EVALUATION OF CEREAL CROP RESIDUES: INFLUENCE OF SPECIES, VARIETY AND ENVIRONMENT ON NUTRITIVE VALUE

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FINAL REPORT
covering the period
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CONTENTS

Objectives of the Report

a. (i) To identify and quantify the major factors responsible for decreasing the nutritive value of different species and varieties of sorghum and millet crop residues.

(ii) To quantify the effects of environment on the nutritive value of cereal crop residues.

To carry out feeding trials involving growth and metabolism studies to evaluate improved selected varieties of sorghum and millet for feeding ruminants.

b (i) To assess the effects of traditional methods of storage and utilisation on the phenolic content of sorghum stover.

To study the phenolic content of sorghum stover during the last few weeks of grain maturation using contrasting varieties.

Objectives a(i), a(ii) and a(iii) are from the original contract, (Section 8a) with a(i) being narrowed from the original to include only sorghum and millet residues. Objectives b(i) and b(ii) were added as a six months extension to the programme and covered the period from October 1990 until March 1991.

2 Work carried out in this period

The complex mixtures of phenolic compounds found in extracts from sorghum samples were poorly separated by existing high pressure liquid chromatography (HPLC). Some considerable time at the beginning of the contract was therefore committed to the development of more satisfactory analytical methods. The use of counter current chromatography (CCC) with existing HPLC procedures, post column derivatisation and diodearray detection markedly increased our ability to separate and identify phenolic components.

The amounts of phenolic compounds and their distribution through sorghum plant components (ie leaf blade, leaf sheath, stem) was examined in a number of experiments. Particular attention was paid to differences between sorghum varieties of both bird and non-bird resistant types. The effects of environment on the production and accumulation of phenolics was followed in some experiments involving a number of different varieties and four different growing sites. Other factors potentially influencing phenolic content of sorghum which were also examined were time of harvesting (or utilising) the stovers, and the effect of period of storage between harvesting and feeding.

Relationships between the phenolic contents of sorghum stover and its nutritional value were examined using *in vitro* assessment of nutritional quality, chemical analyses of other plant components and near infra red (NIR) spectral analysis. The toxicity of phenolic extracts to rumen bacteria was examined together with their effects on rumen microbial fermentation characteristics. New methods were also developed for both these aspects of the work.

Experiments, all conducted in Ethiopia, examined differences in intakes and performance of oxen receiving stovers from different varieties of sorghum with comparisons between bird and non-bird resistant types. These production type experiments were extended to sheep and to goats and were enlarged to incorporate feeding strategy work. These included the amount of feed offered and the physical form of the feed.

The chemical composition and nutritional value (assessed by *in vitro* techniques) of a number of African and Indian varieties of millet were assessed. The content of phenolic compounds found in these samples was very low.

3. Results of findings obtained by the project

A. To Identify and Quantify the Major Factors Responsible for Decreasing the Nutritive Value of Different Species and Varieties of Sorghum Crop Residues

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Phenolic Compounds and their Relationship to *in vitro* Digestibility of Sorghum Leaves from Bird-and Non Bird Resistant Varieties

Preliminary studies concerning the separation and identification of phenolic compounds in sorghum has been extended. Considerable advances have been possible since the development during the course of this contract, of a post column derivitization procedure for the UV-Vis spectroscopic characterisation of phenolic compounds (Mueller-Harvey & Blackwell, 1991) Sorghum leaf blades and sheaths were examined after full grain harvest for soluble phenolics and relationships between HPLC peaks and *in vitro* digestibilities were examined. Full results and details of methodology and experimentation of the latest work is presented in typescript in Appendix B7.

Main findings were:

Apigenin and luteolin together with their 7-0-glucosides, p-coumaric acid, butin and apigeninidin were identified. This is the first reported finding of butin in sorghum tissues.

Derivatives of luteolinidin, chalcone and flavanone and/or dihydroflavonol together with several other derivatives of cinnamic acid, apigenin, luteolin and apigeninidin were also detected.

The composition of phenolics was clearly different between leaf blades and leaf sheaths. The phenolic content of leaf sheaths from bird resistant and non-bird resistant varieties were also different.

Biosynthesis of flavonoids appears to diverge at the flavanone/dihydroflavonol stage between bird resistant and non-bird resistant varieties

Several negative correlations were found between HPLC peaks and *in vitro* digestibilities. These were highly significant with butin and significant with several luteolin derivatives but only with one apigenin derivative. Butin in turn was highly negatively correlated with colorimetric measurements of 3-desoxyanthocyanidins. This may suggest that butin rather than 3-desoxyanthocyanidins (as earlier reported) is implicated in reducing dry matter digestibility.

2. <u>Near Infra Red Spectroscopy (NIRS) of Sorghum Crop Residues</u>

Sorghum leaf blade and leaf sheath which had been analysed for phenolic components and *in vitro* digestibility were analysed by NIRS. Differences between averaged bird resistant and non-bird resistant spectra are shown in Fig 1. NIRS demonstrates that leaf blades and sheaths of bird resistant varieties contain more phenolic than non-bird resistant varieties: a characteristically shaped difference peak occurs at 1664nm. Detrended spectra of several phenolic standard compounds also give similarly shaped broad peaks between 1638 and 1674 nm., indicative of the presence of polyphenolic compounds (condensed tannins). The multiple correlations between various phenolic parameters and NIR spectra were assessed by regression analysis. Table 1 shows that extractable phenolics from leaf blades and sheaths, which absorb at 280nm (A_{280}) are very well explained. For leaf sheaths the correlograms of lignin, NDFD and *in vitro* digestibilities with NIR spectra clearly demonstrated that the more lignified plant materials are less digestible (Fig. 2). Wavelengths at 1200, 1700 and 2260 nm ...

absorbing at 280nm, 550nm and neutral detergent insol... red pigments (mainly cinnamic acid derivatives and flavonoids) were also well correlated with several NIR wavelen... (Fig. 3); In leaf blades lignin does not correlate with digestibilities ... O... ll phenolic levels in blades also lower than in sheaths and their correlation with NIR spectra were of lower order

Conclusion

Comparisons of ... of various phenolic and lignin ... there are several clear differences suggesting that NIRS may be used to differentiate between ...

Microbiology and Nutritional Value

The effect of phenolic compounds in bird-resistant sorghum crop residues, on the growth of specific rumen bacteria were examined *in vitro*. This involved the impregnation of glass microfibre discs with aqueous acetone extracts from sorghum stovers whic... then placed ... agar ... determined ... of i... . Eleven ... tested against these organisms and the resul... *reptococcus* ... showed no evidence of toxicit... *ibrio fibrosolvens* ... the extracts and *Bacteroides succinogenes* was inhibited by seven. T... of phenolics required for these inhibitions were considerably greater than one would ... the rumen of animals receiving sorghum diets suggesting that phenolic content was not the ... mechanism ... soluble phenolics are tested the assessment of the effect of all phenolics on residue digestibility. Amounts of ground substrates were mixed with 90ml of growth medium in gas-tight anaerobic culture bottles (5 replicates). Each bottle was inoculated with 10ml of strained rumen contents and fermented for 5 days at 39°C. A pressure transducer with LED digital readout meter was used during incubations to monitor the accumulation of fermentation gases in culture bottles. The transducer was connected via a three-way syringe valve to a syringe needle and a plastic syringe. Gas pressures was read from the LED meter after inserting the syringe needle through the bottle closure. Corresponding gas volumes were determined by withdrawing the syringe plunger until pressure in the culture bottle had returned to ambient as shown by the display unit Following measurement, the transducer assembly was withdrawn from the culture bottle and the displaced gas was discarded. Pressures and volumes were recorded at 3 to 24h intervals during a 120h fermentation period, with more frequent readings during the initial 40h of incubation. Gas volumes from individual cultures were plotted against corresponding gas pressures and the data subjected to regression analysis (Fig. 4) Cumulative gas production was then determined by summation of predicted (regression corrected) gas volumes from replicate cultures (Fig. 5) a two-pool model of the general from

$$Y = A[\exp(-k_1 t) - 1] + B[\exp(-k_2 t) - 1]$$

was used to fit data points and produce the solid lines shown in Fig. 5. From this equation, values for the rate constants k_1 and k_2 (both per h) and pool size parameters (A and B) could be determined. This procedure has been applied to leaf blade, leaf sheath and stem fractions of bird-resistant and non-bird resistant varieties of sorghum which differed in their polyphenolic chemistry. Digestion studies have been carried out with crop residues before and after the removal of soluble polyphenolics enabling the partitioning of effects. The effects of type and amount of nitrogen availability has also been shown to be of importance (Fig.6) and has also been investigated by the new procedure. Whilst the actual practical experimentation has been completed, data processing is still progressing. This material will be reported in a Supplementary Report as soon as possible.

B. To Quantify the Effects of Environment on the Nutritive Value of Cereal Crop Residues

(I. MEULLER-HARVEY; M S DHANOA, A B McALLAN, IGER, Hurley;
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Varietal Differences Amongst Sorghum Residues in Relation to their Phenolics HPLC - Fingerprints and Responses to Different Environments.

Earlier work on environmental effects has been reported (Progress Report Oct. 1987 - Oct. 1989) which has been extended and is further reported here. Sorghum crop residues (14 varieties) were harvested at grain maturity from two matched varietal trials conducted at Melkasa (elevation 1500 m) and Debre Zeit (elevation 1900m) and from other marked trials at Melkasa and Duckam (elevation 1900m). Full results and details of the methodology and experimentation for this work is presented in Appendix B6 as a typescript submitted for publication. In brief the main findings were:-

Environment had greater effects on phenolic composition than plant variety. Some effects were however, due to variety

Most varieties seemed to give a strong environment x genotype interactions however, the phenolic compositions of two bird resistant varieties were more stable in different environments.

Differences between bird resistant and non-bird resistant varieties were clearly expressed in leaf phenolics at some but not all sites. All varieties had similar stem phenolics.

A strategy is suggested to be used in breeding programmes for the selection of bird resistant varieties with improved digestibilities.

