The use of the *in vitro* gas production technique to identify *in vivo* digestive interactions of sheep fed low quality roughage diets supplemented with high quality forages

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Abstract

This study aimed at investigating and comparing feed interactions observed *in vitro* and *in vivo* between two roughages and two high quality forages. Twenty four wether lambs were used to determine intake, digestibility and rate of passage of barley straw (BS) or meadow hay (hay) supplemented with alfa A (AA) or wheat feed (WF) at two levels of supplementation. Samples of feed from the feeding trial were evaluated as individual feeds and as mixtures by *in vitro* gas production in nitrogen-rich (NR) and nitrogen-free (NF) media. A production trial was also conducted with 56 lambs to determine the effect on intake, live weight gain and feed conversion efficiency of supplementing hay with AA at two levels of supplementation.

Supplementation of BS with AA or WF increased total daily dry matter intake at both levels. Intake of BS was not altered significantly by AA or WF supplementation. Hay intake was decreased significantly at both levels of AA supplementation. WF supplementation led to a non significant increase in hay intake at the lower level and a significant decrease at the higher level. The digestibility of supplemented roughage diets was increased by supplementation. Positive interactions, increasing digestibility by up to 8.7%, were observed for supplemented BS. The relationships between digestibility and level of supplementation were assessed for linearity. A quadratic relationship was found for BS+WF ($P<0.001$), indicating that highly significant interactions occured between these feeds, while there was some indication of a deviation from linearity for BS+AA ($P=0.097$ for quadratic relationship). There were no indications of significant deviations from linearity for hay + either supplement.

In vitro gas production in NR medium showed that WF was the most rapidly fermentable feed, with AA and hay having similar fermentation rates initially, and BS being slowly fermented. BS, and to a much lesser extent hay, were more slowly fermented in NF than NR medium indicating that their fermentation was nitrogen limited. Comparisons of the fermentation of feed mixtures in NR and NF media indicated that fermentable protein and carbohydrate reached approximate balance at the following levels of supplementation: BS + AA 0.5; BS + WF 0.3 to 0.4; hay + AA 0.1; hay + WF 0.3. Positive interactions in gas production were observed between BS and both supplements in both media, but were greatest between BS and WF in NF medium (up to 51% at 96 h incubation). Interactions between BS and WF were generally greater than those observed between other feed combinations. Interactions between hay and AA were modest and not significant ($P>0.05$) in the NR medium. Interactions were observed between hay and WF in both media. In general there was a good agreement between the ranking of the scale of interactions of feeds measured *in vivo* and *in vitro*, although the *in vitro* gas production method appeared to be more sensitive to interactions between feeds than was found *in vivo*.

The *in vitro* rate constant (b) was highly linearly correlated ($R^2 = 0.91$, $P<0.001$) to total dry matter intake for all feeds and feed mixtures except for wheat feed when fed alone. The lag time (T+) showed potential as a predictor of *in vivo* digestibility of roughages and supplemented roughages.

In the production trial a substitution effect occured, resulting in a reduction in daily hay DMI at both levels of supplementation. Increasing supplementation resulted in a increased live weight gain and improved feed conversion efficiency.

Key Words:
in vitro gas production, supplementation, feed interactions, straw, hay, intake, digestibility

Introduction

Ruminants in many less developed countries (ldcs) may consume poor quality roughages such as straws, stovers and senescent native pasture as a major part of their diet, particularly during the dry season when high quality forages are in short supply. The majority of these roughages are high in fibre, low in protein and the intake of digestible nutrients often is not enough to meet maintenance requirements. Intake and digestibility of poor quality roughages may be increased by supplementing diets with high quality forages, by-products and concentrates to correct nutrient deficiencies. Supplementation of poor quality roughages is potentially of great benefit in many ldc feeeding systems. The response to supplementation can be attributed to an increase in the supply of nitrogen (Lyons *et al* 1970) and/or readily fermentable carbohydrate (Orskov 1995, Silva 1985) resulting in an increase in rumen cellulolytic micro-organisms and therefore enhanced fibre degradation. Interactions between feeds do not always result in positive interactions, negative interactions on fibre digestion have also been observed mainly with supplements rich in fermentable starch and sugars (Moss *et al* 1992). However, such supplements are rarely used for ruminants in ldcs and supplementation would be expected to give rise to positive interactions.

Studies in both tropical and temperate regions have assessed *in vivo* responses of supplementing straw and hay with a wide range of different supplements. Ben Salem *et al* (1996) studied the effect of increasing level of spineless cactus (*Opuntia ficus indica* var.*intermis*) on intake and digestion of sheep fed straw based diets. Spineless cactus provided a fodder rich in energy and supplementation resulted in increasing straw intake. The beneficial effect of spineless cactus was attributed to an increase in supply of easily fermentable organic matter as well as NH_3-N. Manyuchi *et al* (in press) studied the responses of supplementing veld hay with Napier hay, concluding that supplementation resulted in an increased intake of veld hay. The response to the napier supplement was not entirely due to increasing nitrogen intake. Napier grass was supplemented with gliricidia and leucaena by Abdulrazak (1996), increasing supplementation with gliricidia resulting in a linear decrease in napier grass intake whereas with leucaena intake of napier grass was not depressed. Rumen ammonia levels increased linearly with supplementation. Silva and Ørskov (1988) studied the effect of five different supplements on the degradation of untreated barley straw fed to sheep, concluding that the supplements high in digestible cellulose and hemicellulose such as sugar beat pulp had the greatest effect on increasing the rate of straw degradation. Silva *et al* (1989) reported that supplementation of straw with sugar beat pulp and fish meal increased digestibility and that they had the best effect when fed in combination.

At present the main way of measuring responses of low quality forages to supplementation is by conducting expensive and lengthy animal feeding trials which are often prohibitive. As a consequence of this, the ability to predict animal performance using simple, cheap and reliable *in vitro* techniques is of considerable important in animal nutrition. A number of *in vitro* techniques have been used to determine the nutritive value of feeds. One of the most commonly used in the Tilley and Terry (1963) technique. This is an end point digestibility method and does not provide information on the digestion kinetics of the feeds. The polyester bag (*in sacco*) method is also used to provide information of the rate and extent of degradation of feed constituents and has been well correlated with animal performance (Ørskov *et al.*, 1988: Reid *et al.*, 1988). There are a number of drawbacks to using this technique which are (a) the number of samples which can be analysed is often limited since samples have to be incubated *in vivo* and (b) it assumes that all DM losses are due to fermentation which is not always the case. In recent years there has been increasing interest in the use of *in vitro* gas production techniques to predict *in vivo* responses. Gas production data produced using the Menke gas production method has been shown to correlate well with *in vivo* measurements (Blummel and Ørskov 1993; Khazaal *et al* 1993) although most of the work has been conducted using single feeds. The gas production technique developed by Theodorou (Theodorou *et al* 1994) is a simple and relatively inexpensive technique which has the potential to study feed interactions *in vitro* (Wood and Manyuchi, in press; Sampath *et al* 1995). Interactions have been defined as the percentage difference between the gas production observed for the mixture and that predicted when the roughage and supplement are fermented alone (Prasad *et al* 1994). Although feed interactions have been shown to occur *in vitro* and *in vivo* (Prasad et al 1994) very few comparisons have been conduced to date.

The aim of this work was to investigate the interactions between feeds *in vitro* using the Theodorou gas production technique and compare these with *in vivo* parameters (intake, dry mater digestibility and rate of passage) of barley straw (BS) and meadow hay (hay) supplemented with the commercial alfalfa/lucerne based feed alfa A (AA) and wheat feed (WF) at different levels of supplementation.

Samples from the feeding trial were run in both a nitrogen rich (NR) medium (Theodorou 1994) and nitrogen free (NF) medium (Menke *et al.*, 1979). The medium used in the standard Theodoru technique is not nitrogen limiting, therefore the response of the forage to the level of supplement is not dependent on the nitrogen contained within the feed. This is probably not an important factor in temperate feeding systems where nitrogen is not generally limiting but is more important in tropical situations where deficiency of nitrogen in diets is often a constraint. A production trial was also conducted to study the effect on intake, live weight gain and food conversion efficiency of supplementing hay with AA.

Materials and Methods

Digestibility Trial

Animals

Twenty four crossbred (Kent x Suffolk) wether lambs were used to determine intake, digestibility and rate of passage. They were housed in individual metabolism crates with free access to water and a mineral block. At the start of the experiment the lambs had an initial mean live weight of 31 kg (s.d. 2.91).

Diets

Lambs were fed either hay or BS supplemented with AA or WF. The BS and the hay were chopped with a motorized cutter. The supplements, AA and WF were fed at a fixed rate (g/Kg metabolic LW) in combination with the roughages at 2 levels of supplementation (low and high, 0.25 and 0.5 DM basis, respectively). There were 12 dietary treatments. The roughages were offered *ad libitum* either alone or with the 2 rates of supplementation. The supplements were also offered *ad libitum* when fed as the sole feed. Animals were fed in two equal meals at 9.30 and 14.30 with the supplement being fed with the first meal.

Experimental Procedure and Design

Lambs were offered four dietary treatments during four consecutive periods, each of 21 days (14 day preliminary period and 7 days experimental period) in an incomplete block design with each treatment being received by 8 animals.

Marker administration and sample collection

On day 13 of the preliminary period animals were dosed with an indigestible marker at 08.30. Each animal received 35g of hay or BS mordanted with chromium $Na_2Cr_2O_7$ (Uden *et al* 1980) or AA or WF if the animals were being offered *ad libitum* supplement. The feeds (35g) were mixed with 100mls of diluted molasses (20mls molasses/80 mls water). Grab samples of faeces were collect from the rectum at 8,12,16,22,26,30,34,38,46,50,54,60,72,78,83,97,103,108,122,132 hours. Fresh samples were dried, ground and analysed for chromium.

Intake and Digestibility Measurements

During the 7 day experimental period the amounts of feed offered and refused were recorded daily and samples bulked for each animal. Supplements were always consumed completely. At the end of the measurement period samples of feed offered and refused were sub sampled dried and then milled. The total daily output of faeces was collected and measured. Approximately 100 g of the daily output of faeces for each animal was frozen before being bulked, sub sampled, dried and milled.

Production Trial

Animals
Fifty six crossbred (Kent x Suffolk) wether lambs were used in a production trial to determine intake and live weight gain. They were housed in pens in pairs, male and female, with free access to water and a mineral block. At the start of the experiment the lambs had an initial mean live weight of 25.1 kg (s.d. 2.94).

