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An *in vitro* assessment of nutritive value of nine roughages supplemented with concentrates containing graded levels of ground linseed "*Linum usitatissimum*"

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Abstract

Effect of supplementing ground linseed (LS) plus concentrates (C)=0%LSC-8%LSC) [39.32,37.32,35.32, 33.32,31.32% noug cake=Guizoita abyssinica+58.99% wheat bran+1.69%salt+0,2,4,6 and 8% LS, respectively, as fed basis] to Roughages=R (barley straw=BS, Coffee parchment=CPa, coffee pulp=CPu, haricot bean haulm=HBH, Maize stover=MaS, finger millet stover=MiS, Rhodes grass hay=RGH, teff straw=TS and wheat straw=WS) on overall degradability of dry mater (IVDMD) and organic matter (IVOMD], production of short chain fatty acids (SCFA), microbial protein (MCP) and metabolizable energy (MEr) and relative feed values (RFV) were assessed in vitro. Ground LSC and R were mixed at 1:3 ratios. About 200mg of each mix was incubated in duplicate *in-vitro* with rumen fluid [obtained from 3 sheep fed on Rhodes grass (*Chloris gayana Kunths*) hay and concentrate] an aerobically with a mineral buffer solution in to a calibrated glass syringe @100 ml and flushed with CO_2. Syringes were pre-warmed (39°C) for 1h, 30ml of rumen-buffer mixture was added into each syringe, incubated in the water bath and gently shaken on an hourly basis during the first 8 h of incubation. Data was recorded at 0,3,6,12,24,48,72 and 96 hrs of incubation. Average gas volume readings were fitted to exponential equation:

$$Y = a + b\left(1 - e^{-ct}\right)$$

Where Y, volume of gas produced at a given time't' (ml); a, gas produced from soluble fractions (ml); b, gas produced from insoluble but with time fermentable fraction (ml); c, rate of fermentation (ml/hr); t, time at measurement (h). MEr, SCFA, digestible OM (DOM) and MCP were estimated from 24h gas volume:-

MEr (kj/kg DM)= 46 Gv (ml/200 mg DM per 24h)+7 CP+22.4 EE+1242; SCFA (mmol L-1)=0.0239 x Gv–0.601; DOM (g/kg DM)=OM (g/kg DM) x IVOMD (%); MCP (g/kg DM)=DOM x 0.032);and RFV=88.9- 0.78 x ADF x 120/NDF/1.29

Where Gv, CP, EE, OM, IVOMD, ADF and NDF are gas volume (ml 200mg-1DM), crude protein, ether extract and organic matter (g kg-1 DM) and acid detergent fiber and neutral detergent fiber (%DM) of incubated samples. CP, IVDMD and IVOMD were higher in LSC-R than R's alone but lower than in LSCs indicating that values of R's were improved by mixing with LSCs. Roughages (BS, HBH, MaS, MiS, TS and WS) had higher IVOMD than IVDMD which were influenced by inclusion of LS but not by levels. General trend in improvement of IVDMD and IVOMD of the LSC-R were recorded at 2 and 4% of LSC. However at higher concentrations of LS general tendencies of decline were observed. Production of MCP of R was improved by mixing with LSC; while highest MCP was obtained at 0%LSC which also had highest DOM content indicating linseed had less impact on the parameters. Lowest ME was from TS (5.96 MJ/kg DM) and highest from most Rs (from 7.02 to 7.30 MJ/kg DM), while CPa and BS had intermediate values. ME content of BS (6.81 to 7.08 MJ/kg DM), MiS (7.02 to 7.50 MJ/kg DM) and WS (5.96 to 6.58 MJ/kg DM) improved by 2%LS. SCFA of roughages increased over incubation periods and WS with lower SCFA (0.52 mMol/l) was raised to 0.63mMol/l with levels of LS; and that of CPu with highest SCFA (0.71 mMol/l) diminished to 0.52 mMol/l. RFV of Rs (compared to good quality alfalfa hay=100% RFV) was improved ($p<0.05$) when supplemented with LSC. CPa and CPu had higher RFV than the rest. Best results of SCFA, MCP, ME and RFV were obtained by mixing roughages with 2% LSC, however mixing with 4% LSC has also moderately influenced the traits.

Keywords: concentrate, gas test, linseed, metabolizable energy, roughages, short chain fatty acid

Introduction

One of the major constraints influencing the animal production in Ethiopia is inadequate nutrition, both in terms of quality and quantity. Crop residues constitute more than 90% of the feed resources available for ruminant livestock in most of the developing countries[1] and Ethiopia being no exception. Livestock in Ethiopia are generally reared on natural pasture and crop residues which are of low feeding value, thus leading to lower productivity. During the dry season livestock lose their body condition quite rapidly as the as the scanty feed resources are unable to even meet their basic requirements.[2–4] Most roughages are deficient in protein, minerals and vitamins, are highly lignified, of low digestibility and, therefore, unable to provide even their basic nourishment.[5] Efficient utilization of agricultural and agro-industrial byproduct as feed could help improve the nutritional status of livestock.[6] Limitations of nutrient supply from natural pasture could be improved by supplementing them with concentrates. Inclusion of nutritious feed such as linseed containing concentrates can improve the nutritive status of roughages by supplying the basic nutrients viz. protein, energy and essential fatty acids.[7] Studies indicate that linseed depresses methane production in the rumen, and thus reduces the energy loss and improves energy utilization of ruminant livestock.[8] In general, linseed or its byproducts such as linseed cake are generally used as a supplement to cereal crop residues for feeding ruminants. Although linseed is used as a supplement to crop residues for feeding ruminants and abundant amount of linseed is produced in Ethiopian, limited information is available when linseed is given with crop residues. Evaluations using *in vitro* techniques are relatively simple and economical and allow evaluations of feed. Energy, rumen fermentation characteristics and microbial biomass synthesis.[9] The study was conducted with the objectives of evaluating the effects of supplementing some crop residues (wheat straw, barley straw, teff straw, finger millet stover, haricot bean haulm, maize stover) coffee by-products (coffee parchment and coffee pulp) and grass (Rhodes grass hay) with a concentrate containing graded levels of ground linseed on *in vitro* dry matter and organic matter digestibility, total short chain fatty acids (SCFA) productions, microbial protein production and metabolizable energy (ME)value of the roughages and the concentrate-roughage mixtures.