C. Effect of Harvesting of Different Stages of Growth and Long Term Storage on the Phenolic Composition in Sorghum Stover

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Background

Leaf stripping of sorghum for animal feeding during plant growth is common practice in some parts of Africa, as is storage of stover for some months after harvesting before feeding. It is essential to know if either practice has any effect on the phenolic content of the plant and hence on its nutritional value.

Experimental

Three bird-resistant varieties of sorghum (Seredo, X/35;24, and Ikinyaruka and one non-bird resistant variety (Melkamesh) were grown in Ethiopia, elevation 1500m. Samples (five neighbouring plants) were collected at 50% flowering stage, black layer stage and full harvest. Plots were triplicated for all varieties. Harvested leaves were fractionated into leaf blade (LB) and leaf sheath (LS) which were air dried and ground to pass a 1mm sieve. Phenolics were solvent extracted and analysed on HPLC using standard methods.

Generally, and in line with previous studies, there were large differences in the shape of the chromatograms (composition and concentration) between LB and LS of each of the sorghum varieties used. The difference between varieties appeared to be greater in LS than in LB (Figs. 7 & 8). From the chromatograms of x/35;24 or Serado varieties (Figs. 7 & 8) it can be seen that large differences exist between the 50% flowering stage compared with the other two stages, especially in the LS fraction. In the case of LS of x/35;24 at 50% flowering stage an as yet unidentified (25min. elution time) peak and luteolin (33min. elution time) were the strongest peaks, whereas in the black layer and harvested stages, apigenidin (24 min. elution time) and an unidentified compound

(18 min. elution time) were the strongest. In LS of Seredo (Fig. 8) the concentrations of luteolinidin and apigenidin 14-15 min. elution time) were greatest and luteolin fell to a smaller peak at the 50% flowering stage. However luteolinidin and apigenidin peaks became smaller than that of luteolin at the black layer and full harvest stages.

In LB of x/35;24 and Seredo, luteolin -7-0 glucoside was the major compound at all stages of growth There was however, a difference in peak distribution between the 50% flowering stage and the other harvesting stages. The differences could be seen where several peaks (12-16 mins. elution time) (corresponding to caffeic acid or flavones) became smaller (Seredo) or disappeared altogether (x/35;24) at the black layer or full harvest stages.

Storage of the samples at room temperature (15°C) for three months after full harvesting did not appear to influence the phenolic content or composition of either LS (Fig. 9) or LB.(Fig. 10)

Conclusions

Sorghum leaves and sheaths harvested for ruminant feeding before the plants reached the black layer stage may be of different (reduced ?) nutritional quality compared with those harvested at later stages.

Storage of leaf blade or leaf sheath fractions from different varieties of sorghum plants did not influence the content of phenolic components.

N.B. These data are largely qualitative at the moment. There is still a considerable amount of data processing required before the material will be suitable for publication.

Feeding and Growth Trials with Different Sorghum Residues

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Background

Farmers in Ethiopia and other African countries are being encouraged to plant new varieties of sorghum which are more bird-resistant than traditional varieties. Unfortunately bird-resistant varieties are known to contain polyphenolic compounds which may have adverse effects on intake and digestion of stover. To investigate this the following series of experiments were performed.

1. Effect of Stover Variety and Amount Offered on Growth. Intake and Selection

Ethiopian Lowland rams, approximately 15 months of age were used. Experiment 1a used 48 individually-fed rams and six treatments in a 3 x 2 factorial randomised-block design. Each animal was fed 100g/day of decorticated cottonseed meal (DCSM), containing 64gN/kg, and offered either 25, 50 or 75/kg body weight daily of one of two varieties of sorghum stover; these were Melkamash, a non bird resistant variety, and MW 5020, a bird resistant variety. Stovers were crudely chopped and offered twice daily, refusals being collected before each meal. A 21 day diet adaptation period was followed by a 75 day experimental period. Measurements made throughout the experiment included body weight, feed intake and composition of the stover offered and refused. In experiment 1b, the same treatments were applied to measure digestibility in 24 of the rams. Fourteen days were allowed for adaptation followed by 21 days experimentation. Measurements made were feed intake and faeces output.

Results of the effects of the amount of stover offered on intake and growth are shown in Table 3. Intake of stover and rate of growth increased significantly ($P<0.01$) when the rate of offer of stover was increased from 25 to 50g/kg liveweight/d. Further increasing the stover allowance to 75g/kg liveweight /d did not result in any further significant increase in intake or weight gain. There was no significant difference in intake or growth due to variety of stover, contrary to expectations. There was no significant interaction between rate-of-offer and variety of stover. Table 4. shows the effects of amount on offer on the composition of the refusals. It can be seen that the stover offered contained more nitrogen and less ADF than the stover refused. Preliminary results from fractionating the offered and refused stover showed that animals were selecting for leaf blade and leaf sheath against stem; particularly in the non bird-resistant variety Melkamash. The animals did not appear to be selecting leaf sheath in the bird resistant variety MW 5020 which may be related to the fact that the MW 5020 leaf sheath is highly pigmented and contains greater amounts of polyphenolics than either leaf blade or stem.

Results from the digestibility experiment 1b are shown in Table 5. The intake found above on increasing offer rate from 25 to 50g.kg liveweight was confirmed. Total dry matter intake was significantly higher than when MW 5020 variety was offered. There were no significant varietal effects on dry matter digestibility but there were significant varietal effects on nitrogen, NDF and ADF digestibilities, all of which were lower in the bird resistant variety MW 5020. The lower digestibilities observed with MW 5020 were as expected because of the high polyphenolic content whereas in contrast the higher intakes of MW 5020 were unexpected. The higher content of leaf in MW 5020 may help explain this apparent anomaly.

Conclusions

Increasing the amounts of stover offered from 25 to 50g/kg liveweight increased average dry matter intake by about 26% and growth rate by about 72% equally with both varieties but the amount of refusal increased from 7 to 42% of that offered. No further improvement was found with further increasing rate offered. There was evidence that rams selecting for leaf and against stem and that selection was facilitated by increasing the amount of stover offered.

Contrary to expectations, intake and growth were not inferior when rams were fed bird-resistant MW 5020 stover compared with non-bird resistant Melkamash stover. Lower digestibilities of components of ME 5020 were as expected whereas the higher intakes of this variety were not.

2. <u>Effect of Chopping and Amount Offered on Growth, Intake and Selection</u>

In the above experiments the sorghum stover was offered in the chopped form. The present experiment investigated the effect of chopping *per se* with stover offered at 20 or 50g/kg LW (Liveweight)/d.

Forty-eight Menz Highland rams of approximately 17kg initial liveweight and 15 months of age were used. These were fed 113g dry matter/ram/d of cottonseed cake and *ad libitum* sorghum stover (non-bird resistant variety (Dinkamesh). Both cottonseed cake and stover were offered twice/d with water *ad libitum*. The experiment lasted for 56 days using a 2 x 2 factorial arrangement involving long (unprocessed) or chopped stover offered, *ad libitum* at 25 or 50g DM/kg LW/d involving four groups each of three rams per treatment. Animals were weighed weekly and the amounts of stover consumed daily were recorded. Offered and refused stover were fractionated into leaf blade, leaf sheath and stem.

For the parameters measured there were no significant ($p < 0.05$) interaction between stover form and amount offered therefore the data presented are main effect means. Both chopping the stover (Table 6) and increasing the amount offered (Table 7) improved stover intake and animal growth rate. It can also be seen that the rams were selecting for leaf and leaf sheath although a substantial amount of stem was consumed even when the stem was in the long form. It can also be seen that at the higher offer rate almost half of the amount offered was refused.

Conclusions

Both chopping stover and increasing the amount offered increased intake and growth. Selectivity increased with increasing amount of stover offered. The data offer strategies for feeding stover to sheep to alleviate dry-season feeding shortages and at the same time provide residues for other purposes such as fuel.

3 Effect of Amount Offered on Growth, Intake and Selection of Goats and Sheep

The greater intakes and selection of sorghum stover observed above when sheep were offered increased *ad libitum* amounts offered resulted in improved growth rates In view of the importance of goats in smallholder tropical-agriculture and the fact that goats are considered to be selective feeders, the present experiment compared the response of goats and sheep when offered increasing *ad libitum* quantities of sorghum stover.

Sorghum stover (bird resistant variety (Seredo) was chopped and stored for one month before use. Twenty four Galla buck goats (11-13 months old) and twenty four Ethiopian Lowland rams (14-16 months old) were used. After an adaptation period of 21 days the experimental period continued for a further 75 days in a 2 x 3 factorial design with 8 replicates. Both goats and sheep were individually penned and fed 155g cottonseed cake dry matter/animal/day and offered one of three *ad libitum* amounts of sorghum stover (25 50 or 75g/kg LW/d). Water and salt lick were available *ad libitum* throughout. Animals were weighed weekly and amounts of stover offered and refused were recorded daily. Samples of offered and refused stover were fractionated into leaf blade, leaf sheath and stem in order to assess selectivity.

For the parameters intake and growth there were no significant ($p<0.05$) interactions between species and amount of stover offered. Main effect means are shown in Tables. Ram growth rates (Table 8) were more than twice those of the bucks but mature weight differences (sheep 27-32kg; goats 18-26kg) would contribute to this. Both species contributed similarly (c.f. absence of significant interaction) in that intake and growth rate increased with increasing amounts of stover offered (Table 9). The largest response occurred to increasing the amount of stover offered from 25 to 50g/kg LW/d. The composition (g/kg) of the stover offered was leaf blade, 84; leaf sheath, 237; stem, 616. Mean leaf content (g/kg) of refusals from the 25, 50 and 75 offer rates were; goats, 1, 2, 5; sheep, 0.1, 0.5, 12. Leaf sheath contents of refusal were; goats, 40, 67, 113; sheep 29, 60, 145 Both sheep and goats selected for leaf blade and leaf sheath.

Conclusions

The results indicated that both goats and sheep are capable of selective feeding leading to increased intake and growth when they are offered increasing *ad libitum* amounts of chopped sorghum stover. For the genotypes used, the growth rate of the sheep was more than double that of the goats.