Diets

Lambs were fed hay supplemented with AA. The hay was fed unchopped. AA was fed at a fixed rate (g/Kg metabolic LW) in combination with the hay at 2 levels of supplementation (low and high, 0.25 and 0.5 DM basis, respectively). There were 4 dietary treatments. The hay was offered *ad libitum* either alone or with the 2 rates of supplementation. AA was also offered *ad libitum* when fed as the sole feed. Animals were fed in two equal meals at 9.30 and 14.30 with the supplement being fed with the first meal.

Experimental Procedure and Design

Lambs remained on the same treatment throughout the length of the trial which lasted 74 days. Lambs were weighed at the start and finish of the experiment and periodically in between. The amounts of feed offered and refused were recorded daily and samples of feed offered were bulked. Supplements were always consumed completely. At the end of the measurement period samples of feed offered were sub sampled, dried and then milled.

Laboratory procedures

Chemical Analysis

Dry matter, organic matter and crude protein were assayed by the methods of the Association of Official Analytical Chemists (1984). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Goering and Van Soest (1970). Chromium was determined by atomic absorption according to the method of William *et al* (1962).

In Vitro Gas Production

Samples of the feeds offered in the digesibility trial which had been dried and ground to pass through a 1mm dry screen were used in the *in vitro* gas production experiments. The proportions of roughage to supplement used *in vitro* were similar to those *in vivo*. The gas production technique of Theodorou *et al* (1994) was used. Samples (1g) were fermented in 100 ml serum bottle in both nitrogen rich (NR) and nitrogen free (NF) media inoculated with fresh rumen fluid. The NF medium was described by Menke *et al* (1979). Feed mixtures were fermented in triplicate, single feeds in six replicates, for each individual fermentation run. Gas readings were recorded at time intervals up to 96 hours at which time the residues were recovered by filtration to be dried and weighed to determine dry matter disappearance (DMD). All feeds and feed mixtures were fermented in two fermentation runs, a total of four runs being conducted. For selected bottles the medium after incubation was analysed for volatile fatty acids (by gas chromotography, ADAS Wolverhampton).

Computation of Data and statistical analysis

Data from the *in vitro* gas production with the single feed ie BS, hay, AA and WF was used to predict values for gas production of feed mixtures assuming no interactions between feeds. Predicted values were calculated by summing the gas produced from the roughage and the supplement multiplied by the proportion of each which had been fermented. A linear relationship between the proportion of substrate and gas production had been demonstrated by Theodorou *et al* (1994). Percentage interactions were calculated using the equation:

$$\frac{100 \times (\text{experimental value} - \text{predicted value})}{\text{predicted value}} = \%\ \text{Interaction}$$

The statistical significance of interactions was assessed from the analysis of variance of a quadratic function fitted to the measured parameter verses proportion of supplement data using Genstat. *In vivo* digestibility data was analysed in the same way. Intake data was also evaluated for the significance of the quadratic function, but interactions could not defined in the same way. This was because for supplemented roughages, the supplement was only fed at a fixed amount and therefore supplement intake was not defined by the properties of the feed.

The differences in gas production between the NR and NF media were calculated.

For correlation between *in vivo* and *in vitro* data, gas production parameters were obtained by fitting the mathematical model of France et al. (1993) and relationships investigated using regression analysis using the computer programme SPSS.

Results

Digestibility Trial and Gas Production

Level of Supplements

The supplements, AA and WF where intended to be fed at a fixed rate (g/Kg metabolic LW) in combination with the roughages at 2 levels of supplementation (0.25 and 0.5 DM basis). The levels of supplementation achieved were:

	AA low	AA high	WF low	WF high
Barley straw	0.32	0.52	0.31	0.47
Meadow hay	0.22	0.37	0.20	0.38

The proportion of supplement consumed with BS was greater than that consumed with the hay for the corresponding level of supplementation, reflecting the relativey low intake of BS compared with hay.

Chemical Composition of the Roughages and Supplements

BS had the lowest CP content (Table 1) of all the feeds at 31.6 g/kg DM. Both AA and WF had a similar crude protein content of approximately 160 g/kg DM. BS had the highest ADF value (521 g/kg DM) whereas WF had a relatively low value (177 g/kg DM).

In Vivo Dry Matter Intake

Supplementation of BS with AA and WF increased total daily dry matter intake at both levels (Table 2). At the lowest level of supplementation with AA there was a 10 percent increase in daily BS dry matter intake whereas at the higher level a 12 percent decrease in BS dry matter intake was observed, although these increases did not achieve statistical significance ($P>0.05$). With WF at the lower level of supplementation there was a 15 percent increase in daily BS dry matter intake and this was maintained at the higher level of supplementation with a 21 percent increase in BS dry matter intake, although again neither increase achieved statistical significance ($P>0.05$). As with BS, supplementation of meadow hay with AA and WF increased total daily dry matter intake at both levels. At both levels of supplementation with AA there was a significant ($P<0.05$) decrease in daily hay dry matter intake with increasing supplement. With the WF there was non significant increase in daily hay dry matter intake at the lower level of supplementation with a significant ($P<0.05$) decrease at the higher level of supplementation. The relationships between total DMI and level of supplement was best described by a quadratic expresion for BS+AA, BS+WF and hay+WF ($P<0.01$) but not for hay+AA ($P>0.05$).

In Vivo digestibility and Interactive Effects

BS had the lowest digestibility (0.46) with WF having the higher digestibility of the 2 supplements (0.65). Hay and Alfa A had digestibilities of 0.58 and 0.61 respectively (data given in Table 3). Supplementing BS with AA and WF resulted in an increase in digestibility. At the lower level of supplementation there was a 16 percent and 22 percent increase in digestibility when supplementing with AA and WF respectively. There was only a marginal increase in digestibility between the low and high level of supplementation. Supplementation of hay with AA had little effect on digestibility at either level, the digestibilities of the two individual feeds were also similar. At the higher level of supplementation with WF there was a 6 percent increase in digestibility compared to the digestibility of hay.

Table 3 also shows the derived values for the percentage differences between predicted (assuming no interactions between feeds) and observed digestiblities. For the BS supplemented with AA there was 4.8 percent positive difference (i.e. increase in digestibility) between the observed and the predicted values at the lower level of supplementation which fell to 1 percent at the higher level of supplementation. For BS+WF at the lower level of supplementation there was a 8.7 percent positive difference between the observed and the predicted values which reduced to 5.4 percent at the higher level of supplementation. A quadratic relationship was found for BS+WF ($P<0.001$), while there was some indication of a deviation from linearity for BS+AA ($P=0.097$ for quadratic relationship). There were no indications of significant ($P>0.05$) deviations from linearity for hay + either supplement.

Gas Production Characteristics of Individual Feeds

The cumulative gas production curves for BS, hay, AA and WF when fermented alone in the NR medium, are shown in Figure 1. The initial rate of gas production was greatest for WF. Hay and AA had very similar initial rates of gas production with BS having the slowest rate. At the end point of the fermentation (96 hours), the gas production was greatest for the hay, followed by WF, BS and AA respectively. The France equation parameters are presented in Table 4. Figure 2 shows the gas production profiles of the BS and hay fermented in NR and NF media. There was no difference in the profiles for the AA and WF between the 2 media. The results shows that the rate and extent of degradation of BS was significantly less in the NF medium indicating that the BS was nitrogen deficient. The difference in the hay gas production between the 2 media was less, indicating that the hay was marginally nitrogen deficient.

Fermentability and Interactions of Feed Mixtures

The mean cumulative gas production and dry matter disappearance of BS supplemented with AA and WF, at the proportions achieved during the *in vivo* digestibility trial, are shown in Table 5. These data were used to derive the values for the percentage differences (interactions) between predicted and observed gas production and DMD which are also included in this table. For BS supplemented with AA in the NR medium interactions were generally small and fell after 27 h incubation. The quadratic component of the relationship between gas production and level of supplement was statistically significant ($P<0.05$) for incubation times up to 39 hours (inclusively), after which gas production ceased to achieve significance. Interactions were larger in the NF medium and increased with incubation time. Interactions were similar for both levels of supplementation up to 48 hours after which interactions were proportionately greater at the lower level of supplementation. For BS supplemented with WF the interactions were greater than with the AA. In the NR medium interactions appeared marginally larger at the higher level of supplementation compared to the lower level with interactions declining after 27 h. In the NF medium interactions increased with time and were similar for both levels of supplementation up to 48 h when there was a marginal decline at the higher level of supplementation. Figure 3 illustrates the relationships between *in vitro* gas production in NR and NF media at selected incubation times and the level of supplementation of BS by WF.

The mean cumulative gas production, dry matter disappearance and the percentage differences between predicted and observed for gas production and DMD for hay supplemented with AA and WF, at the proportions achieved during the *in vivo* digestibility trial, are shown in Table 6. For the hay supplemented with AA there were no statistically significant ($P>0.05$) indications of interactive effects on gas production observed in either media, as illustrated by the approximately linear relationships between gas production and proportion of supplement in Figure 4. There were significant ($P<0.01$) interactive effects on gas production observed when hay was supplemented with WF. In the NR medium interactions appeared similar at both levels of supplementation. In the NF medium interactions appeared greater at the higher level of supplementation.

Differences in Gas Production For Feed Mixtures Between NR and NF Media

The differences in the cumulative gas production after 48 hours between the NR and the NF media for the various feed mixtures are shown in Figure 5. With the BS supplemented with AA there was almost a linear decrease with increasing supplementation up to a level of about 0.5 suggesting that fermentable nitrogen and carbohydrate were in balance at this level. With the WF there was a steep decline up to a supplement level of 0.3-0.4 indicating that the WF and BS were in balance at this level. The difference between the NR and NF media for the hay were modest. With the AA it appeared that there was little further response to supplementation after an inclusion of 0.1 and for WF there was a response up to a supplement level of 0.3.

Correlations between in vivo and in vitro parameters

Table 7 presents the R^2 values for the linear regressions of *in vivo* total dry matter intake and digestibility against the France *et al.* (1993) parameters for the corresponding feeds and feed mixtures fermented *in vitro*. Total dry matter intake was highly significantly correlated with digestibility (R^2 = 0.69, P<0.001). Regression analysis of the *in vitro* France parameters showed that, in NR medium, intake was most strongly related to the rate constant (b) (R^2 = 0.43, P<0.05). However, very strong linear correlation (R^2 = 0.91, P<0.001) was found between total dry matter intake and the rate constant (b) when all feeds and feed mixtures, except for WF when fed alone, were included in the regression. In NF medium the correlations were broadly similar but slightly more strongly related to the *in vivo* parameter (R^2 = 0.52 and 0.93, respectively, for all data and all data excluding WF). The data and fitted relationship are given in Figure 6. Digestibility was related most strongly to the lag time (T+) (R^2 = 0.73, P<0.001). This was in part due to a very close relationship between T+ and digestibility for straw and supplemented BS diets (R^2 = 0.99), however including the hay and supplemented hay diets together with the BS diets (i.e. excluding the two supplements when fed by themselves) there was also a very strong linear correlation (R^2 = 0.97). A strong relationship was also found between digestibility and the second measure of the lag time, T-, although it was less strong than the relationship with T+. In NF medium, BS fed alone had a very long lag time compared with all the other diets and correlation between T+ and digestibility was poor (R^2 = 0.48) with all data included.