Material and methods

Experimental site, sample collection and processing

The *in vitro* studies and 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. Linseed, wheat bran, noug cake, coffee byproducts and crop residues were purchased from local markets of Hawassa. The crop residues (Table 1) and concentrate (Table 2) were dried in an oven at 65°C for 72hours. Samples were then milled using Wiley Mill to pass through a 1.0 mm screen for the *in vitro* gas test and proximate analysis

Table 1 The roughages used for the *in-vitro* gas test

No	Feed stuff	Abbr.	Species name
1	Barley straw	BS	*Hordeum vulgare L.*
2	Coffee parchment	CPa	*Coffee arabica*
3	Coffee pulp	CPu	*Coffee arabica*
4	Haricot bean haulm	HBH	*Phaseolus vulgaris L.*
5	Maize stover	MaS	*Zea mays*
6	Finger millet stover	MiS	*Eleusine coracana*
7	Rhodes grass hay	RGH	*Chloris gayana K.*
8	Teff straw	TS	*Eragros tisteff*
9	Wheat straw	WS	*Triticum aestivum*

Table 2 Composition of the concentrates (LSC, % as fed) and experimental design (LSC mixed with the roughages at 1:3 ratio)

Ingredients	Ingredients of LSC (%, as fed)				
	LSC1	LSC2	LSC3	lSC4	LC5
Wheat bran	58.99	58.99	58.99	58.99	58.99
Linseed	0	2	4	6	8
Noug cake	39.32	37.32	35.32	33.32	31.32
Salt	1.69	1.69	1.69	1.69	1.69

	Roughages								
LSC	BS	CPa	CPu	HBH	MaS	MiS	RGH	TS	WS
LSC1	LSC1-BS	LSC1-CPa	LSC1-CPu	LSC1-HBH	LSC1-MaS	LSC1-MiS	LSC1-RGH	LSC1-TS	LSC1-WS
LSC2	LSC2-BS	LSC2-CPa	LSC2-CPu	LSC2-HBH	LSC2-MaS	LSC2-MiS	LSC2-RGH	LSC2-TS	LSC2-WS
LSC3	LSC3-BS	LSC3-CPa	LSC3-CPu	LSC3-HBH	LSC3-MaS	LSC3-MiS	LSC3-RGH	LSC3-TS	LSC3-WS
LSC4	LSC4-BS	LSC4-CPa	LSC4-CPu	LSC4-HBH	LSC4-MaS	LSC4-MiS	LSC4-RGH	LSC4-TS	LSC4-WS
LSC5	LSC5-BS	LSC5-CPa	LSC5-CPu	LSC5-HBH	LSC5-MaS	LSC5-MiS	LSC5-RGH	LSC5-TS	LSC5-WS

LSC, linseed+concentrate (LSC1, 0%LS, LSC2, 2%LS, LSC3, 4%LS, LSC4, 6%LS and LSC5, 8%LS); BS, barley straw; CPa, coffee parchment; CPu, coffee pulp; HBH, haricot bean haulm; MaS, maize stover; MiS, finger millet straw; RGH, Rhodes grass hay; TS, teff straw; WS, wheat straw

Experimental design and treatments

The experimental design is shown in Table 2. The 9 roughages were mixed with each of the concentrates, containing five levels of ground linseed, giving rise to 45 combinations. The roughage to concentrate ratio used for *in-vitro* analysis was 3:1. The *in-vitro* gas tests were done in duplicate and thereafter averaged.

In-vitro gas test

Rumen liquor was obtained from three rams fed on Rhodes grass (*Chloris gayana Kuths*) hay and concentrate in the morning, prior to feeding using a vacuum pump connected to a glass vacuum container and a semi-flexible oro-ruminal probe, flushed with CO_2, the collected liquor was filtered through three layers of cheesecloth. The filtered

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rumen fluid was pooled and mixed (1:2, v/v) with an anaerobic mineral buffer solution as earlier described[10] and further revised.[11] 200mg (of each) of the air dried and ground (passed through 1.00 mm sieve) sample was incubated *in-vitro* with rumen fluid in to a calibrated glass syringe of 100ml.[12] All the samples were assessed in duplicate and then averaged. The piston of the syringe was then lubricated with pure oil to ease its movement and to prevent the escape of the collected gas. The syringes were pre-warmed (39°C) for 1h, before the addition of 30ml of rumen fluid-buffer mixture into each syringe, thereafter; the filled syringes were gently shaken on an hourly basis for the first eight hours of the incubation. Readings (gas collected) from the syringes were recorded at 0, 3, 6, 12, 24, 48, 72 and 96h of incubation. The average gas volume readings (a, b and c) were fitted to the exponential equation:

$$P = a + b\left(1 - e^{-ct}\right)$$ [13,14]

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 fermentable fraction (ml); a+b, potential gas production, c, rate of gas production (ml/hr); t, time at measurement (h).Gas volume from 24h was used to estimate metabolisable energy (ME), organic matter digestibility (OMD) & total SCFAs.[15,16]

I. IVOMD(g/kgDM)=8.89GV(ml/200mgDM per 24h)+0.448CP(g/kgDM)+0.651 crudeash (g/kgDM)+149

II. MEr(kJ/kg DM)=146 GV (ml/200mg DM per 24h)+7 CP (g/kg DM)+22.4EE (g/kg DM)+1242

III. SCFA m mol L-1)=0.0239 x GV–0.601

Where GV, CP and EE are gas volume (ml 200 mg-1 DM), crude protein and ether extract (g kg-1 DM) contents of the incubated samples, respectively. Digestible organic matter (DOM) and microbial crude protein (MCP) productions were then calculated using the following equations: Digestible organic matter (g/kg DM) =OM (g/kg DM) x IVOMD (%)

Microbial crude protein (g/kg DM)=DOM x 0.032[17]

Microbial N production I at $t1/2$=diet N+ΔNH3–N–NDFN at $t1/2$;[18]

Where ΔNH3–N=NH3–N in 0h blanks-NH3–N in diet incubations at $t1/2$.[19]

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.