E. Nutritional Evaluation of Millet Residues

(A B McALLAN, IGER; E WELTZIEN, ICRISAT, INDIA)

Background

In certain regions of the world pearl millet is not only an important source of food grain but also an essential source of animal feed. In normal years the stover will be fed to ruminants and in certain regions will constitute the major source of feed. In very dry years the green crop may be harvested and fed if there is little chance of any grain production. Little is known about the variability of the nutritional value of millet crop residues but there are indications that farmers perceive differences in stover quality and that these differences are reflected in market price. Preliminary studies (Progress Report 1987-89) with twelve African millet samples indicated that variation in digestibility between varieties was less than that found in sorghum but that the nutritive value of the leaf sheath and stem was well below maintenance requirements for cattle. The present study was an examination of the chemical composition and potential nutritional value of a wider range of phenotypes of improved and local (Indian) varieties and landraces.

Experimental procedure

The experiment was grown at ICRISAT centre Patancheru, India. Twenty four varieties were grown in a rectangular lattice design with four replications. Each plot had two border and one central guard row providing two sub-plots of two 4m rows. Plants were partitioned into leaf blade, leaf sheath and stem which were dried at 55°C with the stems split to facilitate drying. The samples were ground through a 10mm screen and the two sub-samples from the same plots were pooled.

Results

The varieties used in this experiment are described in Table 10. Chemical components of those samples are presented in Table 11. Leaf blade had significantly greater ash content than sheath ($p<0.05$) or stem ($p<0.001$) and leaf sheath ash was significantly higher than that of stem ($p<0.001$). These differences were reflected in significant differences in organic matter contents: Significant differences in the contents of the other components measured (total nitrogen, ADF and NDF) were found between the different morphological components of the millets. Variation in the range of a particular chemical constituent was greatest for nitrogen and ash and least for organic matter. In general greatest variation in chemical composition was found in leaf blade and least variation in stem.

Digestibility characteristics of the millet samples are presented in Table 12. There were no significant differences in digestibilities between plant parts; Unlike the earlier study with African millets which found that the range in digestibility of NDF was less than eight percentage units the range in digestibility of organic matter was approximately fourteen percentage units for both leaf blade and leaf sheath. The range in organic matter digestibility of the stem was about seven percentage units, similar to that found for NDF earlier. Examination of the most and least digestible components of some varieties by post column derivitisation and by high pressure liquid chromatography showed only very low levels of phenolics. The overall digestibility of millet crop residues still generally appears to be less than that of sorghum residues

The number of varieties used in the present studies was quite large. However, there were insufficient numbers of particular types to identify characteristic differences in digestibility. The range in digestibility of millet crop residues needs to be investigated further to identify whether plant characteristics such as time to maturation, stover yield, extent of tillering etc influence crop residue digestibility. Differences in digestibilities in the present work compared with the earlier data should be examined further in order to determine whether these are due to varietal or environmental effects.

Table 1 CORRELATIONS BETWEEN PHENOLIC PARAMETERS AND NIR SPECTRA

	Leaf sheaths R^2	Leaf blades R^2
Digestible NDF	0.62	
In vitro digestibility	0.59	
Lignin	0.72	
Phenolics, A_{280}	0.93	
Insoluble red pigments	0.78	
Nitrogen	0.83	0.93

Table 2 <u>EXTRACT TOXICITY FOR THREE RUMEN MICROORGANISMS</u>

Variety and fraction	Streptococcus bovis	Butyrivibrio fibrisolvens	Bacteriodes succonogenes
Susa-sheath	-	+	+
5Dx160-sheath	-	+	+
3KK724-sheath	-	+	+
IS8686-sheath	-	+	?
X35 24-leaf	-	±	+
IS8686-leaf	-	±	+
IS8686-leaf	-	+	+
81 ES1P17-leaf	-	±	-
Seredo-leaf	-	±	?
Susa-stem	-	±	-
DX135/13/1/3/1	-	+	+
Acacia nilotica	+	+	+
Quebracho tannin (control)	+	+	+
Tannin acid (control)	+	+	+
Negative control (70% aquous Acetone	-	-	-

Table 3 EFFECTS OF AMOUNT OFFERED ON STOVER INTAKE AND GROWTH RATE

Variety	Melkamash (non bird resistant)						MW 5020 (bird resistant)						Level of significance		
Offer-rate of stover (g/Kg LW/d)	25		50		75		25		50		75		Variety (v)	Offer rate(o)	VxO
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.			
Number of rams	8		8		8		8		7		6				
Weight (kg)	14.5		15.5		16.0		17.5		13.5		15.0				
Stover intake (g DM/kg LW/d)	22.4	1.57	28.6	1.65	29.3	2.70	24.4	0.44	30.0	0.66	30.7	3.02	NS	**	NS
Stover refused (% of intake)	10.3	6.3	42.8	3.3	60.9	3.6	2.6	1.8	40.1	1.3	59.1	4.2	NS	**	NS
Growth (g/d)	33.5	12.7	63.4	13.0	76.7	9.1	45.4	12.6	70.2	9.0	76.7	14.9	NS	**	NS

** $p<0.01$

Variety	Melkamash (non bird resistant)								MW 5020 (bird resistant)							
Stover	Offered		Refused						Offered		Refused					
Offer rate (g/kg LW/D)			25		50		75				25		50		75	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Nitrogen (g/kg DM)	5.3	1.0	3.6	0.3	3.6	0.3	3.7	0.3	6.0	0.4	3.9	0.3	4.2	0.3	4.3	0.2
ADF (g/kg DM)	391	12	451	18	492	8	402	10	341	17	426	21	385	6	385	13
Leaf (g/kg)	167		2		20		29		250				49		102	
Leaf sheath (g/kg)	252		3		43		128		220				120		254	
Stem (g/kg)	498		960		897		804		420				762		612	

Table 5

EFFECTS OF AMOUNT OFFERED ON DIGESTIBILITY

Variety	Melkamash (non bird-resistant)						MW 5020 (bird resistant)						Level of significance		
Offer rate of stover (g/kg LW/d	25		50		75		25		50		75		Variety (v)	Offer rate (o)	Vx0
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.			
Number of rams	4		4		4		4		4		4				
Weight (kg)	23.5		25.0		24.5		25.5		23.6		26.0				
Total intake (g DM/d)	593	61	748	32	742	46	673	22	808	29	884	48	**	**	NS
Digestibility															
DM (%)	62.7	2.6	59.2	0.7	57.5	6.5	59.8	2.3	54.7	12.9	59.7	6.2	NS	NS	NS
NDF (%)	64.6	3.6	59.6	1.4	58.5	3.3	59.5	2.7	48.3	10.6	55.0	0.6	*	NS	NS
ADF (%)	65.4	3.8	60.6	3.1	62.5	3.2	59.0	2.0	49.9	13.1	49.5	7.0	**	NS	NS
Nitrogen (%)	62.8	1.7	60.9	1.5	64.2	4.8	58.3	2.3	53.9	14.3	58.8	4.1	*	NS	NS

Table 6

MAIN EFFECT MEANS FOR FORM OF STOVER

Factor	Form (F)		s.e.	Significance	
	Chopped	Unchopped		F	F x Amount
Initial weight (kg)	16.9	17.1			
Growth rate (g/ram/d)	58.2	43.2	3.98	*	NS
DM intake (per group) Stover (kg/d)	1.34	.11	0.06	*	NS
Leaf (kg/d)	0.05	0.05	0.002	NS	NS
Leaf sheath (kg/d)	0.53	0.39	0.02	**	NS
Stem (kg/d)	0.76	0.67	0.04	NS	NS
Stover refused (% of offered)	24.8	36.6	2.19	**	NS

Table 7 MAIN EFFECT MEANS FOR AMOUNT OF STOVER OFFERED

Factor	Amount offered (A) (g stover/kg M.day)		s.e.	Significance	
	25	50		A	A x Form
Initial weight (kg)	17.0	17.0			
Growth rate (g/ram/d)	38.2	63.2	3.98	***	NS
DM intake (per group)					
Stover (kg/d)	1.03	1.42	0.06	***	NS
Leaf (kg/d)	0.03	0.07	0.002	***	NS
Leaf sheath (kg/d)	0.31	0.62	0.02	***	NS
Stem (kg/d)	0.70	0.73	0.04	NS	NS
Stover refused (% of offered)	16.5	45.1	2.19	***	NS

le 8 MAIN EFFECT FOR SPECIES

actor	Species (S)			Significance	
	Goat	Sheep	s.e.	S	S x Amount
No. of animals	21	23			
Initial weight (M) (kg)	14.5	17.0			
Growth rate (g/d)	21.5	48.2	3.74	***	NS
Stover offered (g/DM/d)	731	752			
Stover refused (g DM/kg DM Offered	369	272	8.3	***	NS
Stover intake (g DM/d)	428	475	11.1	***	NS

Table 9 MAIN EFFECT MEANS FOR AMOUNT OF STOVER OFFERED

Factor	Amount offered (A) (g stover/kg M.d)				Significance	
	25	50	75	s.e.	A	A x Species
No. of animals	15	15	14			
Growth rate (g/d)	19.5	39.8	47.8	4.58	***	NS
Stover offered (g DM/d)	352	757	1143			
Stover refused (g DM/kg DM offered)	103	356	509	10.2	***	NS
Stover intake (g DM/d)	315	487	563	13.7	***	NS

Varieties of Millets Used

Expt. No.	Name	Category
1.	BJ 104	High Tillering hybrid
2.	WC-C75	Open-pollinated with high stover yield
3.	ICTP 8203	Open-pollinated, low stover yield,low tillering
4.	ICMH 451	High yielding, moderate tillering
5.	ICMH 423	High yielding, high tillering
6.	MBH 110	High yielding, low tillering
7.	15P Syn Fodder	)
8.	RAJ 11	) Rajasthan varieties. Landraces
9.	MDH 8601	) and topcrosses
10	Desert type	)
11.	Chadi	)
12.	Ex BORNU	African Tall
13.	Ex BORNU (d2)	Dwarf version of above
14.	NCCI	Nigerian composite
15.	NC(d2)	Dwarf version of above
16.	UUJ IVM	Indian fodder variety
18.	ICMN 85410	Very early dwarf hybrid, high tillering
19.	ICMV 87901	Very early improved variety
20.	ICMH 87951	Topcross of ICMV 87901 on 843A
21.	ICMV 821132	Open pollinating variety with very high stover yield
22.	MC C8	Composite with excellent phenotype for stover
23.	ICMH 85109	Rust resistant hybrid
24.	ICMP 88905	Diverse ICRISAT material
25.	ICMV 87913	Diverse ICRISAT material