Volatile Fatty Acids

The molar proportions of the VFAs produced during fermentation of the single feeds in both NR and NF media are given in Table 8. The proportions were broadly similar in the two media. The fermentation of BS produced more acetic acid and less of the higher molecular weight VFAs relative to the other feeds, while WF produced less acetic acid and more of the higher molecular weight VFAs. The VFA profiles of hay and Alpha A were similar.

Interactions between feeds which altered the VFA profiles of the feed mixtures, compared to that predicted from the fermentation of the individual feeds, were observed. These were strongest for BS supplemented with WF, where there were (in the NR medium) significant quadratic relationships for butyric acid (P<0.01) and acetic acid (P<0.05). Effects on propionic acid were inconsistent and generally low. The relationships between VFA molar proportions and supplementation for BS plus WF in NR medium are given in Figure 7. The extent and significance of the interactions observed are given in Table 9. Similar interactions were observed for BS plus AA, although they did not achieve significance for acetic acid (P=0.079). No significant quadratic relationships were observed for hay and either supplement, nor for any feed combination in NF medium although for BS+WF significance was nearly achieved (P=0.056).

Production Trial

Level of Supplement

The level of supplementation achieved in the production trial was 0.28 for the low level of supplementation and 0.44 for the higher level of supplementation.

Chemical Composition of the Feeds

In comparison to the hay fed in experiment 1 the hay fed in the production trial was marginally lower in crude protein and had higher ADF and NDF values (Table 10). The compostion of the AA was very similar.

In vivo dry matter intake, live weight gain and food conversion efficiency

Supplementation of hay with AA did not increased total daily dry matter intake at the lower level but did at the higher level (Table 11). At the lowest level of supplementation with AA there was a 28 percentage decrease in daily hay dry matter intake and 24 percent at the higher level. Live weight gain (LWG) increased with increasing levels of supplementation and the feed conversion efficiency improved with supplementation.

The relationships between level of supplementation and total DMI, roughage DMI and daily LWG were assessed for linearity by regression analysis. These relationships were best described by polynomial models given below.

	Polynomial	
Total DMI	$y= 2046.1x^2 - 119.78x + 1094.4$	$R^2 = 0.9929$
Forage DMI	$y= 3184.4x^2 - 2007.4x+ 1119.7$	$R^2 = 1$
Daily LWG	$y= 97.584x^2 - 63.086x+ 10.91$	$R^2 = 1$

Discussion

Digestibility Trial and Gas Production

Chemical composition of roughages and supplements

The BS which was used for the *in vivo* and *in vitro* experiments was deficient in protein and contained high proportions of acid detergent fibre and neutral detergent fibre. The hay had a crude protein content of about 8 percent, indicating that it was approximately sufficient in nitrogen. Both AA and WF were protein rich supplements having a crude protein content of 16 percent. They contained appreciably less fibre, as would be expected, in comparison to the roughages.

Gas Production Characteristics of Individual Feeds

WF had the most rapid rate of fermentation which indicated a high level of readily fermentable carbohydrates present within the feed. Hay and AA had similar rates of fermentation with BS having the slowest rate of fermentation. These fermentation characteristics were consistent with the in *vivo* digestibility measurements. Both of the 2 supplements appeared to provide sufficient nitrogen to the microbial population since there was no difference in gas production between the NR and NF. The BS was deficient in nitrogen and the hay was marginally deficient, as suggested by their respective crude protein contents.

In Vivo Dry Matter Intake

Supplementation of BS with AA at the lower level of supplementation (0.32), resulted in a non significant increase in BS daily DMI. Results from *in vitro* studies showed that at this level of supplementation the differences between the NR and NF media (indicating deficiency in fermentable nitrogen within the diet) had been partially overcome. At the higher level of supplementation (0.52) there was a non significant decrease in BS daily DMI, the *in vitro* studies indicating that balance between fermentable carbohydrate and protein was being reached. The *in vitro* studies showed that interactions were occurring, that is that the fermentation of the diet was accelerated, to some extent in the NR medium. It would appear that at the lower level of supplemention there was an increase in the level of fermentable nitrogen in the rumen, increasing the rumen cellulolytic microbe population resulting in enhanced fibre degradation and increased intake of both roughage and total feed. At the higher level, nitrogen had ceased to be limiting and the drop in intake may have been as a result of gut fill, as AA was a less rapidly fermented feed.

With the WF there was a non significant increase in BS DMI at both levels of supplementation. *In vitro* results showed interactions were occurring in the NR medium at both levels of supplementation and that the system was in balance for nitrogen at about a supplement level of 0.3-0.4. This would indicate that the increase in intake at the lower level of supplementation (0.31) was likely to be due to an acceleration of roughage digestion in response to increased fermentable protein and carbohydrate. At the higher level of supplementation (0.47), an additional response to the readily fermentable carbohydrate under conditions which were not limited by fermentable protein probably explains the futher increase in intake. WF is high in readily fermentable carbohydrate and its intake did not appear to be regulated by gut fill.

With hay, at both levels of supplementation with AA there was a decrease in intake of the roughage. The *in vitro* studies indicated that there were no significant interactions occurring between hay and AA in the NR medium and that at 48 h incubation there were no differences in gas production between the 2 media after supplementing with 0.1 of AA. With WF, the intake of hay was maintained at the lower level of supplementation and decreased at the higher level of supplementation, possibly as a result of chemostatic inhibition of hay intake as gut fill may have been of secondary importance for these relatively rapidly fermentable feeds. *In vitro* results indicated interactions in NR and NF media, presumably in response to WF readily fermentable carbohydrate, with nitrogen not limiting at about a level of supplementation of about 0.3.

Digestibility and Interactive Effects

At the lower level of supplementation of BS with AA there was a 4.8 percent positive interaction in digestibility, although the interaction overall did not achieve statistical significance ($P<0.05$). The *in vitro* data suggested that this was probably due to a response to fermentable nitrogen supplied by the supplement. With the WF there are positive significant interactions at the lower and higher levels of supplementation. The *in vitro* data suggested that the responses at the lower level were a nitrogen/carbohydrate response and at the higher level there was an increased carbohydrate response. There were no significant interactive effects in digestibility with hay and AA either *in vivo* or *in vitro*. There were no interactive effects on *in vivo* digestibility with hay and WF, although there were significant interactions *in vitro*. Wood and Manyuchi (in press) found interactions *in vitro* between veld hay and Napier hay and groundnut hay but no interactions were found in *in vivo* digestibility, and suggested that this may have been due to increasing rates of passage with supplementation.

Fermentability and Interactions of Feed Mixtures

Of the 2 supplements used for these experiments it appeared that the WF had the greatest ability to stimulate the rumen-microorganisms to produce interactive effects between feeds. This is probably due no only to the high content of nitrogen in the feed but also a supply of readily fermentable carbohydrate for the cellulolytic bacteria resulting in enhanced fibre digestion. Interactions appeared greatest in the NF medium which would be expected, as the NR medium would eliminate most, if not all, responses to fermentable protein. Interactions were observed in both media showing that interactive effects were not all suppressed by the nitrogen supplied in the media but may be due to the presence of readily fermentable carbohydrates, and possibly also to some extent the supply of amino acids and peptides.

Differences in Gas Production for Feed Mixtures between NR and NF

It appears that the proportion of WF required to alleviate the nitrogen deficiency of the substrate occurs at a level of supplementation of around 0.3 whereas for AA it occured at about 0.5. With the hay differences between the media were much smaller. With the hay a low level of supplement, 0.1-0.3, were sufficient to alleviate the slight fermentable nitrogen deficiency.

Correlations between in vivo and in vitro parameters

The strong linear correlation between the rate constant (b) and total dry matter intake for all the diets (except WF alone) fermented in NR medium probably indicates that the intake of these feeds is determined largely by gut fill. The rate of fermentation of the feeds will also be a major factor in determining gut fill. By contrast, the intake of the highly fermentable WF is probably determined more by chemostatic control, hence lies somewhat removed from the general correlation.

Digestibility appears to be related to the lag time observed *in vitro*. Again, there were indications that the supplements did not lie on the general correlation. It is suggested that both lag time and digestibility of the roughages, and predominantly roughage based diets, is determined by the characteristics of the carbohydrate fraction. However, it is unclear what the physiological significance of the lag time is, so any interpretation must be tentative.

The significant, but relatively less strong, correlations between intake and lag time, and digestibility and rate constant (b), were probably due to the correlation between the two *in vivo* parameters.

Volatile Fatty Acids

The proportions of volatile fatty acids which were produced by the individual feeds were as would be expected. The straw samples were high in acetic acid and low in proprionic acid whereas WF was lower in acetic acid and higher in propionic acid. As with previous results *in vivo* and *in vitro* it appears that the larger interactions occurred with the straw and supplement rather than with the hay and supplements. However, perhaps surprisingly, interactions in VFA proportions in NF medium did not reach statistical significance for any of the mixtures studied even though interactions in gas production were generally higher than in NR medium. This may suggest that these interactions are related to the provision of relatively fermentable carbohydrate rather than nitrogen or fermentable protein.

Production Trial

The results from the production trial show that supplementation with AA resulted in a depression in hay intake at both levels of supplementation, consistent with the findings of the digestibility trial. At the lower level of supplementation there was no effect on total DMI, but there was at the higher level of supplementation. It has been noted that one of the limitations to the use of forage supplements may be that they may cause a substitution of the basal diet (Mosi and Butterworth, 1995). Abdulrazak *et al.* (1996) found that supplementation of napier grass with gliricidia decreased the intake of napier grass. The lack of response to supplementation on hay intake suggests that the CP content of the basal diet did not greatly limit the intake of hay and that once the rumen microbial requirements for nitrogen had been met, supplementation did not have any stimulating effect on the basal diet. Again this was fully consistent with the digestibility and *in vitro* studies.

The substitution effect resulting from increasing levels of AA may have been due to a number of factors. AA is a more palatable feed than hay and is easy to consume. Animals tend to choose feeds which are more palatable and less fibrous, and may be less inclined to eat less palatable feeds when presented with a choice. In the digestibility trial, results show that there was little difference *in vivo* and *in vitro* in the digestibility of hay and AA, particularly at the lower level of supplementation. Therefore consumption of AA would not result in any marked increase in digestibility of the diet; increased intake of AA would result in a reduction in intake of the hay. Research tends to indicate that the greatest benefit to supplementation occurs with basal forages which are low in protein (Minson and Milford, 1967; Egan 1996). As the quality of the diet improved in energy and protein, as expected there is an increase in the daily liveweight gain and an improvement in the feed conversion efficiency. AA had a high content of molasses and CP, therefore as the level of supplementation increased then there would be an improvement in LWG and therefore FCE.

