

## Digestive Characteristics, Ammonia Nitrogen and Volatile Fatty Acids Levels, In Sheep Fed Oaten Chaff Supplemented with Grimmatt Barley Grain, Freeze-Dried or Fresh Barley Sprouts

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**Abstract:** About 4 treatments (control, fresh barley sprouts, freeze-dried barley sprouts and barley grain supplementation) were used in a latin square design. Oaten chaff basal diet was used in testing the assertion that hydroponic barley sprouts gave better animal performance than the grain supplement. Results showed increase in DM intake on supplementation, there were differences ( $p < 0.001$ ) among treatments in DM intake. The increased intake due to sprouts supplementation however, did not translate to better digestibility, microbial outflow and nitrogen retention. Total ammonia concentration was higher ( $p < 0.001$ ) for the fresh barley sprouts supplements than for the barley grain and control suggesting that poor quality roughage yields more rumen ammonia when supplemented with fresh hydroponic barley sprouts. The total ammonia concentration did not however, differ ( $p > 0.05$ ) between the fresh or freeze-dried hydroponic barley sprouts. The total VFA concentrations were higher for the freeze-dried and fresh hydroponic barley sprouts than the control but not different ( $p > 0.05$ ) from the barley grain supplementation in the current study. This suggests that sprouting did not give rise to a higher VFA concentration when poor quality roughage was supplemented. It was concluded from this study that supplementing poor quality roughage (oaten hay) with hydroponic barley sprouts increased DMI and total rumen ammonia concentration. However, there was no confirmation of the presence of a grass juice factor purported to be present in sprouts which gives increased performance.

**Key words:** Oaten chaff, sprouts, digestibility, ammonia, volatile fatty acids, supplementing

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### INTRODUCTION

Barley grain and hydroponic barley sprouts have been in use for feeding ruminants (Peer and Leeson, 1985b; Sneath and McIntosh, 2003). The use of hydroponic barley sprouts has been due to reports indicating that sprouts provide a source of rapidly available nutrients made available due to the action of hydrolytic enzymes releasing readily available amino acids and soluble carbohydrates (Barcelos *et al.*, 2002; Fahey *et al.*, 1997; Plaza *et al.*, 2003). There is also a synthesis of vitamins such as the B-complex, K and C causing an increase in their concentrations; minerals like calcium and sodium tend to have increased concentrations which are however, due to loss in DM.

Yields of wet sprouts ranging from 5-10 times the original weight of dry seed have been reported for different commercial hydroponics sheds. These wet sprouts contain about 15% DM. Dry matter changes with sprouting have been in the range of 9.4-18% reported as losses (Peer and Leeson, 1985a; Hillier and Perry, 1969; Chung *et al.*, 1989). Flynn and O'Kiely (1986) found a 24%

loss in DM. Reports by the current researchers (unpublished) indicate the loss in DM was 21.9% over the 7 days sprouting period. Steeping of grain increases its moisture content and the hydrated seeds subsequently increase enzymatic activities. These are mainly hydrolysing enzymes which break down the carbohydrates, protein and lipids into simpler compounds for cell wall synthesis and the early growth of the young plant. These nutrient changes include an increase in total protein concentration, change in amino acid composition, a decrease in starch concentration, increases in sugars, slight increases in crude fat and crude fibre and slightly higher amounts of certain vitamins and minerals. As has been pointed out by Peer and Leeson (1985a), most of these increases in nutrients are not true increases but simply a reflection of the loss in total DM mainly in the form of carbohydrates due to respiration during sprouting. As total carbohydrates decrease, the percentages of other nutrients increase.

The increases in nutrients mentioned, therefore are not true increases because of the aforementioned reasons. Vitamins, however are known to record true increases in

concentration with sprouting (Finney, 1982; Plaza *et al.*, 2003). The increases in animal production parameters such as milk yield and weight gains as a result of hydroponic barley sprouts feeding have been attributed to the presence of a factor known as the grass juice factor (Cannon and Emerson, 1939; Elvehjem *et al.*, 1934; Kohler *et al.*, 1937; Randle *et al.*, 1940; Stirm *et al.*, 1935). This alleged factor was said to be rich in young rapidly metabolizing plant tissues such as sprouts as opposed to mature plants. A report not in support of the view that hydroponic grain sprouts bring about rapid increase in performance indicated that when beef animals were given sprouts to replace highly nutritious feeds there was no advantage; it was only when the sprouts were given as supplements to protein deficient hay that there was an improvement in efficiency (Thomas and Reddy, 1962; Tudor *et al.*, 2003).