Table 11 CHEMICAL COMPOSITION OF MILLET CROP RESIDUES GROWN AT ICRISAT, India (n = 24)

		as % dry matter				
		Organic Matter	Total-N	NDF	ADF	Ash
Leaf blade	mean	86.1±0.33	1.13±0.032	56.2 ±0.45	29.4 ±0.27	13.9 ±0.33
	range	82.6-88.4	0.85-1.39	51.5-61.3	26.9-31.9	11.6-17.4
Leaf sheath	mean	89.6±0.16	0.42±0.018	68.2 ±0.21	47.7 ±0.33	10.5 ±0.16
	range	87.7-90.9	0.27-0.63	66.1-69.8	45.2-52.9	9.1-12.3
Stem	mean	93.9±0.22	0.25±0.006	74.1 ±0.35	38.8 ±0.18	6.1 ±0.21
	range	91.0-95.3	0.20-0.31	70.4-76.7	37.1-40.8	4.7-9.0

Table 12 DIGESTIBILITY OF MILLET CROP RESIDUES GROWN AT ICRASAT, INDIA (n= 24)

		% DMD[1]	% DOMD[2]	% OMD[3]
Leaf blade	mean	47.2±0.70	40.9±0.73	47.5 ±0.74
	range	39.0-53.3	33.3-46.4	38.4-52.9
Leaf sheath	mean	42.0±0.80	36.4±0.78	40.8 ±0.88
	range	33.1-48.1	27.1-42.5	30.5-47.4
	mean	45.4±0.39	40.7±0.45	42.4 ±0.44
	range	41.9-48.9	37.1-44.2	38.2-46.3

1 Dry matter disappearance)
2 Digestible organic matter disappearance) These terms are defined in Fig.11
3 Organic matter digestibility)

Figure legends

Fig. 1 Differences between average bird resistant and non-bird resistant spectra. Leaf blade), leaf sheath)

Fig. 2 Correlograms of (a) lignin, (b) NDFD and (c) *in vitro* digestibilities with NIR spectra.

Fig 3. Correlograms of extractable phenolics absorbing at (a) 280 nm and (b) 550 nm and (c) neutral detergent insoluble red pigments with NIR spectra.

Fig 4. Regression analysis of head-space gas volume versus gas pressure.

Fig 5. Cumulative gas production with sorghum stem as substrate.

Fig. 6 Effects of polyphenolics from *Acacia nilotica* on the growth of *Streptococcus bovis* at different concentrations of organic nitrogen. (A) no organic nitrogen, (B) half the amount of yeast extract and trypticase, (C) full amount of yeast extract and trypticase with ( ) no polyphenolcs added; ( ) plus polyphenolics extracted from 4.2 mg of leaf; ( ) plus polyphenolics extracted from 5.6 mg of leaf.

Fig 7. Chromatograms (HPLC) of aqueous acetone extracts from leaf blade or leaf sheath fractions of the Ethiopian sorghum variety x/35:24 at different growth stages.

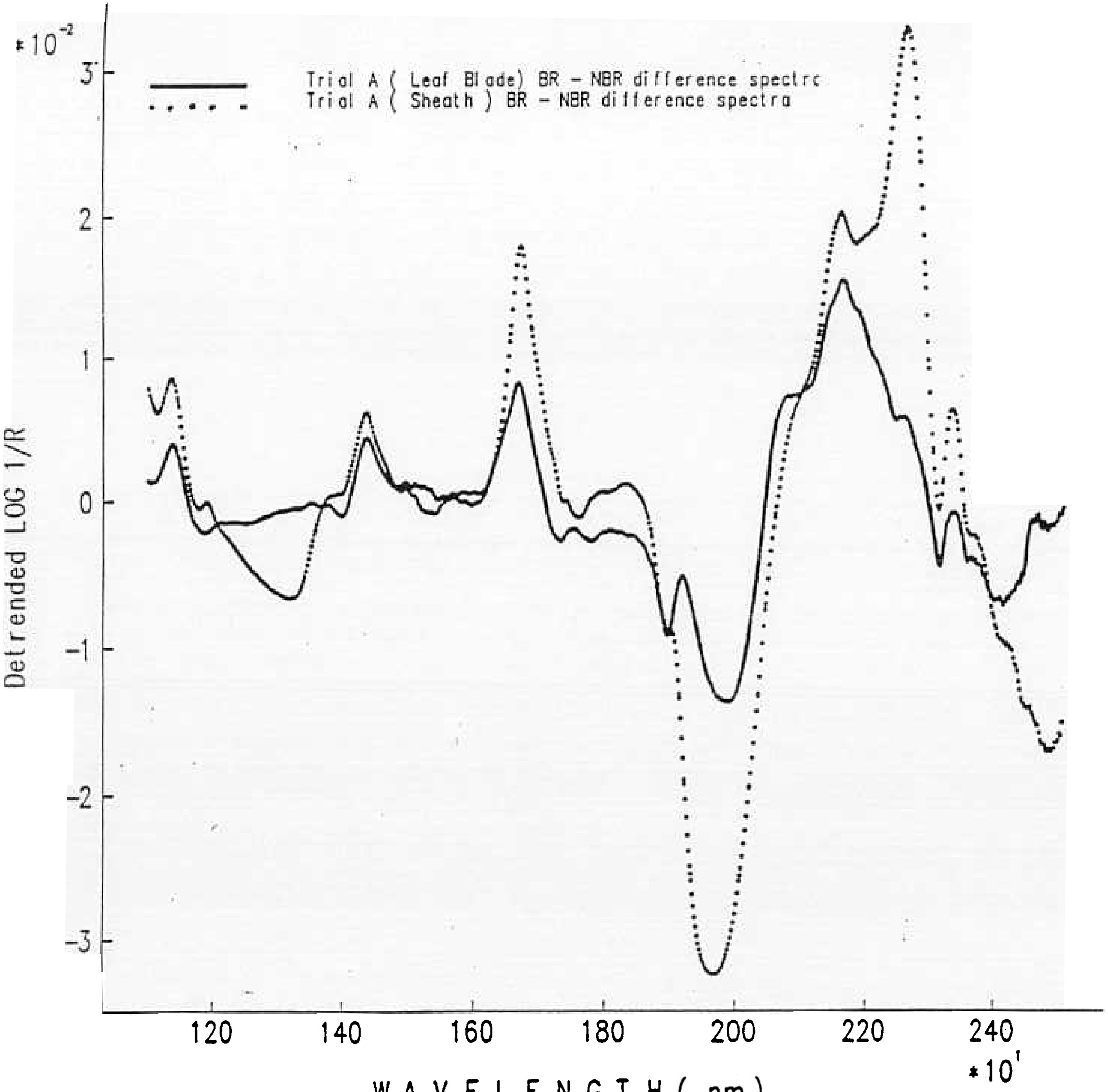
Fig 8. Chromatograms (HPLC) of aqueous acetone extracts from leaf blade or leaf sheath fractions of the Ethiopian sorghum variety Seredo at different growth stages.

Fig. 9 Chromatograms (HPLC)of aqueous acetone extracts from leaf sheath fractions of the Ethiopian sorghum varieties x/35:24; Seredo; Dinkamash and Ikinyaruka at harvest and three months after storage.

Fig 10. Chromatograms (HPLC) of aqueous acetone extracts from leaf blade fractions of the Ethiopian sorghum varieties x/35:24; Seredo; Dinkamash and Ikinyaruka at harvest and three months after storage.

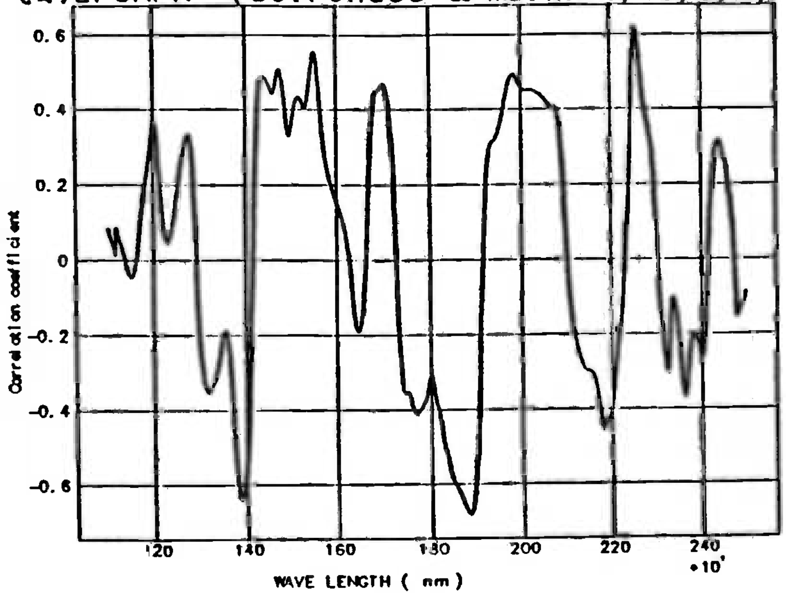
Fig 11. A definition of terms used in the *in vitro* digestibility assay.