Conclusions

Strong linear correlations were found between total dry matter intake and the rate constant (b), and between digestibility and the lag time (T+) for the roughages and supplemented roughage diets. Therefore there were strong indications that the *in vitro* gas production technique may be suitable for the prediction of intake and digestibility of roughage based diets. Positive interactions were found during *in vitro* fermentaton of BS supplemented with AA and WF. Interactions were observed in *in* *vivo* digestibility for BS + WF and supplementing BS resulted in an increase in daily BS dry matter intake. No significant interactions were observed *in vitro* or in *in vitro* digestibility when hay was fermented with AA in NR medium. Some interactions were observed *in vitro* when hay was supplemented with WF, but there were no interactions observed *in vivo*. In general there was a good agreement between the ranking of the scale of interactions of feeds measured *in vivo* and *in vitro*, although the *in vitro* gas production method appears to be more sensitive to interactions between feeds than is found *in vivo*. In the production trial in which meadow hay was supplemented with AA then substitution of the basal forage occured supporting the theory that supplementation has the greatest benefit with basal forages which are low in protein.

It appears that the *in vitro* gas production technique could be a useful tool in identifying and predicting possible digestive interactions between feeds thus reducing the need to conduct lengthy animal feeding trials to assess the effect on poor quality roughages of a wide range of supplements. However, the processes involved in *in vivo* digestion and *in vitro* fermentation are complex, and while they have their similarities there are also fundamental differences. A more mechanistic approach needs to be developed to integrate data from *in vitro* and *in vivo* trials to develop further the use of *in vitro* gas production to predict *in vivo* responses

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Table 1
Chemical compostion of roughages and supplements

	BS	HAY	AA	WF
Nutrient contents g/kg DM				
Organic Matter	939	931	878	938
Crude Protein	31.6	80.2	162	167
Acid Detergent Fibre ADF	521	391	347	177
Neutral Detergent Fibre NDF	828	678	445	486
Ether Extract	0.3	1.7	2.45	35.2

Table 2
The effect of supplementation on in vivo total daily dry matter intake and daily roughage intake, showing the statistical significance of the quadratic relationship between level of supplement and total DMI and of roughage DMI of supplemented roughages compared with unsupplemented roughage

Level of Supplement	**Alfa A Daily DMI**	**Roughage DMI**	**WF Daily DMI**	**Roughage DMI**
STRAW				
No Supplement	384	384	384	384
Level X	618	420 ns	642	442 ns
Level Y	715	340 ns	877	465 ns
100 %	1506	0	1117	0
Sig. of Quadratic	***		**	
HAY				
No Supplement	725	725	725	725
Level X	885	693 *	932	741 ns
Level Y	1077	681 *	1040	646 *
100 %	1506	0	1117	0
Sig. of Quadratic	ns		***	

Table 3
The effect of supplementation on in vivo dry matter digestibility and percentage differences between predicted (assuming no interactions) and observed dry matter digestibility

Straw level of supplement	**AA *in vivo* digestibility**	**% difference (interaction)**	**WF *in vivo* digestibility**	**% difference (interaction)**
no supplement	0.46	0	0.46	0
low level	0.53	4.8	0.56	8.7
high level	0.55	1.0	0.58	5.4
all supplement	0.61	0	0.65	0
Sig. of Quadratic		ns (P=0.097)		***
Hay				
no supplement	0.58	0	0.58	0
low level	0.58	-0.9	0.58	-1.2
high level	0.59	-1.0	0.61	-1.6
all supplement	0.61	0	0.65	0
Sig. of Quadratic		ns		ns

$$\%\text{Difference} = \frac{(\text{observed DMD} - \text{predicted DMD}) \times 100}{\text{predicted DMD}}$$

Table 4
France Parameters for roughages and supplements fermented alone in Nitrogen rich media

France parameter	**Barley straw**	**Meadow hay**	**Alpha A**	**Wheat feed**
Gas pool (A) ml g^{-1} DM	278.2	276.4	229.6	237.9
Rate constant (b) h^{-1}	0.0308	0.0421	0.0570	0.0872
Rate constant (c) $h^{-0.5}$	-0.0648	-0.0438	-0.0716	
Lag time (T+) h	1.08	0.26	0.37	
Lag time (T-) h	1.18	0.28	0.39	

Table 5
Mean values of cumulative gas production (CG) and dry matter disappearance (DMD) at various incubation times and percentage difference between observed and predicted values for BS supplemented with AA and WF fermented in NR and NF media

BS supplemented with AA in nitrogen rich media

	Cumulative Gas Production (ml/g)				P of Quadratic Relationship	Percentage Differences			
Level of BS	1	0.68	0.48	0		1	0.68	0.48	0
Level of AA	0	0.32	0.52	1		0	0.32	0.52	1
12 hrs	38.6	54.5	63.9	80.2	**	0	4.94	6.02	0
27 hrs	104.8	126.9	140.5	157.3	**	0	4.36	6.34	0
48 hrs	188.4	198.0	205.2	206.2	ns	0	1.99	3.81	0
96 hrs	244.7	239.1	240.2	227.7	ns	0	-0.08	1.82	0
DMD	0.653	0.647	0.646	0.643	ns	0	-0.44	-0.27	0

BS supplemented with AA in nitrogen free media

	Cumulative Gas Production (ml/g)					Percentage Differences			
Level of BS	1	0.68	0.48	0		1	0.68	0.48	0
Level of AA	0	0.32	0.52	1		0	0.32	0.52	1
12 hrs	36.8	57.3	66.6	81.8	**	0	11.81	10.59	0
27 hrs	61.7	103.2	124.6	157.0	***	0	11.96	11.97	0
48 hrs	90.4	159.4	184.8	206.1	***	0	25.12	22.76	0
96 hrs	138.7	230.1	233.2	229.6	***	0	37.17	25.44	0
DMD	0.346	0.611	0.634	0.646		0	38.33	26.27	0

BS supplemented with WF in nitrogen rich media

	Cumulative Gas Production (ml/g)				P of Quadratic Relationship	Percentage Differences			
Level of BS	1	0.69	0.53	0		1	0.69	0.53	0
Level of WF	0	0.31	0.47	1		0	0.31	0.47	1
12 hrs	38.1	66.9	84.0	123.2	**	0	3.78	7.52	0
27 hrs	103.9	144.3	164.3	195.4	***	0	9.05	11.8	0
48 hrs	189.5	216.6	230.0	226.8	***	0	7.74	11.1	0
96 hrs	247.2	262.8	272.8	246.1	***	0	6.44	10.6	0
DMD	0.649	0.693	0.727	0.729	**	0	2.91	5.85	0

BS supplemented with WF in nitrogen free media

	Cumulative Gas Production (ml/g)					Percentage Differences			
Level of BS	1	0.69	0.53	0		1	0.69	0.53	0
Level of WF	0	0.31	0.47	1		0	0.31	0.47	1
12 hrs	32.5	67.7	81.7	116.7	**	0	15.54	13.39	0
27 hrs	56.7	123.4	151.8	193.3	***	0	24.62	25.55	0
48 hrs	83.3	186.0	214.1	221.3	***	0	47.52	44.52	0
96 hrs	136.0	253.3	265.4	239.6	***	0	50.66	43.70	0
DMD	0.346	0.661	0.706	0.690		0	46.03	39.14	0

Table 6
Mean values of cumulative gas production (CG) and dry matter disappearance (DMD) at various incubation times and percentage difference between observed and predicted values for Hay supplemented with AA and WF fermented in NR and NF media

Hay supplemented with AA in nitrogen rich media

	Cumulative Gas Production (ml/g)				P of Quadratic Relationship	Percentage Differences			
Level of Hay	1	0.78	0.63	0		1	0.78	0.63	0
Level of AA	0	0.22	0.37	1		0	0.22	0.37	1
12 hrs	80.3	80.5	81.8	86.6	ns	0	-1.38	-0.95	0
27 hrs	164.1	165.1	167.2	164.0	ns	0	0.66	1.94	0
48 hrs	229.4	224.0	223.3	211.1	ns	0	-0.6	0.33	0
96 hrs	268.0	257.6	253.6	231.6	ns	0	-0.91	-0.36	0
DMD	0.732	0.717	0.719	0.654	ns	0	0.31	2.32	0

Hay supplemented with AA in nitrogen free media

	Cumulative Gas Production (ml/g)					Percentage Differences			
Level of Hay	1	0.78	0.63	0		1	0.78	0.63	0
Level of AA	0	0.22	0.37	1		0	0.22	0.37	1
12 hrs	71.4	74.8	77.8	86.6	ns	0	0.03	0.94	0
27 hrs	124.1	135.3	144.8	163.2	*	0	1.94	4.47	0
48 hrs	194.7	204.5	209.1	209.7	**	0	3.28	4.39	0
96 hrs	254.7	253.4	250.5	231.3	**	0	1.55	1.84	0
DMD	0.716	0.720	0.708	0.655	ns	0	2.48	2.16	0

Hay supplemented with WF in nitrogen rich media

	Cumulative Gas Production (ml/g)				P of Quadratic Relationship	Percentage Differences			
Level of Hay	1	0.8	0.62	0		1	0.8	0.62	0
Level of WF	0	0.2	0.38	1		0	0.2	0.38	1
12 hrs	78.0	95.2	105.5	127.7	***	0	8.25	8.88	0
27 hrs	160.8	179.7	186.8	199.0	***	0	6.64	6.56	0
48 hrs	227.3	240.4	244.4	230.5	***	0	5.46	6.97	0
96 hrs	268.1	278.5	282.3	252.8	***	0	5.08	7.64	0
DMD	0.727	0.752	0.771	0.745	***	0	2.99	5.08	0

Hay supplemented with WF in nitrogen free media

	Cumulative Gas Production (ml/g)					Percentage Differences			
Level of Hay	1	0.8	0.62	0		1	0.8	0.62	0
Level of WF	0	0.2	0.38	1		0	0.2	0.38	1
12 hrs	69.2	83.1	96.6	119.6	***	0	4.8	9.31	0
27 hrs	119.0	147.0	171.6	194.8	***	0	9.61	16.1	0
48 hrs	188.6	216.2	234.2	223.5	***	0	10.6	16	0
96 hrs	251.2	267.4	277.6	246.1	***	0	6.87	11.3	0
DMD	0.715	0.740	0.758	0.752	***	0	2.52	4	0