RFV=(DDM (%DM) x DMI (%BW))/1.29.[20]

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

DDM (% DM)=88.9-0.78 x ADF (% DM) and DMI (%BW)=120/NDF (% DM), thus 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) and ether extract (EE) and ash contents.[21] Nitrogen was determined according to Kjeldhal method and protein was calculated as N x 6.25. Neutral detergent fiber (NDF) was determined according to Van Soest et al.,[22] while acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Robertson[23] using the Ankom Fiber Analyzer (Ankom Technology, 2005). Hemicelluloses were computed as a difference between NDF and ADF, and cellulose as a difference between ADF and ADL contents.

Statistical analysis

The rumen fluid was obtained in duplicate from 3 healthy Arsi Bale rams using stomach tube. The data were analyzed using SPSS v 19 for Windows. The means were calculated using descriptive statistics while they were compared using Duncan's multiple range test. The values were considered significant at $P<0.05$ Means for the *in Sacco* analysis of the data were fitted to the equation[13] using SAS 9.0 Institute (2004) software.[24]

Results and discussion

Nutrient content of the feed ingredients, roughage and concentrates

The nutrient content of the feed ingredients used for concentrate making, roughages (crop residues; coffee byproducts and Rhodes grass hay: RGH) and the concentrates are presented in Tables 3. As presented in Table 3 the crude protein (CP) content of the roughages varied between 5.05 (BS) and 10.85% on DM basis (HBH). Except BS, MaS and TS all other roughages have CP content (7.64-8.865DM) very close to the minimum CP requirements (8%) required for the multiplication of rumen microbes, or maintenance CP (7.2%) requirement of ruminants or the minimum CP (7.0%) that limits intakes.[25–27] Thus most of these roughages could provide fairly good amount of CP to ruminants. The CP content of the concentrates was reasonably high when compared to the roughages and thus complimented with the CP value of the roughages when three parts of it was mixed with one part of the concentrates containing graded levels of linseed. The levels of Neutral detergent fiber (NDF) differed among the roughages with values ranging from CPa (39%DM) to WS (76%DM). While, the Acid detergent fiber (ADF) values constituted about two third of the portion of the NDF in the roughages (27 and 45%DM in CPa and WS, respectively). However, ADL constituted more than 2/3 of the ADF of the roughages, which could possibly be related to the over maturity of the crops, coffee berries and the grass when harvested. An increase in the values of lignin in the straw was reported as the finger millet crop matures.[28] The NDF content of MiS as assessed as 62.5% and it was observed to be the least in MiS heads.[29] The study further indicates that the LSC-R mixtures had higher CP values when compared to those of the corresponding Rs alone, however the value was lower than those of the concentrates. The CP content of Rs were lower than that of minimum recommended requirements needed for lactation (120g/kg DM) and growth (113g/kg DM) of ruminants;[30] and also for early (19%CP) and late (13%CP) lactation.[31] It was also less than that (15%CP) required for optimum growth of growing cattle[32] but the values of HBH had slightly lower CP content needed for the minimum growth requirements.

In vitro dry matter and organic matter digestibility and calculated digestible organic matter

The in vitro dry matter and organic matter digestibility (IVDMD, IVOMD) and calculated digestible organic matter contents (DOM, g/kg DM) of the roughages and concentrates are presented in Table 4 and their mixtures in Table 5. As shown in Table 4, CPa had the highest but TS and MiS had the lowest IVDMD. However, CPa had highest whereas CPu had the lowest IVOMD and DOM ($p<0.05$) respectively; and from among those having DOM values in between, BS, HBH and TS had higher DOM than the rest. The IVOMD and DOM contents of the concentrates were only slightly higher than those of most of the roughages.

Table 3 The nutrient content of roughages and concentrate-linseed mixtures that were used for the *in-vitro* trial