Figure 1

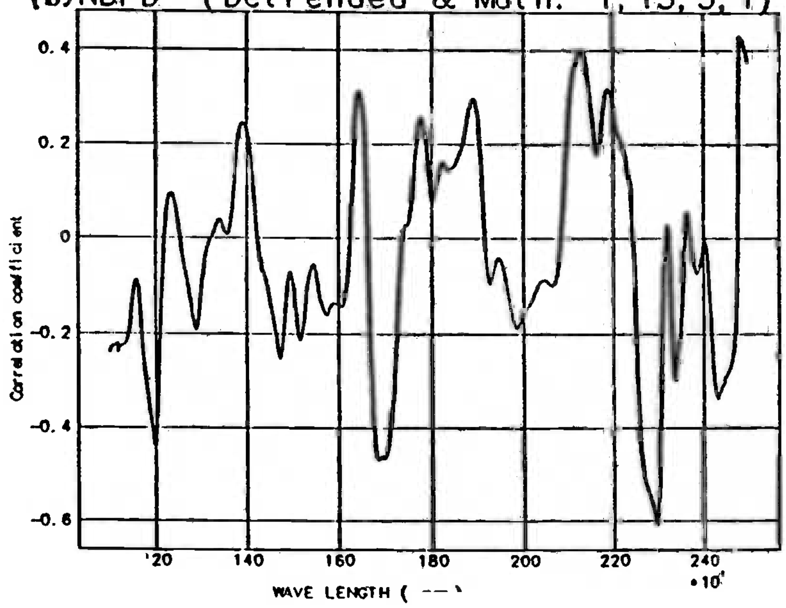


*10^-2
3
2
1
0
-1
-2
-3
Trial A (Leaf Blade) BR - NBR difference spectrc
Trial A (Sheath) BR - NBR difference spectra
Detrended LOG 1/R
120
140
160
180
200
220
240
*10'
WAVELENGTH (nm)

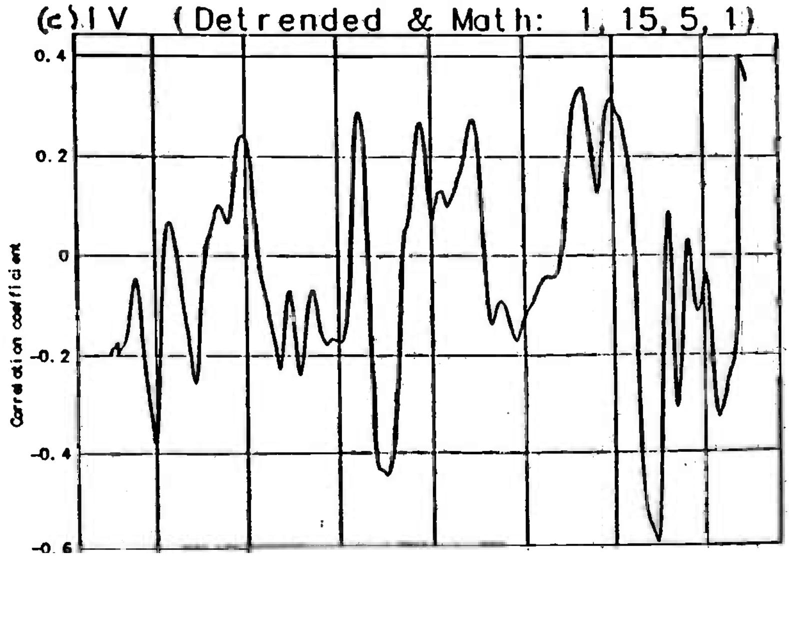
(a) LIGNIN (Detrended & Math: 1, 15, 5, 1)



(b) NDFD (Detrended & Math: 1, 15, 5, 1)



(c) IV (Detrended & Math: 1, 15, 5, 1)



Figure

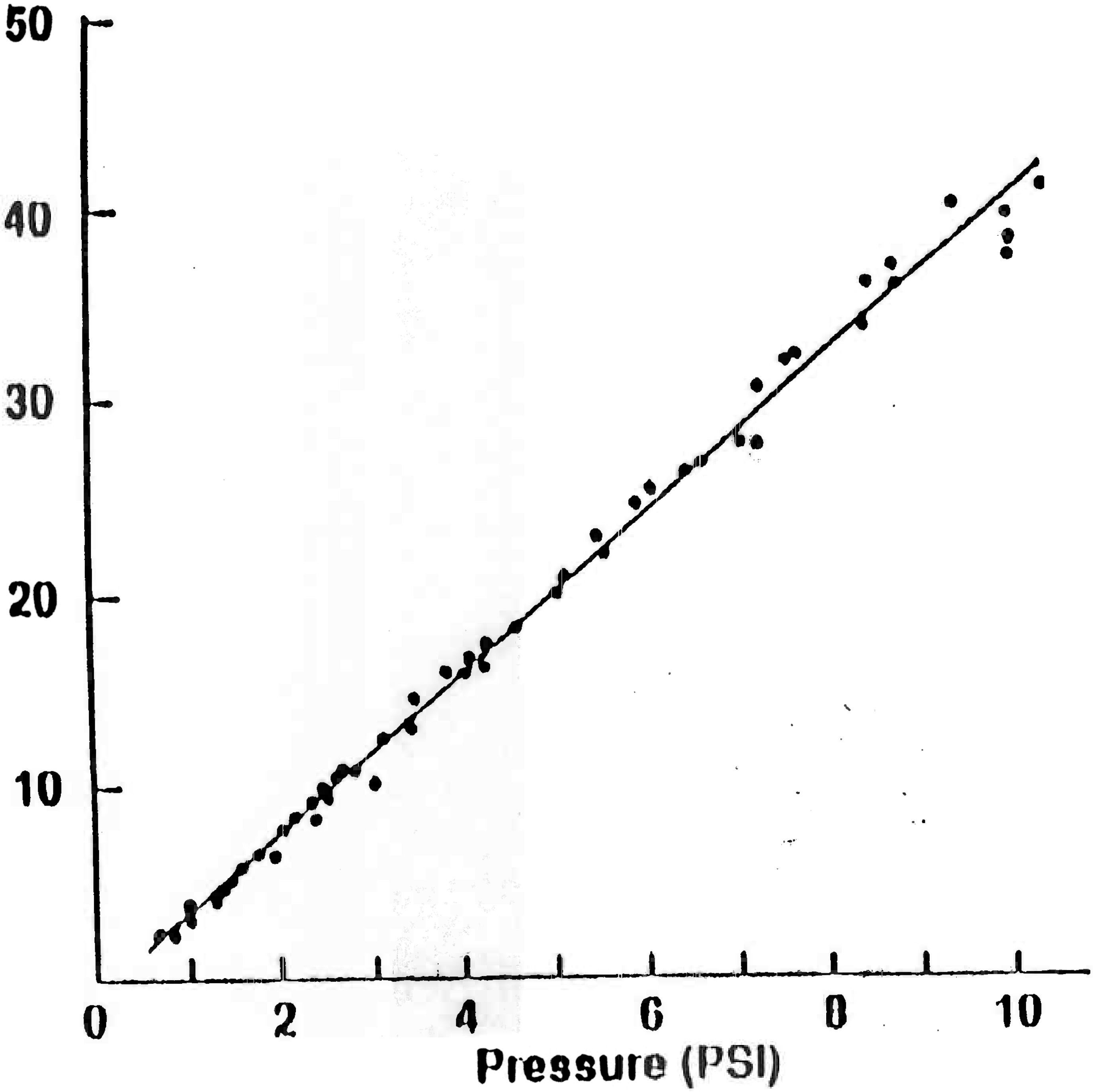


Figure 5

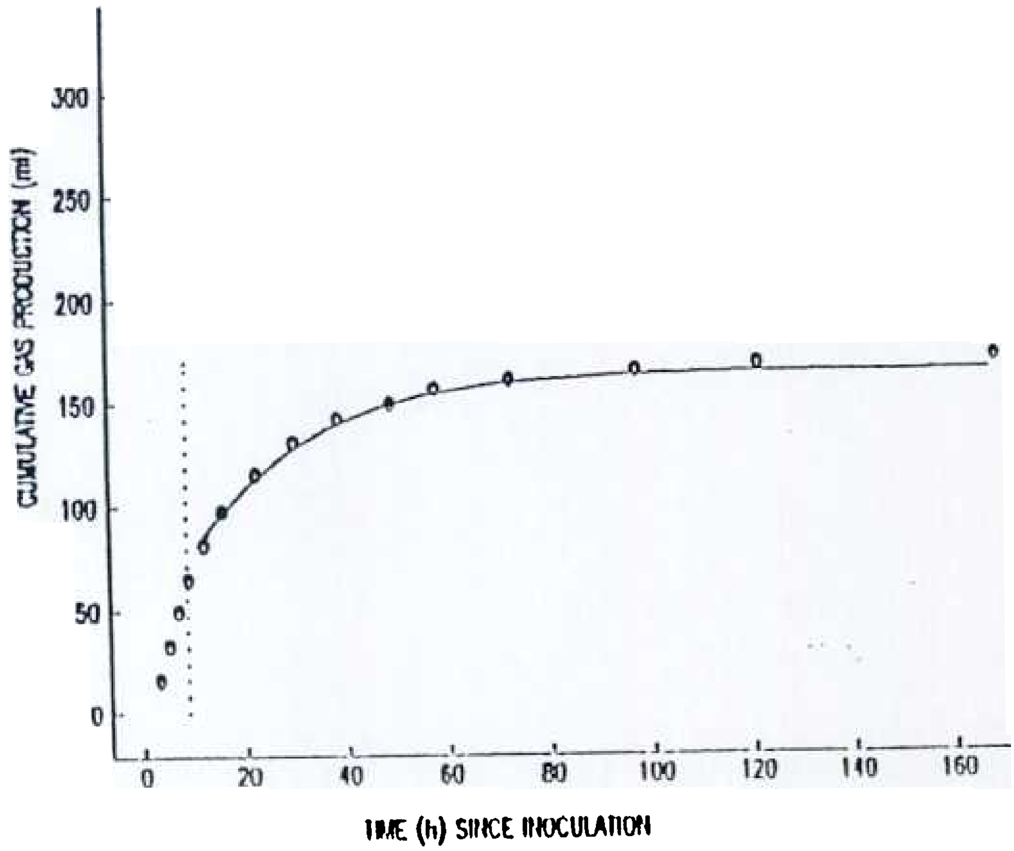

CUMULATIVE GAS PRODUCTION (ml)
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
TIME (h) SINCE INOCULATION