Table 7

Correlations (R^2) between *in vivo* dry matter intake and digestibility and *in vitro* gas production parameters for feeds and feed mixtures in nitrogen rich medium

***In vitro* parameter**	**dry matter intake (g per day)**	**digestibility**
Gas pool A, ml g^{-1} DM	0.29	0.12
Rate constant b, h^{-1}	0.43*	0.58**
Rate constant c, $h^{-0.5}$	0.03	0.01
Lag time T+, h	0.38*	0.73***
Lag time T-. h	0.34*	0.68**

Table 8 Molar proportions of volatile fatty acids (VFAs) for individual feeds fermented in nitrogen rich (NR) and nitrogen free (NF) media

	Medium	Acetic acid	Propionic acid	Butyric acid
Barley straw	NR	0.708 ±0.0045	0.224 ±0.0046	0.068 ±0.0002
	NF	0.685 ±0.0141	0.261 ±0.0120	0.054 ±0.0021
Meadow hay	NR	0.601 ±0.0492	0.318 ±0.0306	0.081 ±0.0186
	NF	0.625 ±0.0036	0.309 ±0.0040	0.066 ±0.0004
Alpha A	NR	0.626 ±0.0388	0.303 ±0.0270	0.071 ±0.0120
	NF	0.633 ±0.0017	0.305 ±0.0032	0.062 ±0.0043
Wheat feed	NR	0.581 ±0.0348	0.337 ±0.0321	0.083 ±0.0081
	NF	0.578 ±0.0359	0.331 ±0.0297	0.092 ±0.0092

Table 9 Interactions (%) between feeds: effects on the molar proportions of volatile fatty acids (VFAs)

Feeds	Medium	Level of supplementation	Acetic acid	sig[a].	Propionic acid	sig[a].	Butyric acid	sig[a].
BS + AA	NR	low	-1.16	ns	-0.01	ns	11.89	**
		high	-0.83	(P=0.079)	-0.48		10.27	
	NF	low	0.96	ns	-2.14	ns	-0.93	ns
		high	-0.27		-2.62		16.48	
BS + WF	NR	low	-1.82	*	-2.35	ns	25.55	**
-		high	-3.30		2.40		19.40	
	NF	low	-2.12	ns	0.45	ns	19.88	ns
		high	-2.40		0.25		21.26	(P=0.056)
Hay + AA	NR	low	2.62	ns	-5.56	ns	2.28	ns
		high	6.02		-9.37		-8.45	
	NF	low	-0.72	ns	1.10	ns	1.70	ns
		high	-0.21		0.85		-1.99	
Hay + WF	NR	low	0.98	ns	-3.43	ns	6.13	ns
		high	3.34		-6.48		0.64	
	NF	low	-0.43	ns	0.58	ns	1.17	ns
		high	0.46		-2.87		8.13	

[a]Significance of quadratic relationships

Table 10
Chemical composition of hay and AA fed in the production trial

	HAY	AA
Nutrient contents g/kg DM		
Organic Matter	944	927
Crude Protein	67.2	164.5
Acid Detergent Fibre ADF	459	358
Neutral Detergent Fibre NDF	791	426

Table 11
Total dry matter intake, live weight gain and feed conversion efficiency of lambs feed meadow hay supplemented with AA.

HAY

Level of AA	Total DMI (g/d)	Roughage DMI (g/d)	Live Weight Gain (g/d)	Feed Conversion Efficiency
No Supplement	1120	1120	10.3	108.5
Level X	1125	807	35.9	31.4
Level Y	1518	852	56.8	26.7
100 %	3013	0	171.0	17.6

Figure 1

Cumulative gas production profiles for barley straw, meadow hay, Alfa A and wheat feed fermented in nitrogen rich media.

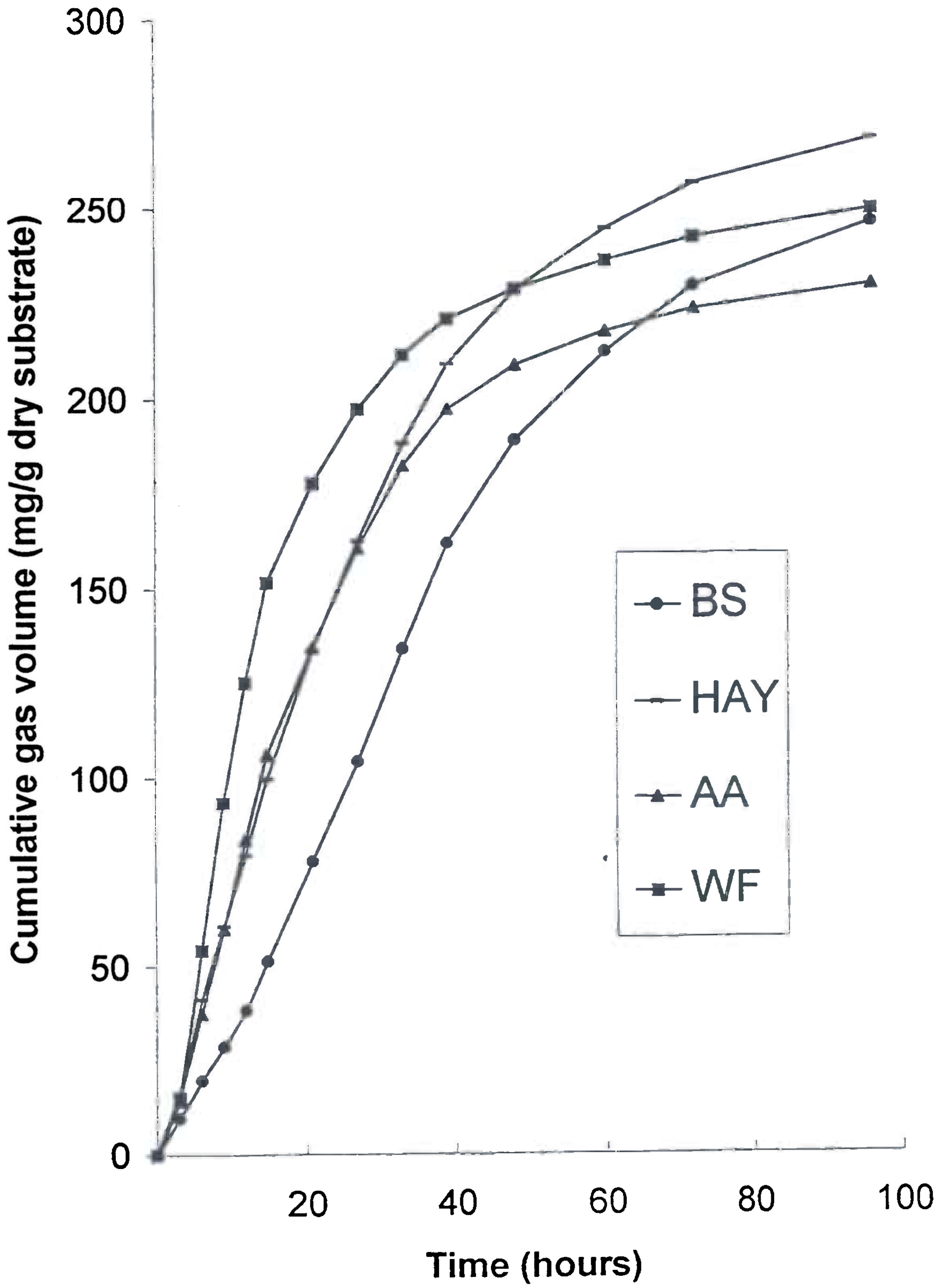


Figure 2

Cumulative gas production profiles for barley straw and meadow hay fermented in nitrogen rich media and nitrogen free media

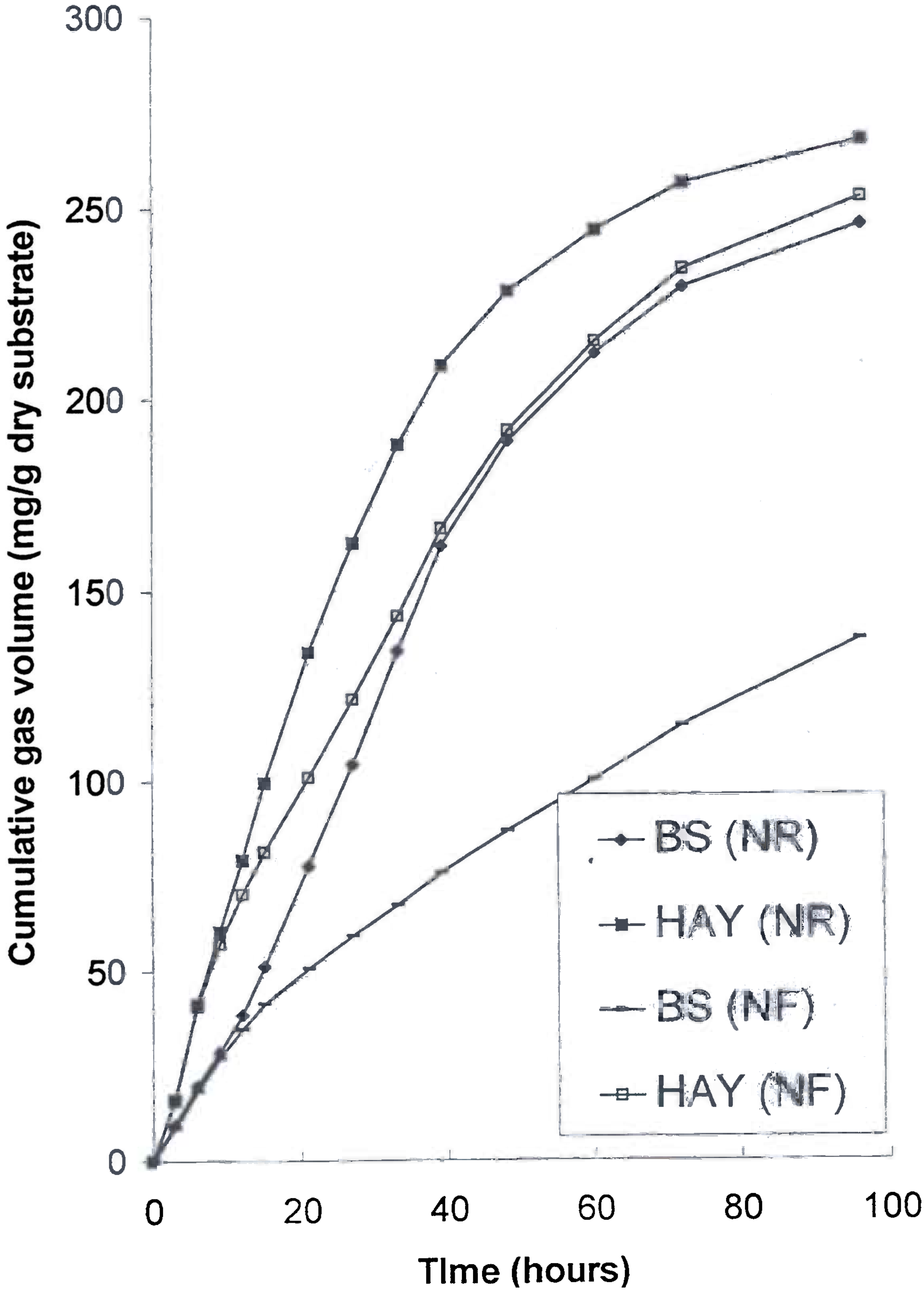


Figure 3

Barley straw supplemented with wheat feed: cummulative gas production at selected times versus porportion of supplement. (a) Fermented in nitrogen rich medium. (b) Fermented in nitrogen free medium.