This study was therefore conducted to verify the proposition that sprouts contain a better profile of nutrients than barley grain and are more readily available to the animal to rapidly improve performance and that sprouts contain a factor (grass juice factor) which gives the animal better performance compared to grain feeding.

## MATERIALS AND METHODS

**Animals and housing:** About 4 Merino sheep (initial weight of 45 kg±5.2) were housed in individual pens in an animal house. Each was fitted with a permanent rumen cannula.

**Diets and sample collection:** The four fistulated sheep above were given oaten chaff *ad libitum* with a mineral supplement, daily and the voluntary intake determined over a period of 14 days in individual pens. After this period, the sheep were subjected to four different treatments in a Latin square design. During the treatment phase, there was a 7 days total urine and faecal collection (in metabolism cages) and also sampling of rumen fluid. Between treatment phases, there was an adjustment period of 14 days before collection of samples. The apparent digestibility of DM, OM and N retention were determined. The rumen fluid profile of VFA (molar proportions and total concentration) and rumen ammonia in the four treatments were determined.

**Experimental design and analysis:** About 4 Merino sheep fitted with permanent rumen cannulae were used for a 4×4 Latin square design. The Latin square design (Steel and Torrie, 1980) of four treatments and four periods was used for the assessment of the three barley treatments (grain, freeze-dried and fresh hydroponic barley sprouts) and a control. The data were analysed using the Analysis of

Variance (ANOVA) and treatment means compared using pair wise comparisons at 5% level of probability (Duncan's LSD).

Minitab 12.1 software was used for data analyses. Intake and digestibility data were analysed using the one-way analysis of variance while the General Linear Model (GLM) was used in repeated measures analysis for data repeated over time (ruminal pH, ammonia and VFA concentrations).

### Analytical methods and calculations

**Estimation of microbial N supply:** Purine derivative (allantoin) conversion to microbial outflow was determined as follows: Microbial N supply for sheep was calculated based on the equation by Chen and Gomes briefly described below:

$$Y = (0.150^{W^{0.75}} e^{-0.25X}) + 0.84X$$

Where:

Y = Purine derivative excretion in the urine

X = Exogenous purines

**Dry matter, organic matter and ash:** The DM content of feed, refusals and faeces was estimated by drying samples in triplicate from each animal in each period in a forced draught oven at 60°C for a minimum of 72 h or until constant weight was achieved. Thereafter the samples were bulked and milled in a Wiley Mill to pass through 1 mm screen and dried overnight in crucibles at 105°C to determine the final DM content. The DM was then combusted in a muffle furnace at 600°C for 3 h to determine both the organic matter and ash content (AOAC, 1990).

**Total N:** The total N content in the feed ingredients, feed refusals, faeces and urine was determined using the automated semi microkjeldahl system (AOAC, 1990).

**Ammonia N:** The concentration of ammonia N in the rumen fluid supernatant was estimated using an autoanalyser (Technicon) according to the method described by Beitz (1974). The proportion of un-ionised ammonia in the total ammonia concentration was calculated using the Henderson-Hasselbalch equation (Siddons *et al.*, 1985) taking into account total ammonia concentration and rumen fluid pH:

$$\text{Un-ionised } [\text{NH}_3] = 1 - (1 / (1 + \text{antilog } [\text{pH} - \text{pK}'\text{a}], \text{pK}'\text{a} = 9.02$$

**Volatile fatty acids:** The total molar concentration (m mol L<sup>-1</sup>) of all VFAs and molar percentages of major VFA (acetic, propionic and butyric) and minor VFA (iso-butyric, iso-valeric and valeric) were estimated in the

rumen fluid supernatants by methods of Erwin *et al.* (1961) using Gas Liquid Chromatography (GLC) (Model CP 3800 GC) and iso-caproic acid as an internal standard. The ratio of lipogenic to glucogenic VFA were determined as described by Maas *et al.* (2001).

**RESULTS AND DISCUSSION**

**Feed DM intake, apparent DM and OM digestibility and nitrogen retention:** The composition of barley grain, freeze-dried barley sprouts and oaten chaff are shown in Table 1 while the mean daily DMI (g day<sup>-1</sup>), total N intake (g day<sup>-1</sup>), N balance (g day<sup>-1</sup>), DM (%) and OM (%) digestibility of the four diets in the current study are shown in Table 2. There was a difference (p<0.001) among the four treatments in feed DMI.