Figure 6

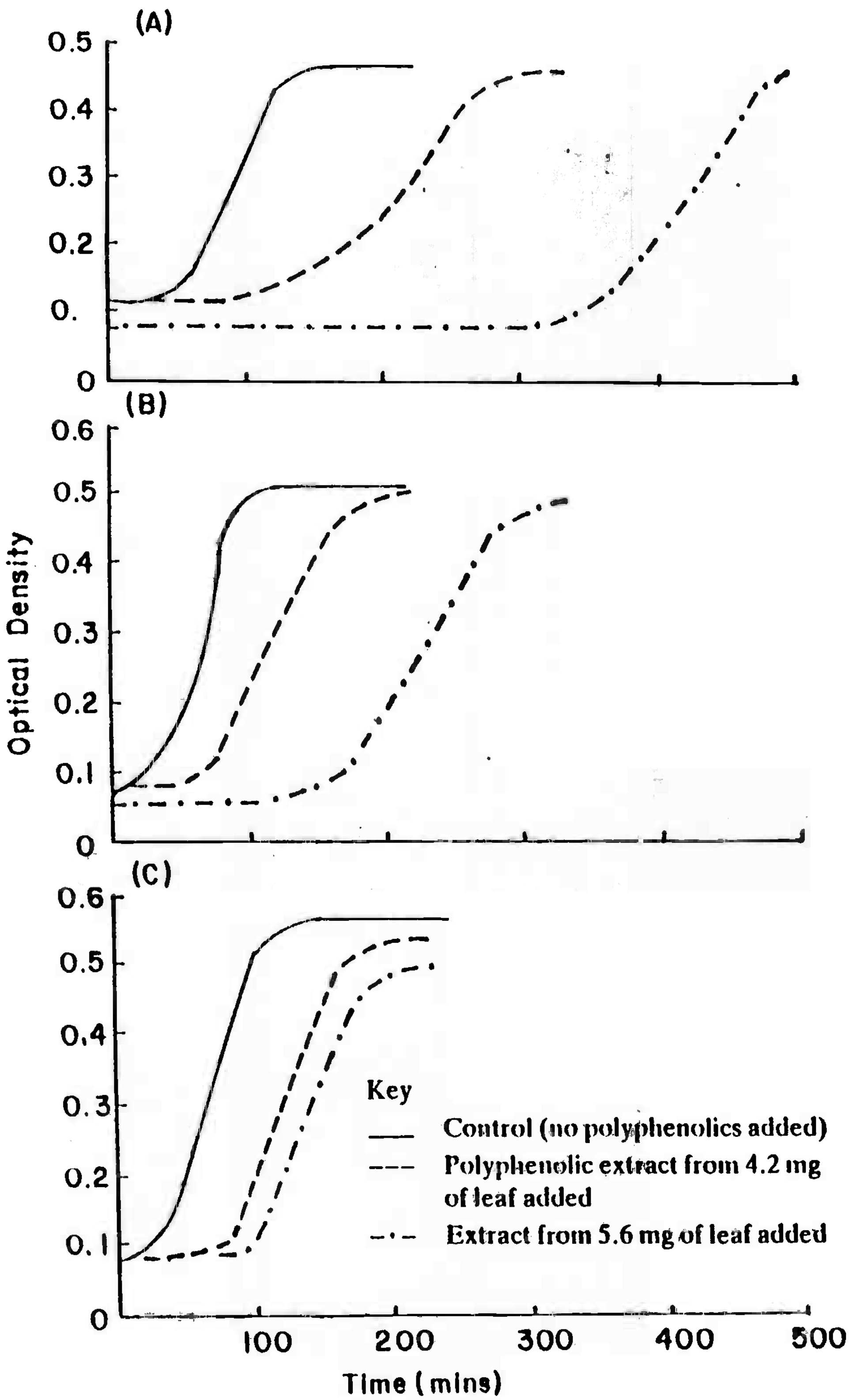

(A)
(B)
(C)
Optical Density
0
0.1
0.2
0.3
0.4
0.5
0.6
100
200
300
400
500
Time (mins)
Key
Control (no polyphenolics added)
Polyphenolic extract from 4.2 mg of leaf added
Extract from 5.6 mg of leaf added

Figure 7

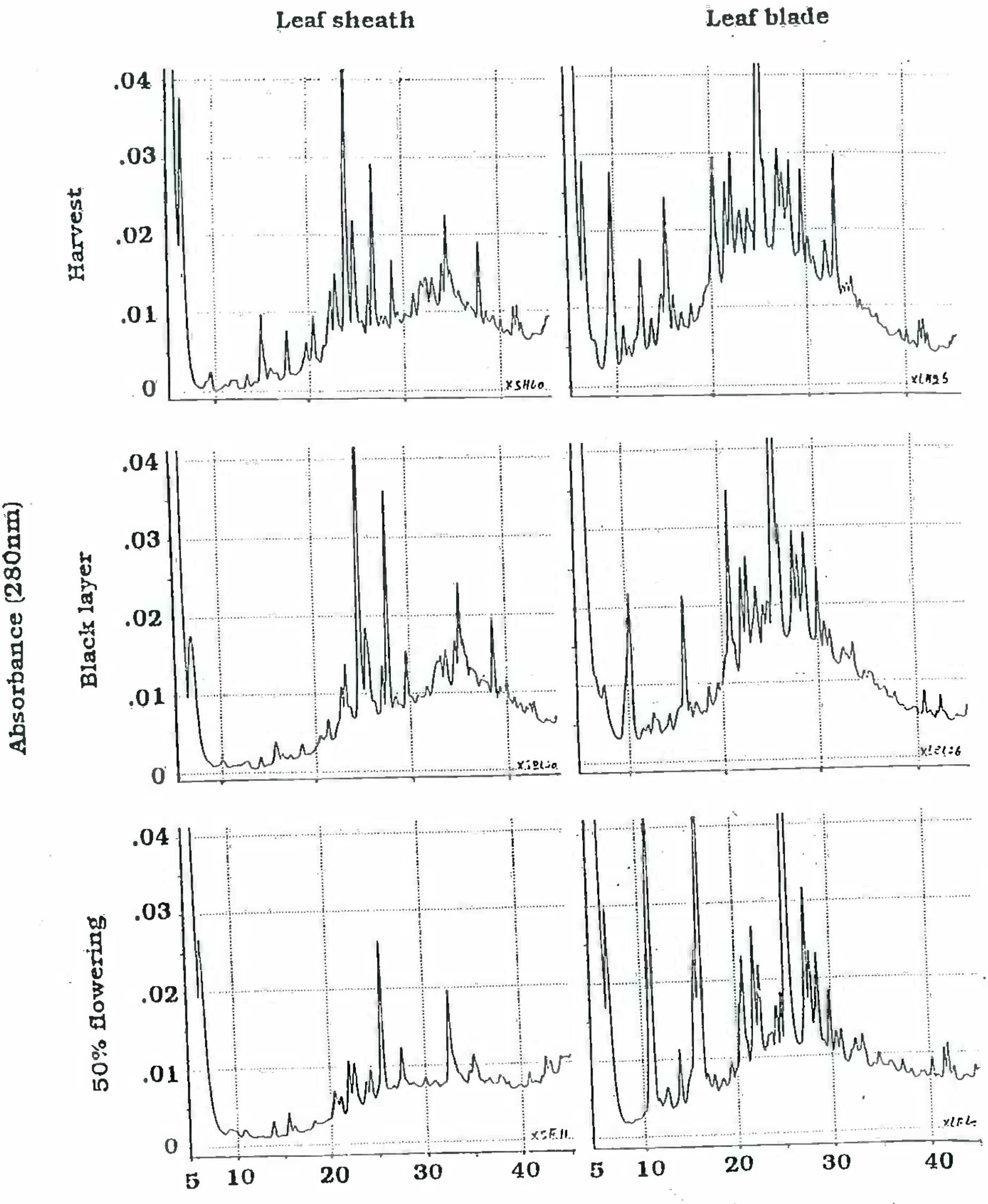
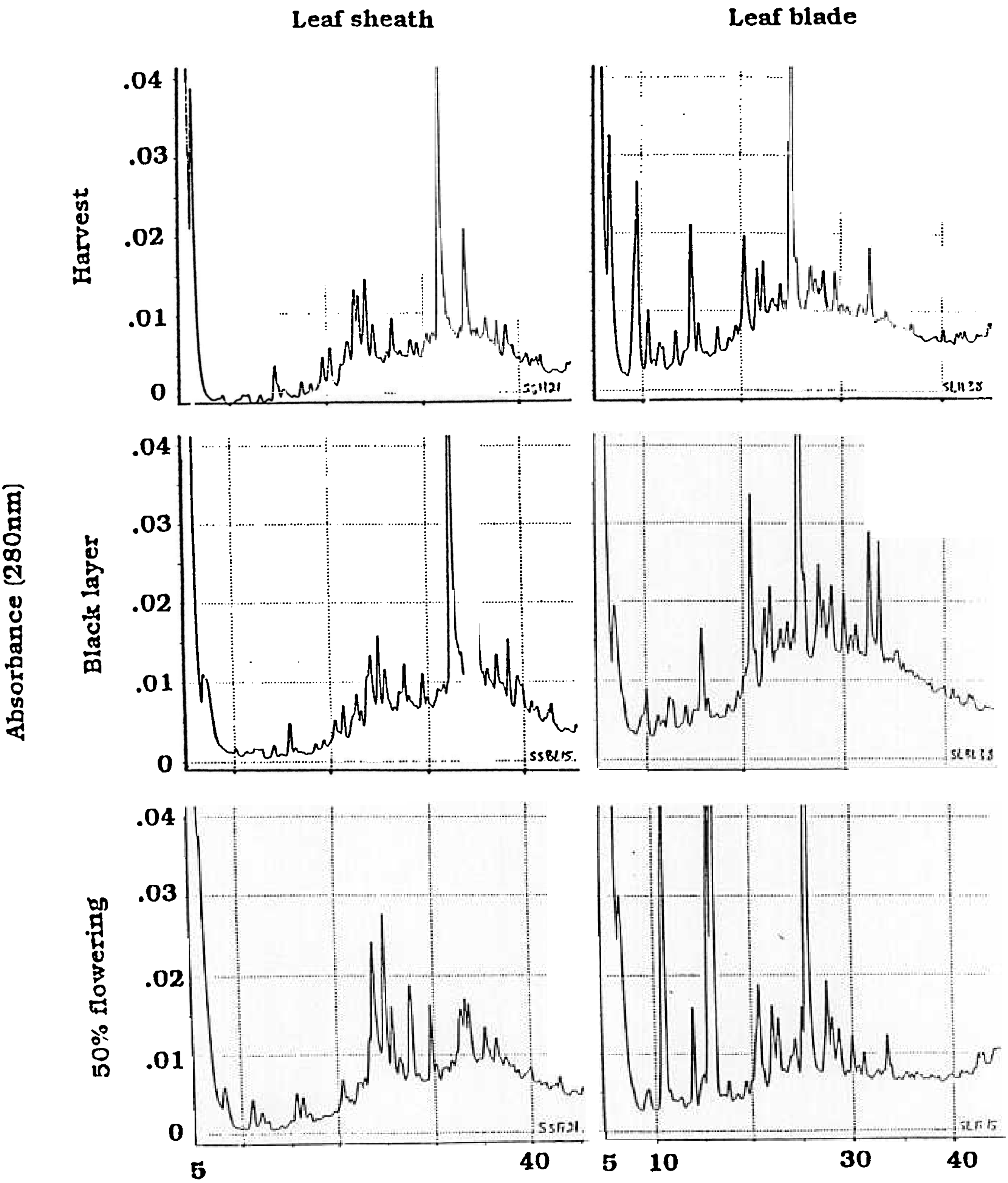
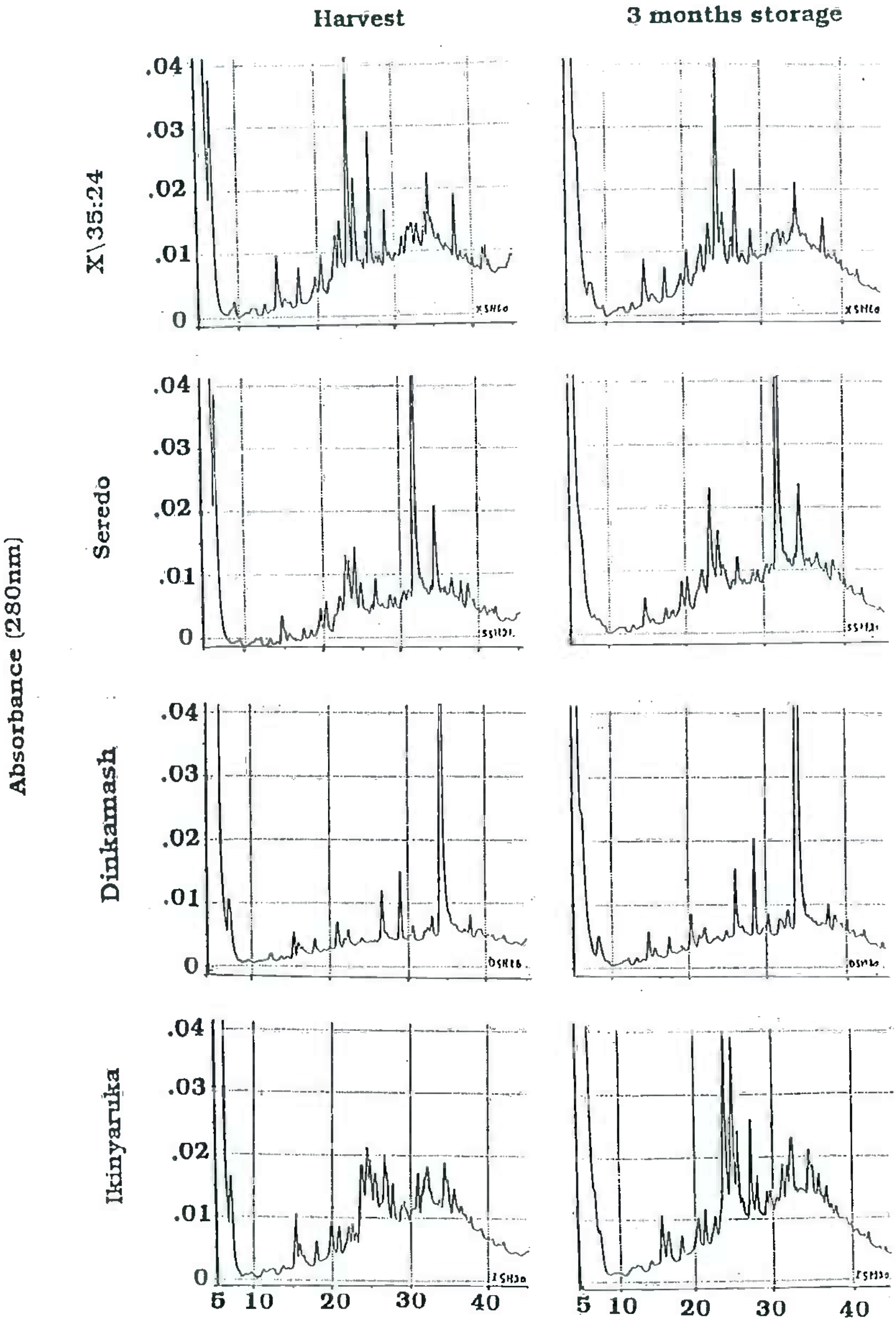