Roughages	DM, %	Nutrient content (% DM)							CP	
		Ash	OM	EE	CP	NDF	ADF	ADL	%DOM	g/MJ ME
BS	96.01^{f}	10.60^{de}	89.94^{ab}	0.085^{cd}	5.05^{a}	69.70^{cd}	39.70^{d}	30.53^{de}	7	9.6
CPa	95.20^{de}	7.98^{b}	92.01^{e}	0.120^{e}	8.78^{e}	39.21^{a}	26.95^{a}	17.05^{a}	11.1	15.2
CPu	92.01^{a}	8.73^{c}	91.26^{d}	0.060^{bc}	8.86^{e}	40.96^{a}	30.34^{ab}	20.13^{ab}	15.2	15.8
HBH	93.49^{c}	9.68^{d}	90.32^{bc}	0.095^{de}	10.85^{f}	56.07^{b}	46.15^{e}	36.15^{f}	14.3	19.2
MaS	94.16^{d}	8.88^{c}	91.11^{d}	0.090^{cde}	5.69^{b}	70.84^{d}	34.09^{bc}	24.09^{bc}	8.6	10.5
MiS	95.50^{ef}	9.20^{cd}	90.80^{cd}	0.035^{bc}	7.64^{d}	67.23^{cd}	34.92^{cd}	24.92^{bc}	11.6	13.8
RGH	94.63^{de}	8.79^{c}	91.20^{d}	0.070^{cd}	7.43^{cd}	65.75^{c}	31.35^{ab}	21.35^{ab}	11.4	13.9
TS	95.32^{ef}	7.32^{a}	92.68^{f}	0.02^{a}	6.82^{c}	71.24^{d}	37.83^{cd}	27.83^{cd}	9	12.6
WS	92.68^{b}	10.31^{e}	89.69^{a}	0.030^{ab}	7.72^{d}	75.70^{e}	45.27^{e}	35.27^{ef}	12.3	15
Concentrate-linseed mixtures										
LSC1	92.31^{bc}	7.19	92.82	0.15^{a}	17.00^{ab}	51.51	20.13	5.95	21.3	24.6
LSC2	91.15^{a}	8.04	91.96	0.21^{b}	18.69^{a}	50.76	19.7	6.11	23.5	27
LSC3	92.38^{bc}	7.49	92.52	0.23^{b}	20.24^{c}	46.18	19.13	5.4	25.5	28.3
LSC4	92.93^{c}	7.32	92.69	0.24^{bc}	19.71^{bc}	48.18	20.12	5.39	24.1	26.8
ISC5	91.39^{ab}	7.02	92.99	0.27c	20.65^{c}	44.52	19.14	5.71	25.3	27.4

Means for roughages and concentrate-linseed mixtures within columns with different superscript letters were significantly different ($p<0.05$).
ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; EE, ether extract; LSC, linseed containing concentrate; NDF, neutral detergent fiber; OM, organic matter; BS, barley straw; CPa, coffee parchment; CPu, coffee pulp; HBH, haricot bean haulm; MaS, maize stover; MiS, finger millet straw; RGH, Rhodes grass hay; TS, teff straw; WS, wheat straw

Table 4 *In vitro* DM and OM digestibility, gas volume, ME, short chain fatty acids, digestible organic matter, microbial crude protein production and relative feed value of the crop residues and concentrates

Roughages	Parameters								
	IVDMD %	IVOMD %	DOM g/kg DM	GV ml/200 mg DM	ME MJ/kg	SCFA mMol/l DM	MCP		RFV %
							g/kg DM	g/kg DOM	
BS	38.59^{b}	80.70^{e}	723^{d}	27.2^{ab}	5.25^{ab}	0.59^{ab}	23.1^{d}	31.95	47.57^{b}
CPa	62.03^{e}	86.16^{e}	788^{f}	30.7^{f}	5.79^{f}	0.67^{g}	25.2^{f}	31.98	39.03^{a}
CPu	56.36^{d}	64.37^{a}	583^{a}	29.6^{e}	5.62^{e}	0.64^{ef}	18.7^{a}	32.08	35.17^{a}
HBH	51.10^{c}	84.51^{e}	759^{e}	29.7^{ef}	5.65^{ef}	0.65^{fg}	24.3^{e}	32.02	33.95^{a}
MaS	34.08^{ab}	72.50^{cd}	661^{c}	28.4^{cd}	5.43^{cd}	0.62^{cde}	21.1^{c}	31.92	53.97^{c}
MiS	32.58^{a}	73.38^{d}	660^{c}	28.9^{de}	5.52^{de}	0.63^{def}	21.1^{c}	31.97	51.16^{bc}
RGH	36.61^{ab}	71.44^{c}	650^{bc}	27.7^{bc}	5.34^{bc}	0.60^{abc}	20.8^{cd}	32	54.29^{c}
TS	32.90^{a}	82.19^{e}	762^{e}	28.2^{bcd}	5.40^{bcd}	0.61^{bcd}	24.4^{e}	32.02	50.29^{bc}
WS	36.71^{ab}	69.63^{b}	628^{b}	26.5^{a}	5.16^{a}	0.57^{a}	20.1^{b}	32	45.49^{b}
Concentrate	**IVDMD**	**IVOMD**	**DOM**	**GV**	**ME**	**SCFA**	**MCP**		
LSC1		85.83	797	30.5^{a}	6.92^{a}	0.67	25.5	31.99	
LSC2		86.63	797	30.5^{a}	6.92^{a}	0.69	25.5	31.99	
LSC3		85.8	794	31.0^{ab}	7.16^{ab}	0.68	25.5	32.16	
LSC4		88.2	818	32.0^{ab}	7.36^{bc}	0.71	26	31.78	
ISC5		87.63	815	33.0^{c}	7.53^{c}	0.72	26	31.9	

Means for household wastes and concentrates within columns with different superscript letters were significantly different ($p<0.05$).
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; RFV, relative feed value; SCFA, short chain fatty acid; BS, barley straw; CPa, coffee parchment; CPu, coffee pulp; HBH, haricot bean haulm; MaS, maize stover; MiS, finger millet straw; RGH, Rhodes grass hay; TS, teff straw; WS, wheat straw

Table 5 *In vitro* digestibility, gas volume, ME, short chain fatty acids, digestible organic matter, microbial CP production and relative feed value of the concentrate-roughage mixtures