The diets supplemented with hydroponic barley sprouts (T3 and T4) had higher average daily DMI than the control and the diet supplemented with barley grain (T1 and T2, respectively). There were no differences (p>0.05) in the mean daily DMI between the control and barley grain supplementation. However, there were differences among the two treatments supplemented with hydroponic barley sprouts. The treatment with freeze-dried barley sprouts supplementation gave a higher (p<0.001) mean daily DMI than the fresh barley sprouts supplementation. From Table 2, the apparent DM and OM digestibilities were not different (p>0.05) among the means of all the treatments. The DM digestibility ranged from 57.5% for the diet T3 to 61.3% for both T1 and T2. The OM digestibility ranged from 57.2% for T3-61.4% for T2. The N balance (g day<sup>-1</sup>) did not differ (p>0.05) among the means of the four treatments as a result of the different supplementations given. The overall effect of the supplementation showed no negative nitrogen balance for any of the treatments during the course of the experiment. Live weights of all the animals were recorded at the beginning and end of each of the four periods but were not included in the tables of results. Each period did not last >3 weeks and this was considered not sufficient to bring about noticeable changes that could be reported.

**Total VFA concentrations, proportions and pH of rumen fluid:**

The total VFA concentrations, the molar percentages and pH are shown in Table 3 while the trend of total VFA concentration over time for the four treatments is shown in Fig. 1. There were differences (p<0.01) in total VFA concentration among treatments in response to type of supplementation given while there was no time x treatment interaction (p>0.05) for any of the VFA concentrations. There was also a significant effect of time on the concentration of total VFA and for the molar proportions of the VFA except for butyric acid. All the supplemented treatments (except barley grain supplementation) had higher total VFA concentrations than the control treatment. The supplemented treatments (barley grain and the two barley sprouts supplementations) were not different from each other in total VFA concentration, although the treatments with sprouted supplements tended to be higher.

There was a significant time effect on VFA concentration except for butyric acid. The trend in total VFA concentration as shown in Fig. 1 shows a gradual

Table 1: Composition of barley grain, barley sprouts and oaten chaff

Composition	DM (%)	CP (%)*	OM (%)*	Ash (%)*
Barley grain	92.3	13.9	90.3	2.0
Barley sprouts (freeze-dried)	91.6	15.9	87.3	4.3
Oaten chaff	92.6	8.1	87.1	5.5

\*As percentage of DM

Table 2: Total DMI, DM and OM digestibility, nitrogen balance and microbial outflow in sheep fed oaten chaff (T1), T1+barley grain (T2), T1+freeze-dried barley sprouts (T3), T1+fresh barley sprouts (T4)

Components	T1	T2	T3	T4	Significance
Total DMI g day <sup>-1</sup>	1000.0 <sup>a</sup>	993.0 <sup>a</sup>	1187.0 <sup>c</sup>	1087.0 <sup>b</sup>	***
Total DMI g kg <sup>-1</sup> W <sup>0.75</sup> day <sup>-1</sup>	55.6 <sup>a</sup>	54.8 <sup>a</sup>	65.0 <sup>c</sup>	59.4 <sup>b</sup>	***
DM digestibility (%)	61.3	61.3	57.5	61.0	NS
OM digestibility (%)	60.2	61.4	57.2	60.8	NS
Total N intake (g day <sup>-1</sup> )	12.8	12.2	15.9	14.7	NS
Fecal N (g day <sup>-1</sup> )	4.6	4.6	6.3	4.9	NS
Urine N (g day <sup>-1</sup> )	4.7	5.4	5.6	6.5	NS
Nitrogen balance (g day <sup>-1</sup> )	3.5	2.2	4.1	3.3	NS
Microbial outflow (g day <sup>-1</sup> )	4.4	4.5	5.0	5.0	NS

\*\*\*(p<0.001), NS = Not Significantly different (p>0.05), Means with different superscripts a,b,c within same row differ significantly (p<0.001)

Table 3: Concentrations of rumen fluid ammonia and VFA, molar proportions of VFA and rumenpH of sheep fed oaten chaff (T1), T1+barley grain (T2), T1+freeze-dried barley sprouts (T3), T1+fresh barley sprouts (T4)