Leaf sheath
Leaf blade
Absorbance (280nm)
Harvest
Black layer
50% flowering
.04
.03
.02
.01
0
5
10
20
30
40

Figure 8



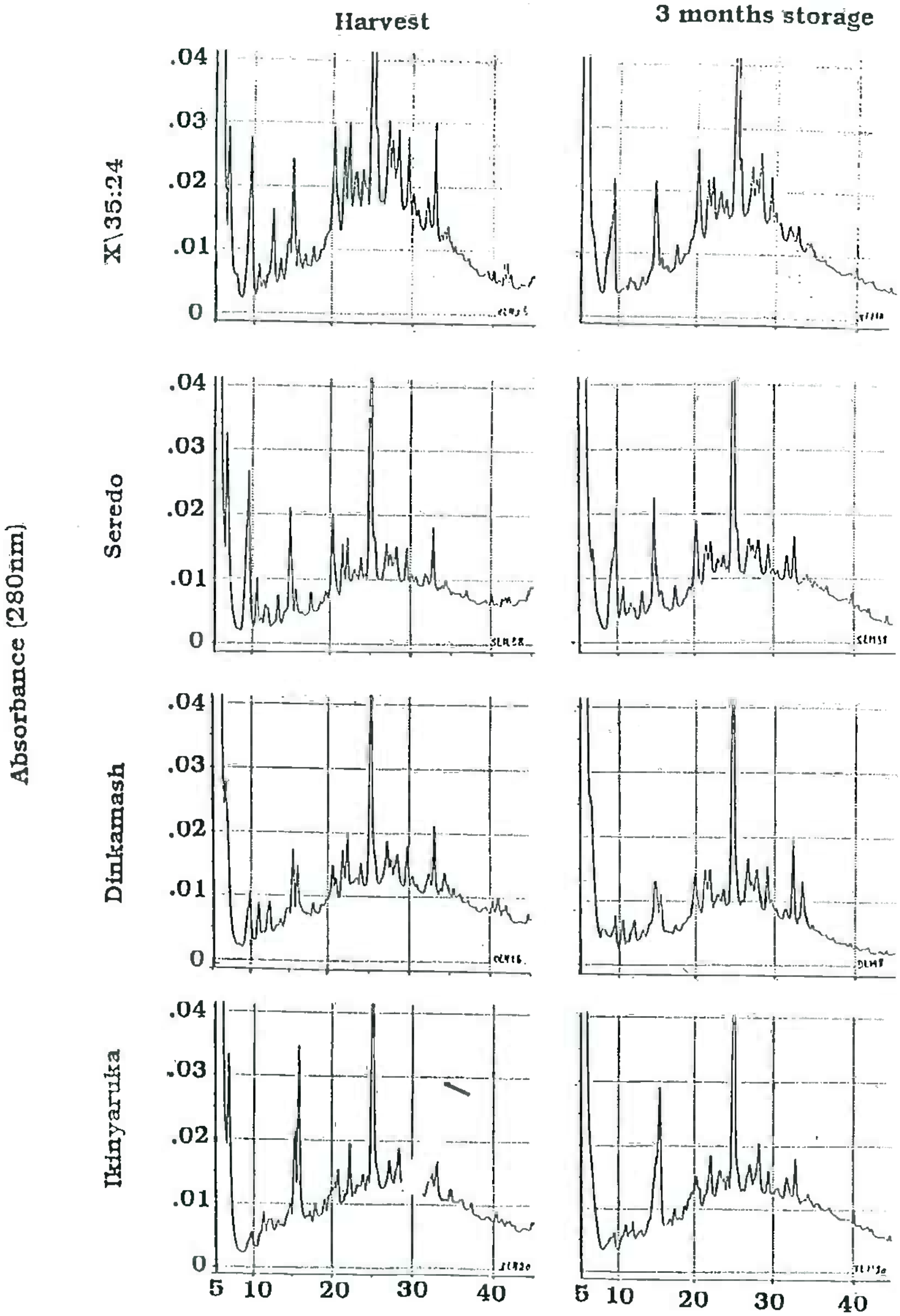
Leaf sheath
Leaf blade
Absorbance (280nm)
Harvest
Black layer
50% flowering
.04
.03
.02
.01
0
5
40
5
10
30
40

Figure 9



Harvest
3 months storage
Absorbance (280nm)
X\35:24
Seredo
Dinkamash
Ikinyaruka
.04
.03
.02
.01
0
5
10
20
30
40