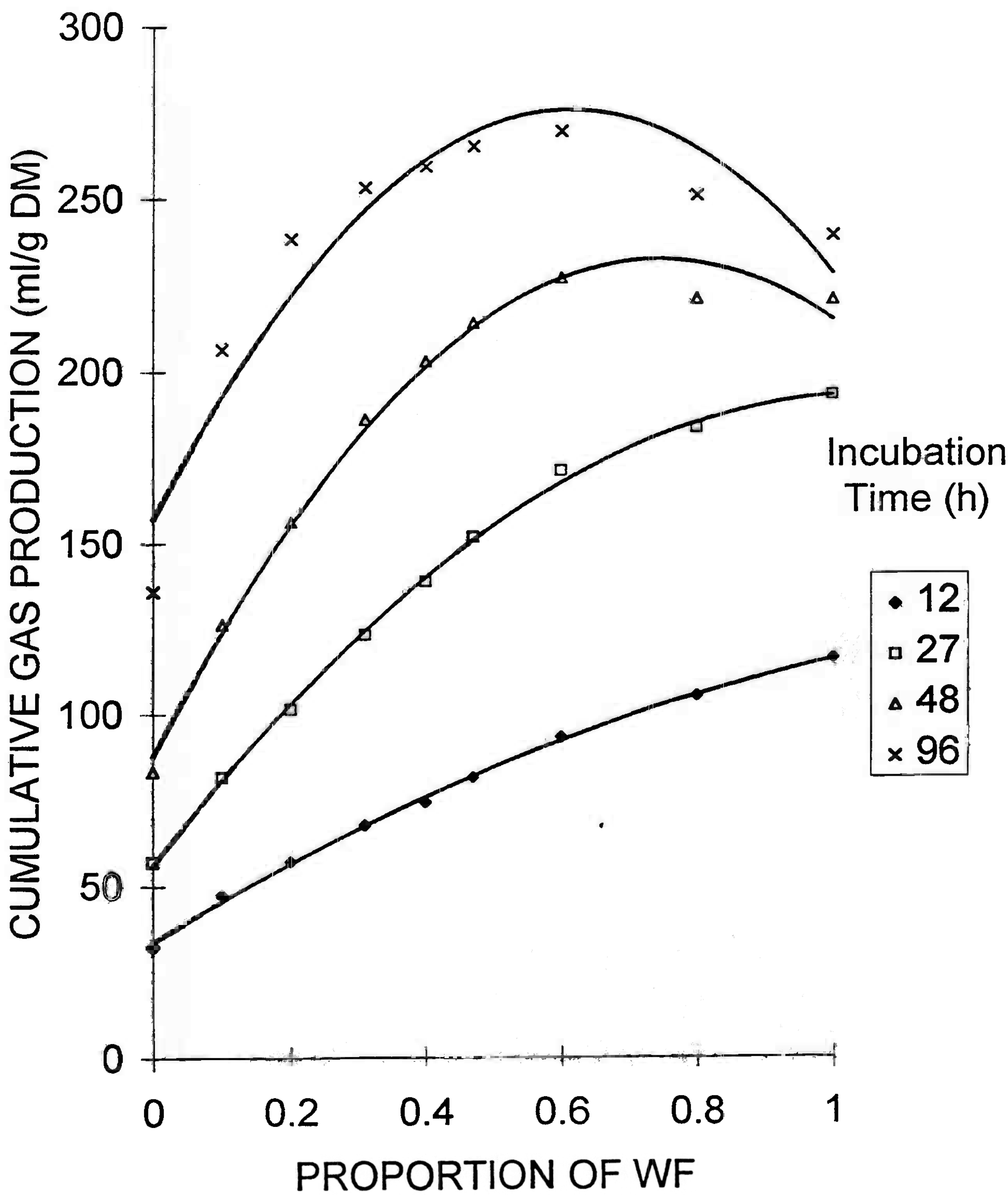
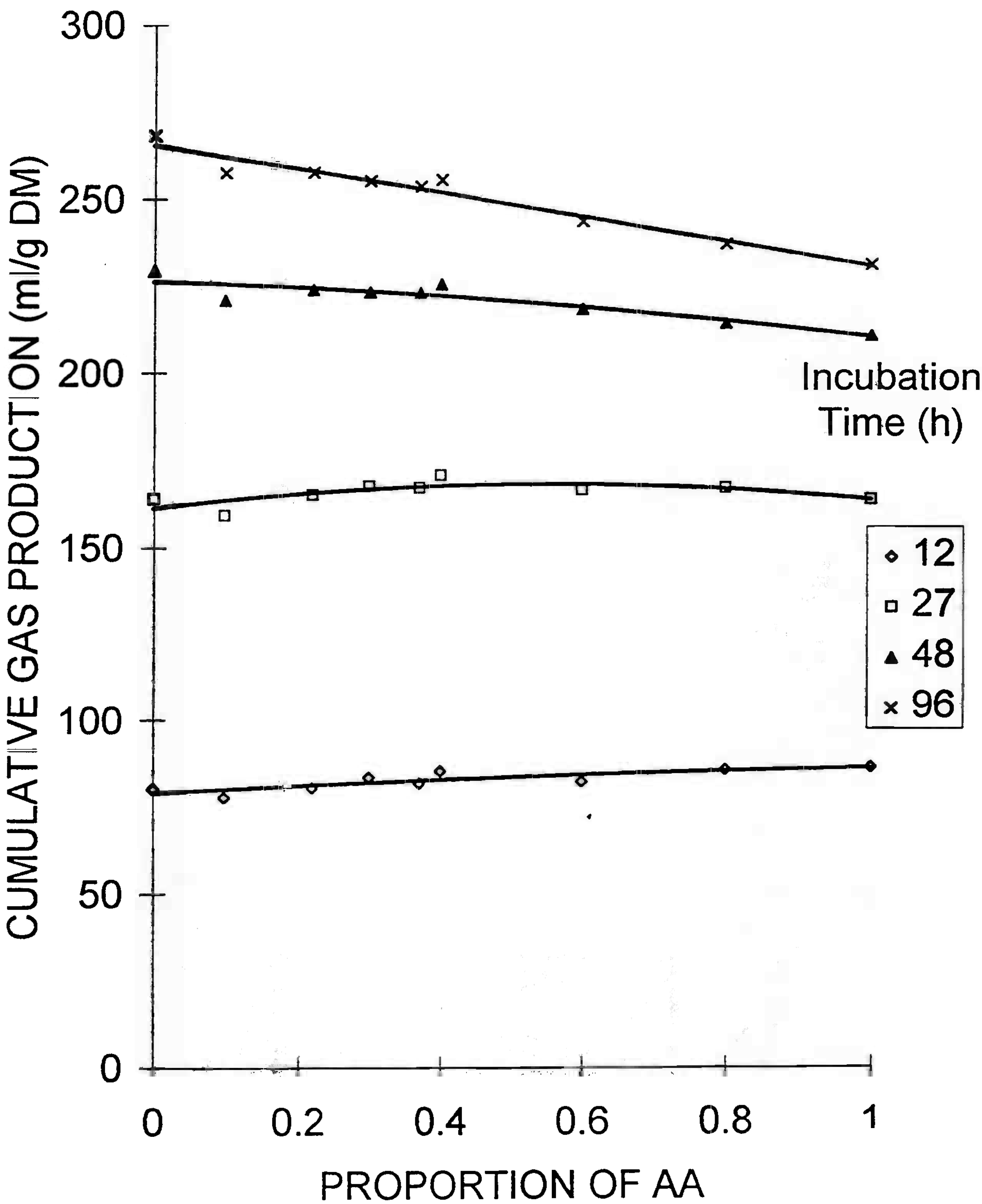
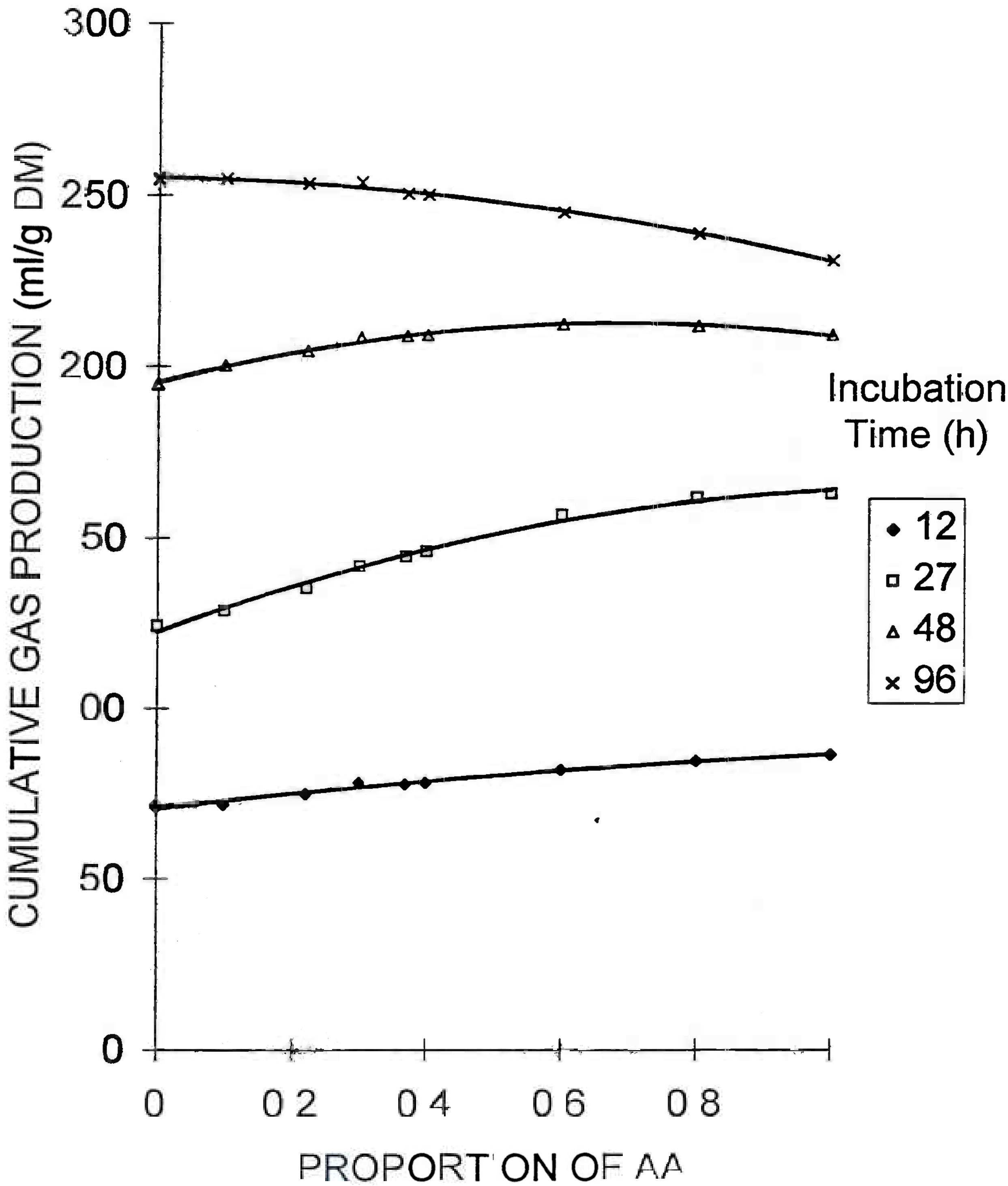