Parameters	T1	T2	T3	T4	SEM	Significance level
Ammonia concentration (mg N L <sup>-1</sup> )	125.00 <sup>a</sup>	126.00 <sup>a</sup>	149.00 <sup>ab</sup>	172.00 <sup>b</sup>	26.00	***
Non-ionised NH <sub>3</sub> -N (mg L <sup>-1</sup> )	0.24	0.22	0.22	0.22	0.12	NS
Total VFA concentration (m mol L <sup>-1</sup> )	84.40 <sup>a</sup>	89.60 <sup>ab</sup>	96.30 <sup>b</sup>	98.50 <sup>b</sup>	10.68	**
Acetate (%)	63.70	61.60	61.50	61.20	2.62	NS
Propionate (%)	23.50	24.00	25.20	24.20	2.30	NS
Butyrate (%)	10.30 <sup>a</sup>	11.30 <sup>ab</sup>	10.60 <sup>a</sup>	11.90 <sup>b</sup>	1.07	***
Minor VFA (% <sup>†</sup> )	2.53 <sup>a</sup>	3.08 <sup>b</sup>	2.65 <sup>ab</sup>	2.77 <sup>ab</sup>	0.48	*
Glucogenic VFA (% <sup>††</sup> )	26.00	27.10	27.90	26.90	2.36	NS
Lipogenic VFA (% <sup>†††</sup> )	52.70	52.80	51.80	53.40	2.36	NS
Glucogenic:Lipogenic	0.33	0.34	0.35	0.33	0.04	NS
pH	6.16 <sup>a</sup>	6.07 <sup>ab</sup>	6.00 <sup>ab</sup>	5.97 <sup>a</sup>	0.17	*

<sup>†</sup>Sum of isobutyric, isovaleric and valeric, <sup>††</sup>Sum of propionic, isobutyric, isovaleric and valeric, <sup>†††</sup>Sum of acetic and butyric, \*(p<0.05), \*\*(p<0.1)\*\*\* (p<0.001), NS = Not Significantly different (p>0.05), Means with different superscripts a, b, c, within the same row differ significantly

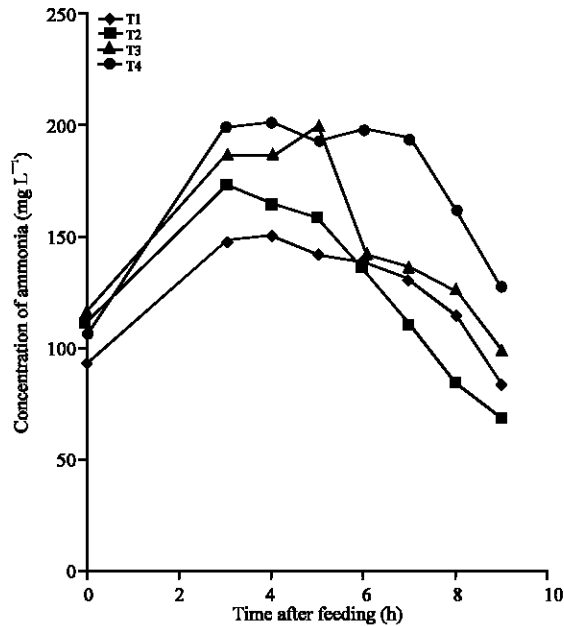


Fig. 1: Total VFA concentration in rumen fluid of sheep fed oaten chaff (T1), oaten chaff + barley grain (T2), oaten chaff+freeze-dried barley sprouts (T3) and oaten chaff+fresh barley sprouts (T4)

rise from 3 h after feeding till 7 h after feeding when a decline commenced. The peak concentration of the VFA lasted between 5 and 7 h after feeding. The trend in concentration looked similar for all the treatments over time. The mean molar proportions of acetic and propionic acids did not differ ( $p > 0.05$ ) among the four treatments while butyric acid differed ( $p < 0.001$ ) so also the minor VFA (isobutyric, isovaleric and valeric) ( $p < 0.05$ ). There was no difference in mean butyric acid concentration for the control, barley grain and freeze-dried barley sprouts supplementation. The fresh sprouts supplementation, however, gave a higher ( $p < 0.001$ ) concentration of butyric acid than the control and freeze-dried barley sprouts supplementation; the fresh sprouts supplementation, however did not differ ( $p > 0.05$ ) from barley grain supplementation. The highest mean concentration for minor VFA in barley grain supplementation was different from control but not from the sprouts treatments (freeze-dried or fresh). The mean concentrations of glucogenic, lipogenic and the ratio of glucogenic: lipogenic VFA did not differ ( $p > 0.05$ ) due to treatment. The pH values were different with the highest pH value of 6.16 in the control different from the lowest pH value of 5.97 for fresh barley sprouts supplementation. The pH values tended to follow the pattern of total VFA production with the highest VFA levels having the lowest pH value and vice versa.