LSC-R mix	Parameters								
	IVDMD %	IVOMD %	GV, ml/200mg DM	ME, MJ/kg DM	SCFA, mMol/l	DOM, g/kg DM	MCP		RFV%
							g/kg DM	g/kg DOM	
0%LSC-BS	45.14^{e}	83.51^{hijk}	29.02^{defghijk}	5.56^{cdefghijkl}	0.63^{fghi}	751^{p}	24.03^{hijklm}	32	49.51^{ij}
2%LSC-BS	44.56^{e}	80.28^{e}	26.92^{cd}	5.32^{cd}	0.58^{d}	702^{f}	22.46^{de}	31.99	51.84l^{mn}
4%LSC-BS	43.20^{d}	80.78^{ef}	27.25^{de}	5.29^{c}	0.59^{de}	738^{l}	23.62^{ghijk}	32.01	47.07^{g}
6%LSC-BS	55.72^{k}	81.45^{efg}	27.68^{def}	5.35^{cde}	0.61^{def}	709^{g}	22.69^{def}	32	49.91^{ijk}
8%LSC-BS	57.37^{l}	68.08^{b}	19.01^{a}	4.08^{a}	0.39^{a}	599^{a}	19.18^{a}	32.02	47.03^{g}
0%LSC-CPa	58.92^{mn}	89.13^{qr}	31.94^{jklm}	6.03^{jklmn}	0.68l^{mnop}	786^{v}	25.14^{nopqrs}	31.98	46.69^{g}
2%LSC-CPa	60.11^{mn}	72.69^{c}	31.44^{hijklm}	5.94^{hijklmn}	0.68l^{mnop}	766^{r}	24.50^{klmnop}	31.98	68.94^{s}
4%LSC-CPa	62.66^{op}	83.70^{ijkl}	32.30^{klm}	6.04^{klmn}	0.68l^{mnop}	793^{x}	25.39^{pqrs}	32.02	40.55^{cd}
6%LSC-CPa	59.21^{mn}	88.65^{pqr}	32.94^{m}	6.12^{n}	0.68l^{mnop}	759^{q}	24.28^{ijklmn}	31.99	42.87^{ef}
8%LSC-CPa	69.59^{r}	76.35^{d}	32.02^{jklm}	5.97^{ijklmn}	0.68l^{mnop}	790^{wx}	25.29^{opqrs}	32.01	35.34^{a}
0%LSC-CPu	58.92^{mn}	88.72^{pqr}	31.40^{klm}	6.05^{lmn}	0.71^{pq}	824^{C}	26.36^{tu}	31.99	46.71^{g}
2%LSC-CPu	60.06^{mn}	72.32^{c}	21.76^{ab}	4.54^{b}	0.46^{b}	632^{c}	20.21^{b}	31.98	68.93^{s}
4%LSC-CPu	62.41^{op}	83.64^{ijkl}	29.10^{defghijkl}	5.54^{cdefghij}	0.63^{ghij}	830^{D}	26.54^{u}	31.98	40.55^{cd}
6%LSC-CPu	58.70^{m}	88.77^{pqr}	32.43l^{m}	6.08^{mn}	0.72^{q}	739l	23.64^{ghijk}	31.99	41.76^{de}
8%LSC-CPu	68.66^{r}	76.08^{d}	29.20^{defghijkl}	5.58^{cdefghijkl}	0.51^{c}	673^{d}	21.53^{c}	31.99	35.55^{b}
0%LSC-HBH	62.03^{o}	86.46^{no}	31.45^{hijklm}	5.93^{ghijklmn}	0.70^{nopq}	793^{x}	25.38^{pqrs}	32.01	42.32^{ef}
2%LSC-HBH	62.03^{o}	86.46^{no}	28.63^{defghij}	5.54^{cdefghij}	0.62^{efgh}	734^{k}	23.49^{fghij}	32	43.61^{f}
4%LSC-HBH	62.03^{o}	86.46^{no}	27.79^{def}	5.39^{cdef}	0.61^{def}	799^{y}	25.58^{qrst}	32.02	38.68^{b}
6%LSC-HBH	62.03^{o}	86.46^{no}	31.35^{ghijklm}	5.94^{hijklmn}	0.69^{nopq}	716^{h}	22.92^{defg}	32.01	38.61^{b}
8%LSC-HBH	62.03^{o}	86.46^{no}	27.96^{defg}	5.44^{cdefg}	0.60^{def}	749^{op}	23.95^{hijklm}	31.98	39.51^{bc}
0%LSC-MaS	53.61^{j}	85.52l^{mno}	31.42^{hijklm}	5.90^{ghijklmn}	0.69^{nopq}	804^{z}	25.70^{rstu}	31.97	54.89^{qr}
2%LSC-MaS	44.94^{e}	82.55^{fghij}	29.27^{defghijkl}	5.55^{cdefghijk}	0.64^{hijk}	744^{mn}	23.82^{ghijkl}	32.02	55.68^{r}
4%LSC-MaS	48.32^{gh}	81.74^{efghi}	28.76^{defghij}	5.51^{cdefghi}	0.63^{fghi}	793^{x}	25.36^{pqrs}	31.98	52.01l^{mno}
6%LSC-MaS	35.22^{a}	76.15^{d}	30.52^{efghijklm}	5.84^{efghijklmn}	0.67^{klmno}	743^{m}	23.79^{ghijk}	32.02	51.65l^{mn}
8%LSC-MaS	40.07^{c}	84.00^{jklm}	29.75^{defghijklm}	5.66^{cdefghijklmn}	0.65^{hijkl}	772^{s}	24.71^{lmnopq}	32.01	49.59^{ij}
0%LSC-MiS	54.11^{j}	87.21^{opq}	30.41^{efghijklm}	5.76^{cdefghijklmn}	0.66^{jklmn}	788^{vw}	25.20^{nopqrs}	31.98	54.81^{qr}
2%LSC-MiS	47.38^{fg}	83.89^{jkl}	30.96^{fghijklm}	5.90^{ghijklmn}	0.68l^{mnop}	801^{yz}	25.64^{qrstu}	32.01	50.96^{jkl}
4%LSC-MiS	46.82^{f}	83.12^{ghijk}	27.70^{def}	5.35^{cde}	0.60^{def}	771^{s}	24.66^{lmnopq}	31.98	51.52^{klm}
6%LSC-MiS	51.26^{i}	85.82^{mno}	29.27^{defghijkl}	5.59^{cdefghijklm}	0.64^{hijk}	729^{j}	23.31^{efghi}	31.98	53.57^{opq}
8%LSC-MiS	60.31^{n}	84.64^{klmn}	29.84^{defghijklm}	5.67^{cdefghijklmn}	0.66^{ijklm}	771^{s}	24.66^{lmnopq}	31.98	51.15^{jkl}
0%LSC-RGH	62.17^{o}	87.27^{opq}	30.32^{efghijklm}	5.76^{cdefghijklmn}	0.66^{jklmn}	782^{u}	25.03^{nopqr}	32.01	54.54^{pqr}
2%LSC-RGH	49.05^{h}	82.91^{ghijk}	28.39^{defgh}	5.47^{cdefgh}	0.62^{efgh}	749^{op}	23.97^{hijklm}	32	50.99^{jkl}
4%LSC-RGH	49.25^{h}	81.62^{efgh}	27.87^{def}	5.39^{cdef}	0.61^{def}	696^{e}	22.28^{cd}	32.01	51.28^{klm}
6%LSC-RGH	66.80^{q}	87.11^{op}	24.24^{bc}	4.85^{b}	0.52^{c}	724^{i}	23.15^{defgh}	31.98	52.53l^{mno}