Figure 4

Meadow hay supplemented with Alpha A: cummulative gas production at selected times versus porportion of supplement. (a) Fermented in nitrogen rich medium. (b) Fermented in nitrogen free medium.

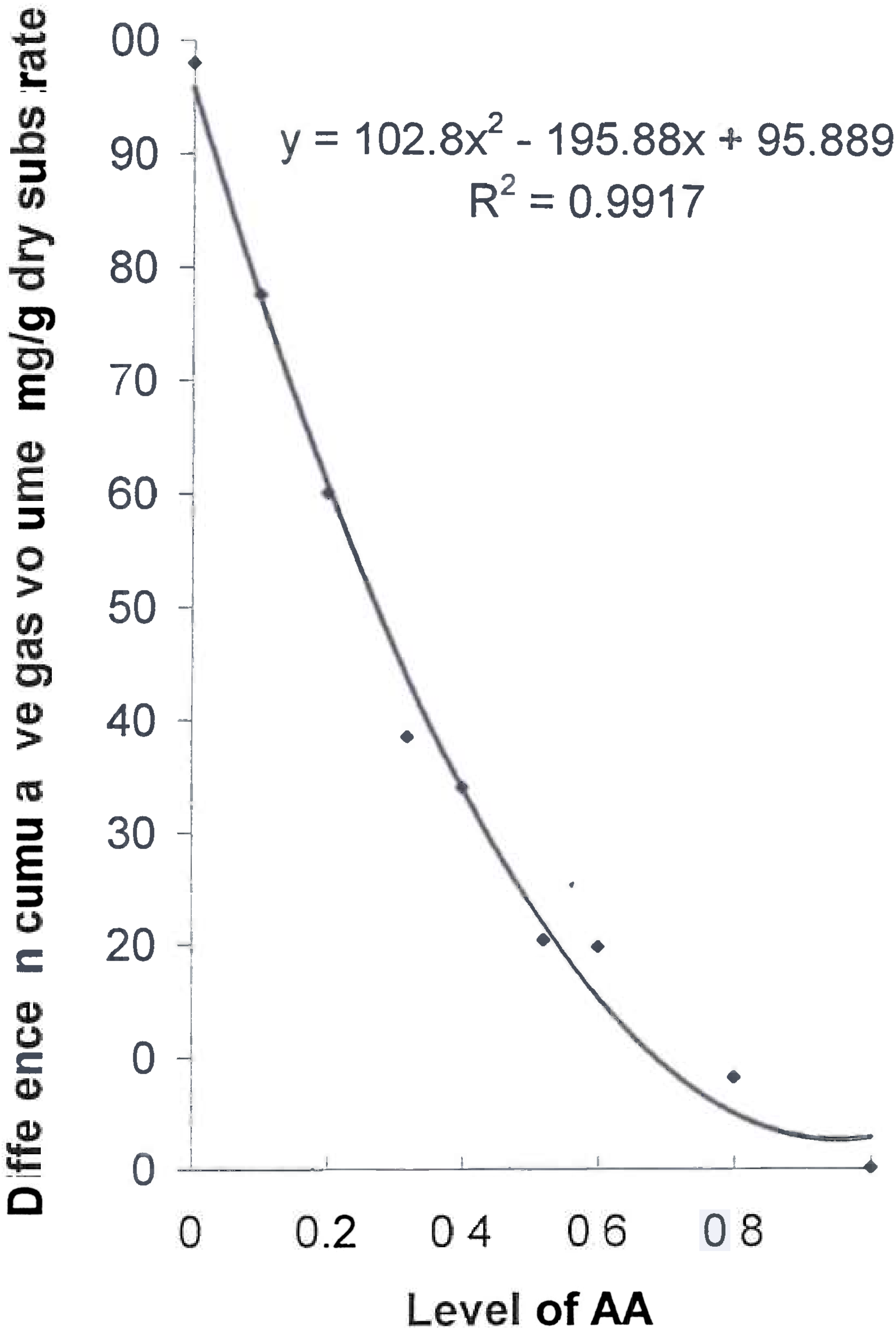


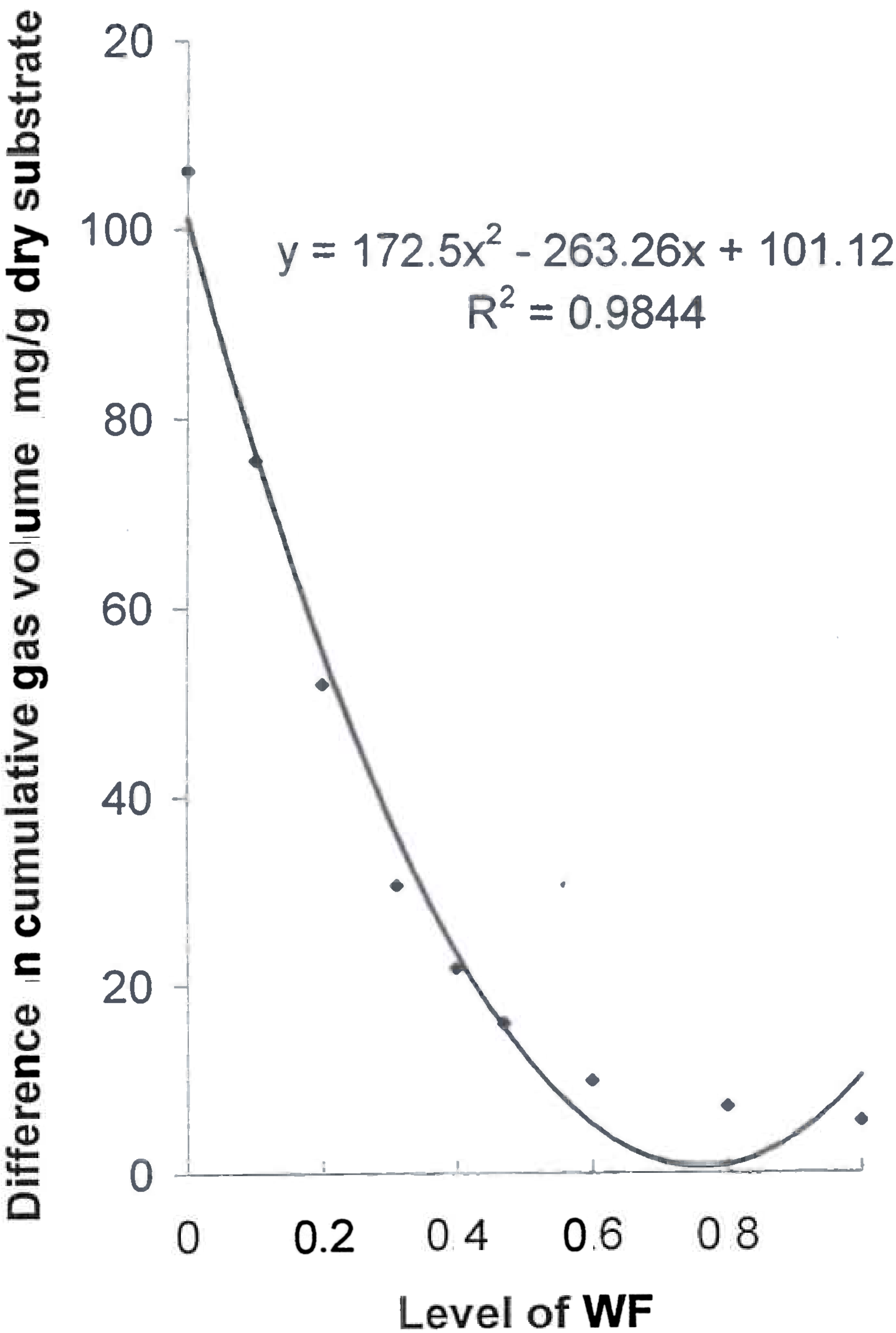


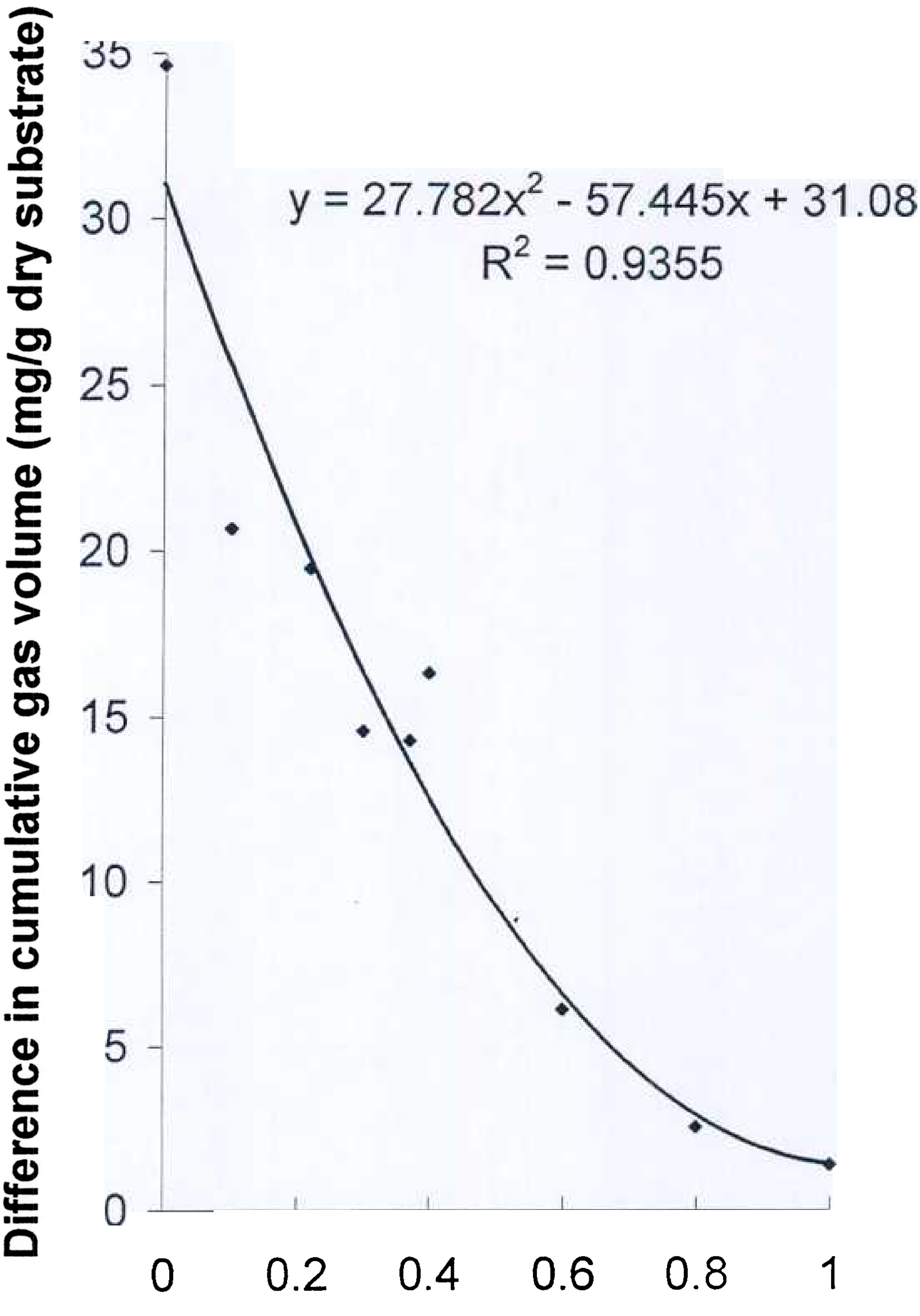
CUMULATIVE GAS PRODUCTION (ml/g DM)
300
250
200
50
00
50
0
0
0 2
0 4
0 6
0 8
PROPORT ON OF AA
Incubation Time (h)
12
27
48
96

Figure 5

Difference in cumulative gas production (ml/g DM substrate) after 48 h incubation between nitrogen rich and nitrogen free media for barley supplemented with AA (a) and WF (b) and meadow hay supplemented with AA (c) and WF (d)




Difference in cumulative gas volume mg/g dry substrate
20
100
80
