buttery, P.J. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis. Journal of Agricultural Science. (Camb.) 120, 251–263.

Sniffen CJ, Ballard CS, Carter MP, Cotanch KW, Dann H M, Grant RJ, Mandebvu P, Suekawa M and Mrtin SA. 2006. Effects of malic acid on microbial efficiency and metabolism in continuous culture of rumen contents and on performance of mid lactation dairy cows. Animal Feed Science Technology, 127: 13-31.

Stern, M.D., G.A. Varga, J.H. Clark, J.L. Firkins, J.T. Huber, and D.L. Palmquist. 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. J. Dairy Sci. 77: 2762–2786.

Stokes, S.R., Hoover, W.H., Miller, T.K., Blauweikel, R. 1991a. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. Journal of Dairy Science, 74: 871-881.

Stokes, S.R., Hoover, W.H., Miller, T.K., Manski, R.P., 1991b. Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. Journal of Dairy Science, 74: 860-870.

Tchinda, B., D. Wegad and R.M. Njwe, 1993. Rumen degradation of elephant grass supplemented with graded levels of perennial peanut by West African Dwarf sheep. In: Lebbie S.H.B., Rey, B. and Irungu, E.K. (Ed.), Small ruminants research and development in Africa. Proceedings of the second biennial conference of the African small ruminants research network AICC, Arusha, Tanzania. 7-11 December 1992. ILCA, Addis Ababa, Ethiopia, pp: 187-190.

Thirumalesh T. and U. Krishnamoorthy. 2013. Rumen Microbial Biomass Synthesis and Its Importance in Ruminant Production. International Journal of Livestock Research 3(2):5-26.

Uttam S, Leticia S, Dennis H, Nicholas H, Lawton S, Gary H and David E.K. 2010. Common terms used in animal feeding and nutrition. The University of Georgia, College of Agriculture and Environmental Sciences, Virginia, USA.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74:3538–3597.

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