Table Continued...

LSC-R mix	Parameters								
	IVDMD %	IVOMD %	GV, ml/200mg DM	ME, MJ/kg DM	SCFA, mMol/l	DOM, g/kg DM	MCP		RFV%
							g/kg DM	g/kg DOM	
0%LSC-TS	63.68^{p}	81.27^{efg}	31.83^{ijklm}	5.97^{ijklmn}	0.70^{opq}	815^{B}	26.08^{stu}	32	54.66^{qr}
2%LSC-TS	82.70^{t}	65.07^{a}	30.98^{fghijklm}	5.81^{defghijklmn}	0.68^{lmnop}	800^{y}	25.58^{qrst}	31.98	52.49^{lmno}
4%LSC-TS	78.84^{s}	81.84^{efghi}	29.03^{defghijkl}	5.54^{cdefghij}	0.63^{ghij}	807^{A}	25.81^{rstu}	31.98	53.32^{nopq}
6%LSC-TS	68.50^{r}	87.35^{opq}	31.32^{ghijklm}	5.88^{fghijklmn}	0.69^{nopq}	747no	23.91^{hijklm}	32.01	52.37^{lmno}
8%LSC-TS	54.03^{j}	83.05^{ghijk}	28.47^{defghi}	5.47^{cdefgh}	0.62^{efgh}	759^{q}	24.29^{ijklmn}	32	49.21^{i}
0%LSC-WS	59.99^{mn}	89.29^{r}	24.16^{bc}	4.83^{b}	0.51^{c}	620^{b}	19.85^{ab}	32.02	49.95^{ijk}
2%LSC-WS	56.07^{kl}	86.93^{op}	29.73^{defghijklm}	5.62^{cdefghijklm}	0.65^{hijkl}	750^{op}	24.01^{hijklm}	32.01	52.101^{mno}
4%LSC-WS	65.75^{q}	87.10^{op}	31.02^{fghijklm}	5.82^{defghijklmn}	0.69^{mnopq}	777^{t}	24.85^{mnopqr}	31.98	48.87^{hi}
6%LSC-WS	57.04^{l}	87.19^{opq}	29.90d^{efghijklm}	5.67^{cdefghijklmn}	0.66^{ijklm}	777^{t}	24.86^{mnopqr}	31.99	48.71^{hi}
8%LSC-WS	37.74^{b}	87.06^{op}	28.88^{defghij}	5.53^{cdefghij}	0.63^{fghi}	761^{q}	24.34^{jklmno}	31.98	47.38^{gh}

Means within columns with different superscript letters are significantly different (p<0.05)

LSC, linseed containing concentrate; IVDMD, in vitro dry matter degradability; IVOMD, in vitro organic matter degradability; GV, gas volume; ME, metabolizable energy; SCFA, short chain fatty acid; DOM, digestible organic matter; LSC, linseed containing concentrate; MCP, microbial crude protein; RFV, relative feed value; MCP (g/kg DM), DOM (g/kg DM) x 0.032[17] AFRC, 1993; BS, barley straw; CPa, coffee parchment; CPu, coffee pulp; HBH, haricot bean haulm; MaS, maize stover; MiS, finger millet straw; RGH, Rhodes grass hay; TS, teff straw; WS, wheat straw

Gas volume (GV)

The results of gas volume are presented in Tables 5 & Table 6. The volume of gas is linearly associated with IVDMD, IVOMD, ME and SCFA. CPa had the highest GV followed by those of CPu and HBH while the GV was lowest from WS, the others were intermediate between the two. The amount of linseed influenced the GV which was enhanced with the amount of concentrates. The amount of gas produced was slightly higher than those of the roughages, thus there was no significant increment in GV when LSC was added to the roughages. In most of the mixtures, 0 and 4%LSC-R produced more GV than the rest of the mixtures.

Metabolizable energy

The ME contents of the roughages, concentrates and their mixtures are presented in Table 4 & Table 5 respectively. The ME contents varied less amongst the roughages (5.25 to 5.79MJ/kg DM for WS and CPa, respectively). The ME content of Rs with LSC improved linearly from 6.92 to 7.52MJ/kg DM with increasing levels of linseed inclusion. The ME content of the roughages improved by mixing them with the concentrates, although the improvement was relatively low at 2% inclusion of LSC, while the values reduced among CPu by inclusion of 2%LSC. The ME content of most of the roughages improved through mixing with LSC (0, 6 and 8%).The highest ME was observed at0% and 6%LSC, however it was contrary when LSC was included at 2 and 4% levels. The ME contents of CPu reduced by inclusion of 2%LSC. The ME content of BS enhanced by the inclusion of concentrate but without linseed (0%LSC).The study further indicated that at most of the levels of LSC supplementation, the ME content of CPu, MaS, RGH, TS and WS improved when compared to those of the roughages. As these roughages were low in ME values, inclusion of LSC at 0,6 and 8%improved the ME values in the concentrates. A general trend of reduction in volume of gas with increasing levels of linseed in the concentrates was also observed. This was further elaborated with the changes in the ME content of the different concentrate mixes. The study further indicates that the concentrates derived on any LSC and those with 6% linseed (6%LSC) had the highest ME values among most of the roughages except those of MiS, RGH and WS.