60
40
20
0
y = 172.5x2 - 263.26x + 101.12
R2 = 0.9844
0
0.2
0.4
0.6
0.8
Level of WF



y = 27.782x2 - 57.445x + 31.08
R2 = 0.9355
Difference in cumulative gas volume (mg/g dry substrate)
35
30
25
20
15
10
5
0
0
0.2
0.4
0.6
0.8
1
Level of AA

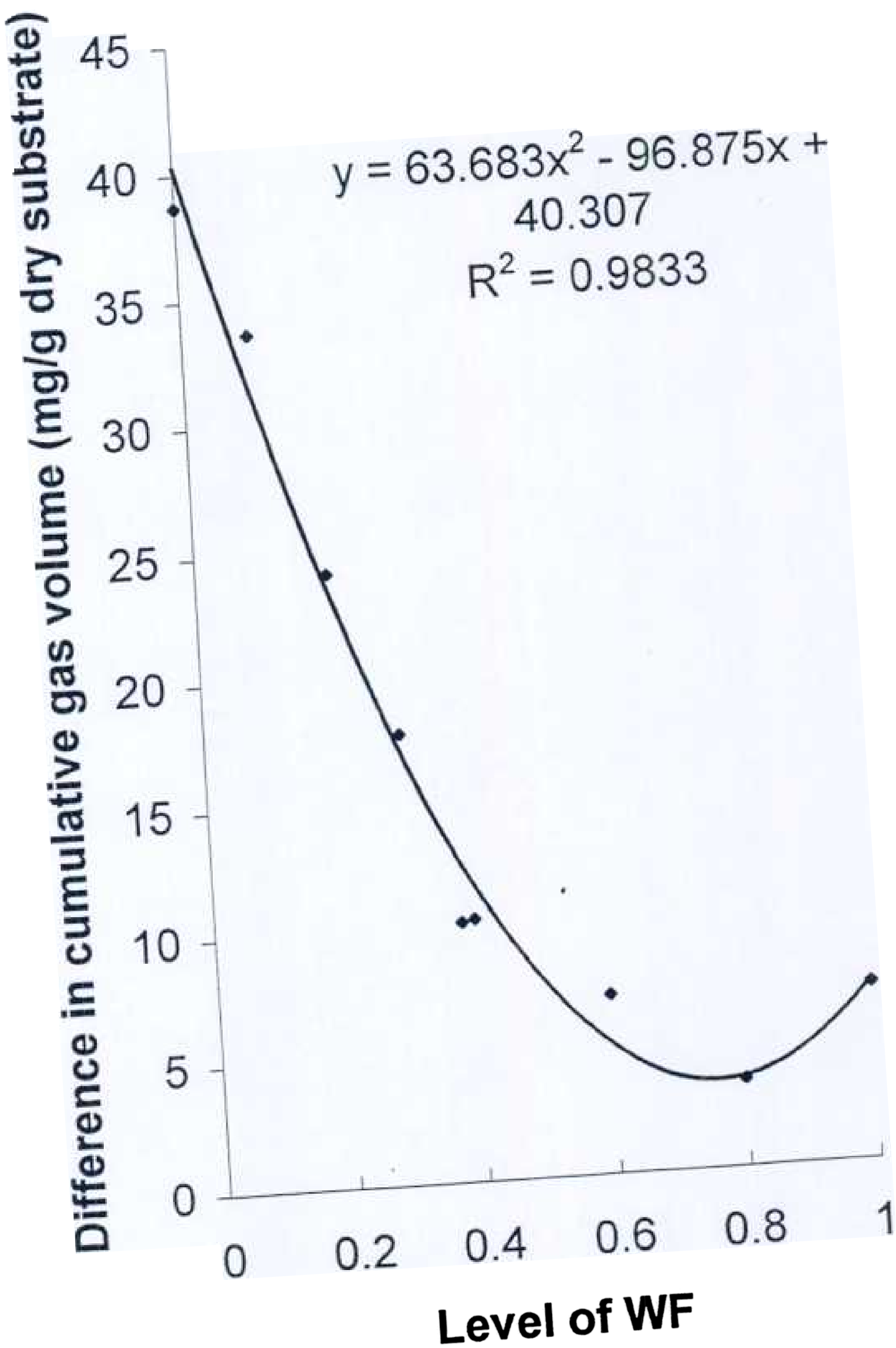

y = 63.683x² - 96.875x + 40.307
R² = 0.9833
Difference in cumulative gas volume (mg/g dry substrate)
45
40
35
30
25
20
15
10
5
0
0
0.2
0.4
0.6
0.8
1
Level of WF

Figure 6

Relationship between total daily dry matter intake and the *in vitro* rate constant (b), excluding wheat feed fed alone

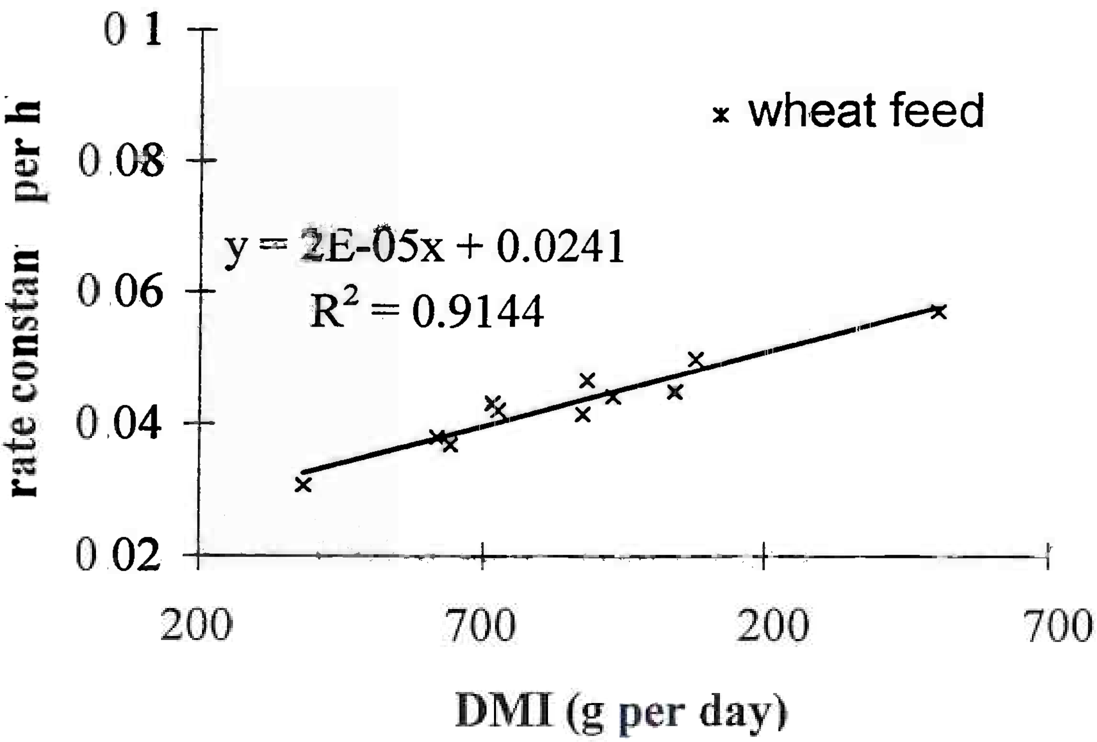


Figure 7
Barley straw (BS) supplemented with wheat feed (WF): volatile fatty acid (VFA) molar proportions ploted against proportion of supplement