Figure 10


Harvest
3 months storage
Absorbance (280nm)
X\35:24
Seredo
Dinkamash
Ikinyaruka
.04
.03
.02
.01
0
5
10
20
30
40

"In Vitro" Digestibility. Definition of Terms.

following diagram is a representation of a forage sample in terms of estible and non digestible fractions of the organic matter and the ash. umn 1 represents the whole sample, column 2 the whole sample broken down o organic matter and ash fractions and column 3 the organic matter and ash ctions broken down to digestible (soluble) and indigestible (insoluble)

1	2	3
Whole Sample (B)	Organic Matter (C)	Digestible Organic Matter (A)
		Indigestible Organic Matter
	Ash	Soluble Ash (D).
		Insoluble Ash

DMD

matter disappearance - All digestible/soluble material as a percentage of e sample.

$$\%DMD = \frac{A + D}{B} \times 100$$

OMD

Organic matter digestibility - Digestible organic matter as a percentage of organic matter.

$$\%OMD = \frac{A}{C} \times 100$$

DOMD

estible organic matter disappearance - Digestible organic matter as a centage of whole sample.

$$\%DOMD = \frac{A}{B} \times 100$$

4. Implications of the results or findings for achieving the objectives of the project

The basic objectives to define anti-nutritional components in sorghum have been achieved to a limited extent. This is partly as a result of the complexity of the polyphenolics in sorghum and the need to extend existing and develop new methods of analytical separation and identification. The success in actually identifying single compounds (e.g. Butin) as having anti-nutritional properties gives plant breeders more specific objectives in attempting to improve the nutritional quality of sorghum stover by breeding techniques. Further work should be able to establish the principal chemical components of sorghum polyphenolics having the greatest anti-nutritional properties. The relationship established between chromatographic peaks separated by the complex procedures of HPLC, the relatively more easily obtainable NIR spectra and nutritional quality are very promising but there is still some considerable work to be done in that area.

The identification of environmental and varietal effects on anti-nutritional factors means that some advice can be given to farmers on selection for best stover quality. However, at the moment, this knowledge covers only a limited number of varieties and sites. Using the information in this report, further studies should be undertaken and other varieties and sites but also including effects on grain yield and stover yield. A survey should establish the major varieties of sorghum grown in Ethiopia and the environmental contributions in the main growing areas with these factors influencing future efforts.

The equivocal effects observed *in vitro* with varieties high in polyphenolics suggest that chemical composition was not the only factor influencing response. The results of the feeding trials reported here have been very enlightening and it appears that the use of particular feeding strategies can maximise the use of sorghum by the animals. The ability to select for leaf and sheath and against stem means that a knowledge of the morphological composition of different varieties is important and this aspect could perhaps be incorporated with further varietal and environmental research suggested above. Increased intakes with increasing amounts on offer, particularly on high polyphenolic containing varieties was an unexpected but important finding and needs further investigation. Preliminary data (not in the report but to follow) indicate that cattle do not have the selection ability of sheep or goats and sequence feeding of sheep/goats with refusals fed to cattle could be economically important. Other feeding strategies such as supplementation with nitrogen, frequency of feeding may also influence stover utilisation. The objectives looking at the stage of growth and effects of storage on the polyphenolic content of maize stover were fully achieved and preclude the need for further work in this area.

It has been established that the levels of polyphenolic compounds in millet stover are too low to account for its poor nutritional value and other factors must be responsible. These factors were not established in this work but it has been shown that millet stover *in vitro* digestibility can be quite variable and needs further examination. No production trials were carried out with millet varieties but if these are considered for future work, the feeding strategies used for sorghum could be a very appropriate starting point.

5. <u>Priority tasks for follow up</u>

a) One full refereed paper, two review articles, three abstracts and one PhD thesis have already been published.

b) Two presentations have been made to the British Society of Animal Production (BSAP) (March 1991) and will be published soon as abstracts. Two full papers have been submitted for consideration for publication in the Journal of Science and Food Chemistry (one of which has recently been accepted) and another invited review on polyphenolics and nutrition is in press. Seven (7) abstracts have been accepted for presentation at the BSAP symposium on "Animal Production in Developing Countries" to be held at Wye College in September. This meeting will be attended by nutritionists and extension service personnel from developing countries. The proceedings will be widely circulated and the infirmation well disseminated.

c) A further interpretation of the results than is presented here is still underway and will lead to the submission of at least three more papers to refereed journals. Further presentations at national and international meetings are planned.

d) Publication list

Published Papers

1. Mueller-Harvey, I., McAllan, A. B. Theodorou, M K, and Beever D.E. (1988). Phenolics in fibrous crop residues and plants and their effects on the digestion and utilisation of carbohydrates and proteins in ruminants. In: Reed J.D., Capper, and Neate P.J.(Eds). *Plant breeding and the nutritive value of crop residues.* Proceedings of a workshop held at ILCA, Addis Ababa, Ethiopia, 7-10 December ICLA, Addis Ababa. pp 97-132.

2. Mueller-Harvey, I. and Blackwell, P.M.S. (1988). Polyphenolics in sorghum crop residues. Bulletin de Liaison no. 14 du Groupe Polyphenolics, Narbonne, France. Compte-rendu des Journes Internationales d'Etude et de l'Assemblee Generale, University Brock Street, Catherines, Ontario, Canada 16-19 August 1988. (Abstract)

3. Mueller-Harvey, I. (1989). Identification and importance of polyphenolic compounds in crop residues. In: '*Physico-chemical characterisation of plant residues for industrial and feed use*' (A. Chesson and E.R. Orskov, Eds). Elsevier Applied Science, London. pp 88-109.

4 Aboud, A.O., Reed, J.D., Owen, E. & McAllan, A.B. (1990). Feeding sorghum stover to Ethiopian sheep: effect of stover variety and amount offered on growth, intake and selection. *Animal Production* 50, 593. (Abstract)

5. Mueller-Harvey, I., Dhanoa, M.S. and Barnes, R.J. (1990). NIRS of sorghum crop residues. In: *Third International Conference on Near Infrared Spectroscopy.* Brussels, Belgium. 25-29 June.

6. Mueller-Harvey, I. and Blackwell, P.M.S. (1991). An improved HPLC post-column derivitisation procedure for the UV-Vis spectroscopic characterisation of phenolic compounds. Phytochemical Analysis **2**, 38-42

7. Aboud, A.A.O. (1991). Strategies for the Utilization of Sorghum Stover as Feed for Cattle, Sheep and Goats. PhD Thesis, University of Reading.

Papers 'in press' typescripts of which appear in the Appendix

1 Osafo, E.L.K., Owen, E., Aboud, A.A.O., Said, A.N., Gill, E.M. & McAllan, A.B. 1991). Feeding sorghum stover to Ethiopian sheep: Effect of chopping and amount offered on growth, intake and selection. Anim. Prod. (in press).

2. Aboud, A.A.O., Owen, E., Reed, J.D., Said, A.N. & McAllan, A.B. (1991) Feeding sorghum stover to Ethiopian goats and sheep. Effect of the amount offered on growth, intake and selection. Anim Prod. (in press)

3. Mueller Harvey, I. and Blackwell, P.M.S. (1991). An improved post column derivitisation procedure using shift reagents for the UV-Vis spectroscopy of phenolic compounds in plant extracts. XVth International conference Groupe Polyphenols. Universite Louis Pasteur. Strasbourg. France 9-11 July.

4. Mueller-Harvey, I., Dhanoa, M.S., Blackwell, P.M.S. and Reed, J.D. (1991). Cluster analysis of HPLC data and *in vitro* digestibilities to describe varietal differences between sorghum crop residues and their responses to different sites. Cost 84bis workshop proceedings. Reggio Emilia, Italy.

5. Mueller-Harvey, I. and McAllan, A.B. (1991). Tannins - their biochemistry and nutritional properties. In: *Advances in Plant Cell Biochemistry and Biotechnology* (I.M Morrison, ed). JAI Press Ltd., London. (in press).

6. Mueller-Harvey, I. and Dhanoa, M.S. (1991). Cluster analysis of HPLC-chromatograms to describe varietal differences between sorghum crop residues and their responses to different sites J. Sci. Food Agric. (in press).

7. Mueller-Harvey, I and Reed, J.D (1991). Phenolic compounds and their relationship to *in vitro* digestibility of sorghum leaves from bird and non-bird resistant varieties. J. Sci. Food Agric. (in press).

<u>Papers to be presented at BSAP Meeting on Animal Production in Developing Copuntries, Wye College, September 1991</u>

Theodorou, M.K., Williams, B.A. & McAllan A.B. (1991). The use of head space gas pressure in batch cultures to aid the determination of the nutritive value of sorghum crop residues.

Mueller-Harvey, I., Dhanoa, M.S. & McAllan, A.B. (1991) Variations in the phenolic components of sorghum crop residues related to varietal and environmental differences.

Mueller-Harvey, I., Reed, J.D. & McAllan, A.B. (1991). Phenolic compounds and their relationship to *in vitro* digestibility of sorghum leaves of bird resistant and non bird resistant varieties.

Khazaal, K., Mueller-Harvey, I., McAllan, A.B., Osafo, E.L.K., Owen, E. & Said, A.N (1991). Effects of harvesting at different stages of growth and long term storage on the phenolic composition of sorghum stover.

Osafo, E.L.K., Owen, E., Said, A.N., Gill, E.M. & McAllan, A.B. (1991). Sorghum stover as ruminant feed in Ethiopia: Effect of cultivar, site of growth, pre-harvest leaf stripping and storage on yield and morphology.

Osafo, E.L.K., Owen, E., Said, A.N., Gill, E.M. & McAllan, A.B. (1991). Feeding sorghum stover to Ethiopian sheep and cattle: Effect of chopping and amount offered on intake and selection.

Aboud, A.A.O., Owen, E., Reed, J.D., Said, A.N. & McAllan, A.B. (1991). Feeding sorghum stover to Ethiopian goats and sheep: Effects of amount offered on intake, selection and performance.

6. Name of Report Author: A.B.McAllan

Signature:

Date: 12th June 199