Short chain fatty acids

The values calculated for the production of short chain fatty acids (SCFA, mMol/l) of the roughages and concentrate-roughage mixtures (LSC-roughage) from the *in vitro* gas test are presented in Tables 4 & Table 5. The findings indicate that the highest SCFA (P<0.05) was recorded from CPa, HBH and CPu while the lowest values were observable from WS, with the other values (p<0.05) lying between the two above mentioned extremes. The concentration of *in vitro* SCFA production of most of the roughages enhanced by mixing them with the linseed-containing concentrates. The study further indicated that higher values of SCFA was recorded in most of the roughages, except those of MiS, RGH and WS, where the higher levels were assessed at 0%LSC and 6%LSC supplementation. It can further be concluded that there was no correlation between the levels of LS with those of SCFA production. The SCFA production of most of the roughages was not significantly influenced by mixing with the concentrates and also with the level of LS. However, the values of the SCFA varied when BS, CPu and WS were mixed with LSC, the findings are in consonance with that of total VFA concentration of steers fed spear grass hay alone which was 54.9mm and did not change significantly when supplemented with *Spirulina platensis, Chlorella vulgaris*, *Dunaliella* salina cells and *cotton seed* meal (52.3, 57.1, 51.3, 61.5 mM, respectively).[33] The findings from Table 3 further indicates that among the studied crop residues the CP values were highest in CPa, CPu and HBH, consecutively they too produced the highest amount of SCFA (Table 5). The present findings varied from earlier reports where significantly higher levels of SCFA was produced by feeds which had higher levels of nitrogen (0.74 to 0.78mMol/250mg) than lower N

(0.74 to 0.78mMol/mg).[19] High concentration of SCFA can also be correlated with higher MCP yield. The findings from the present study indicate that N from different roughages did not influence the SCFA, which are in agreement with total VFA concentration (mM) that did not differ between different diets.[34] The in vivo studies too indicated similar trends in sheep[35] or cows.[36]

Microbial crude protein

The calculated microbial crude protein (MCP) production from the roughages and concentrate-roughage mixtures[17] are presented in Tables 4 & Table 5. he MCP production of the concentrates (calculated from DOM) did not vary significantly (25.5±0.3 for 0%LSC and 26.2±0.4 g/kg DM for 6%LSC), the observations are in accordance with the findings of concentrates containing varying protein levels (16-19% CP in the dry matter).[37] The tables indicate that the increment of MCP was lowest in CPu and WS based diet, while the levels from HBH and CPa were the highest. The findings too show that the CP: ME ratio and also CP: DOM values were quite high in HBH (19.2g CP/MJ ME and 143g CP/kg DOM) which might be attributed to comparatively less energy available for the microbes to convert the CP to MCP and thus influencing the MCP yield which was enhanced by supplementing the diet with the concentrates when compared to rest of the Rs. The study also indicates that the feeding value of the roughages with low MCP (CPu and WS) improved when mixed with the concentrates, this was more when compared to diets with potentially high MCP viz. HBH and CPa. In most of the cases diets containing 2 and 8%LSC led to reduction in MCP production while it improved at 0, 4 and 6%LSC. The linear improvement in MCP was observed from 2- 6 %LSC, thereafter there was a decline which was recorded at 8%LSC. The actual MCP yield, (although not the efficiency, 4-5g MCP/MJME), was improved by supplementing the roughages with the concentrates, however, the levels of LS had variable influence on this parameter. Findings of a study indicated that efficiency of microbes was approximately 10g MP/MJ ME[17] while other findings indicated values between 7 to 14g MP/MJ ME[38] which are much higher than the present findings. Efficiency of microbial production of roughages and LSC-R mixtures was about 32g MCP/kg DOM. The CP content of the roughages was assessed to range between70 and 152g/kg DOM (BS and CPu, respectively) while the corresponding values in the concentrates were between (213 to 255g/kg DOM). The higher CP values in the concentrates improved the efficiency of microbial CP production in the concentrate based diets. The results from different studies have indicated variable efficiencies some of which are in agreement with the present studies for the roughages and LSC-roughage mixtures, while higher and lower values too have been reported by several authors. The result of a study[39] indicated that 30 grams of microbial N/kg in truly degraded organic matter; while it was reported that the supply of microbial protein to the ruminants per unit of feed ingested varied from 14 to 60 g N/kg of digestible OM.[30] Microbial efficiency expressed as grams microbial N/kg DM and organic matter ranged from 21 to 27; and 35 to 44, respectively.[40] Microbial biomass yield in concentrates and forages was assessed to vary between 211 and 303g/kg TDOM, respectively.[41] Among the lactating cows when proportion of roughage was reduced from 40 to 32 %, the organic matter apparently degraded in the rumen (OMADR) yield around 22g of MCP/100g High proportion of roughages in the ration (extremely low energy) yielded only 15 to 20g MCP/100g OMDR, whereas with high levels of concentrates in the ration resulted in14 to 18g MCP/100g OMADR[42] the findings are in close agreement with the present results. 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 106g MCP/kg DOMI, respectively.[33] Although this efficiency is influenced by the intake, the figures, when converted to per unit of DOM, are more or less in agreement with the present MCP values. In a study it was reported that the breakdown of carbohydrate in the presence of adequate ammonia results in enhancement of microbial protein.[43] Thus it can be concluded that with readily soluble and fermentable protein the protein to energy ratio show a decreasing trend in the rumen, the trend was also similar in the roughages, when added with feed containing relatively higher amount of energy. This was what was observed in the roughages when supplemented with the energy rich concentrate. The results are in consonance with earlier findings who also observed that among the several factors influencing the synthesis of microbial protein, the availability and synchronization between energy and nitrogen compounds (N) in the rumen play a primordial role.[44] The findings too are indirectly in accordance with earlier findings who too observed similar enhancements when roughages were supplemented with concentrates. Microbial biomass yield from purified diets vary considerably which might be attributed to the quality of the diets.[45,46] Diets containing up to 31 or even 39% NSC and RDP values from 11.8 and 13.7% on DM basis supported enhanced MCP synthesis when compared to a diet containing 25% NSC and 9% RDP.[47] The efficiency of MCP synthesis was 11 to 20% greater in sheep given diets with CHO source (barley) synchronous with rapeseed meal when compared to those asynchronous with urea.[46] The NRC system assumes that the amount of protein required by the microorganisms is equivalent to the amount of MCP produced in the rumen; however all of the MCP is available to the animals metabolizable protein (MP) therefore the differences.[48] The NRC calculates true protein content in rumen microorganisms as 80% times crude protein, the rest 20% is nucleic acid.[48] The NRC estimate is that only 80% of the true protein in microorganisms is actually absorbed by the animal.[48] 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.[49]

Relative feed value

Calculated relative feed value (RFV) of the roughages and concentrate-roughage mixtures are presented in Tables 4 & Table 5. The results indicate that lowest ($p<0.05$) RFV of the roughages was obtained from CPa, CPu and HBH, while the highest ($p<0.05$) values were from MaS and RGH. In general the RFV of the roughages, which is closely related with ADF and NDF contents, which improved by the addition of concentrates. The findings further indicate that 0%LSCresulted in improvement in almost all the roughages this was followed by inclusion of 2%LSC. With the supplementation of the concentrates, CPa, CPu and HBH had highest increment in the RFV, contrary it was lowest in BS, TS and WS vis-a-vis that of good quality alfalfa hay. The RFV of the other feeds (MaS, MiS and RGH) were not very much influenced by supplementation of LSC. The concentrate without linseed (0%LSC) improved the RFV significantly while there was no improvement in RFV by inclusion of linseed in the concentrate alone contrary there was some reduction in RFV in the roughages. In general no visible trend in RFV was observed with the increasing levels of linseed in the concentrate. Thus, it can be concluded that mixing three parts of the roughages with one part of concentrates improved the overall nutritive value by enhancing the CP, DOM, ME, total SCFA, MCP production and RFV. The level of linseed added to the concentrate up to 4 % had a synergistic effect with the roughages and concentrates and hence could be recommended.

Summary and conclusion

The *in vitro* gas tests conducted on nine different types of roughages supplemented with concentrates containing five levels (0, 2, 4, 6 and 8%) of ground linseed (LSC) revealed significant improvements of the roughage-LSC mix in their CP (smallest CP of BS and MaS (5.05 and 5.69%, respectively) was raised to over 11.71 and 19.85% CP, respectively at 0%LSC) and ME (from WS and BS (5.16 and 5.25 MJ ME/kg DM, respectively) was slightly raised to 5.56 at 0%LSC and 5.82 at 4%LSC, respectively) contents and IVOMD (from CPu and WS (64.37 and 69.63%, respectively) to 88.72 and 89.29% at 0%LSC, respectively); production of SCFA (from CPu and BS (0.57 and 0.59 mMol/l, respectively) it was very raised to 0.71 and 0.63 mMol/l at 0%LSC) and MCP (from CPU (18.7 g/kg DM) it was improved to 26.36 and 26.54 g/kg DM at 0 and 4%LSC, respectively) and RFV (from HBH, CPu and CPa (33.95, 35.17 and 39.03%) it was raised to 42.32, 46.71 and 46.69% at 0%LSC and to 43.61, 48.93 and 48.94% at 4%LSC, respectively). The crude protein and ME contents of the roughages were improved by mixing them with the concentrates. The *in-vitro* gas test revealed that potential OM degradability and thus DOM content was numerically improved; when roughages were mixed with the concentrates although improvements were not significant. This improvement was because of change in the CP and ME value of the roughages due to LSC supplementation. The influence of the level of linseed was variable and in most cases was not significant. The SCFA production of roughages 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. That less gas and SCFA, and more microbial N, were recovered in N-rich versus N-low incubations, together with the findings that TSDOM were unaffected by N level, supports the hypothesis of a shift in partitioning of substrate use from SCFA–gas complex to MCP.[19] The MCP production of the Rs was improved by mixing them with the concentrates; 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 an increased degradability in the rumen and conversion of this degraded protein to microbial protein. The relative feed value (which is very closely related with ADF and NDF contents) of the roughages were improved very much when supplemented with the concentrate. The RFV of the roughages was improved by the addition of concentrate. Coffee pulp, coffee parchment, finger millet straw and teff straw when supplemented with the concentrates had much higher RFV as compared to good quality alfalfa hay which is considered to have 100% RFV. However, barley straw, haricot bean haulm, Rhodes grass hay, maize stover and wheat straw had lower RFV. It can thus be concluded that mixing three parts of the roughages with one part concentrates improved the nutritive value of the mixture in terms increasing CP, ME, total SCFA production. The level of linseed in the concentrate up to 4% or 6% has supported the positive effect of mixing the roughages with the concentrates and could as such be recommended.

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None

Conflicts of interest

The author declares that there are no conflicts of interest.

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