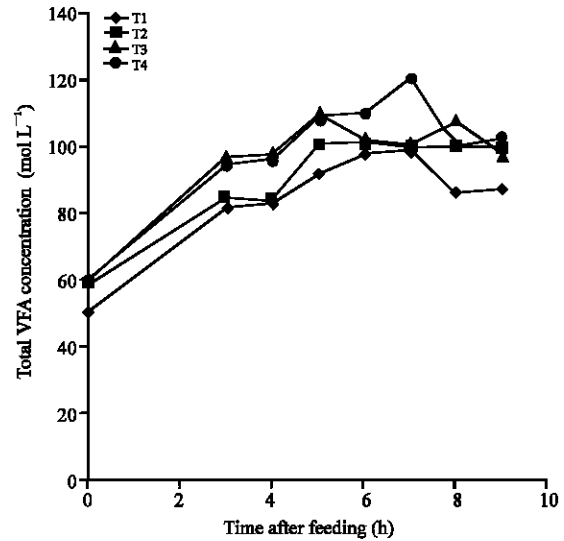


Fig. 2: Rumens ammonia concentration in sheep fed oaten chaff (T1), oaten chaff+barley grain (T2), oaten chaff+freeze-dried barley sprouts (T3) and oaten chaff+fresh barley sprouts (T4)

**Ammonia concentration in rumen fluid:** The effect of barley grain and sprouts treatment on rumen ammonia levels are shown in Table 3 while the trend in concentration of rumen ammonia levels is shown in Fig. 2. Mean ammonia concentrations differed among the treatments ( $p < 0.001$ ). The diets that had hydroponic barley sprouts supplements gave the highest values, however the freeze-dried barley supplement did not differ ( $p > 0.05$ ) from any of the other treatments unlike the fresh barley sprouts supplements ( $p < 0.001$ ). Supplementing with unsprouted barley grain did not give a difference in mean ammonia concentration levels from the control. The two sprouts supplements did not differ from one another in rumen ammonia concentration. Rumens ammonia levels were  $> 50 \text{ mg NL}^{-1}$  at feeding time for all the treatments and were even higher for the rest of the day. The slopes of the curves showing the rise in rumen ammonia concentrations were similar for all the treatments. This was indicated earlier where treatment x time interactions were not different between treatments. There was no difference ( $p > 0.05$ ) in non-ionised ammonia N in the rumen fluid of the sheep in the current study due to type of supplementation given.

**Rumen bacterial outflow:** The microbial outflow ( $\text{g day}^{-1}$ ) in the current study did not differ ( $p > 0.05$ ) due to type of supplementation given on the oaten chaff diet. The sprouts supplements tended to be slightly higher than the

barley grain supplementation and the control treatments but this was not different ( $p>0.05$ ). Generally, microbial protein synthesis depends on the supply of energy sources from fibrous and non-fibrous carbohydrates and also the supply of nitrogen sources. The readily fermentable sources are more effective than other sources (Stern and Hoover, 1979).

**Feed DM intake, apparent DM and OM digestibility and nitrogen retention:** Dry matter intake ( $\text{g day}^{-1}$ ) was higher ( $p<0.001$ ) with sprouts supplements (T3 and T4) in this study than the treatments without sprouts supplements (T1 and T2) as expected. The basal diet was low-protein quality oat chaff hay with a CP content of 8.1% (Table 1). Generally, a concentration of <6-8% CP in the basal forage is considered to be the threshold for a response by ruminant livestock to N supplements (Mathis *et al.*, 2000). The fresh hydroponic barley sprouts have been reported to have highly soluble proteins and amino acids in response to the enzymatic transformations during early plant growth (Chung *et al.*, 1989; Dikshit and Ghadle, 2003). These enzymatic activities in the young plant also cause the breakdown of carbohydrates, proteins and lipids into simpler compounds and cause nutrient changes such as an increase in total protein concentration, changes in amino acid composition, decrease in starch, increase in sugars, crude fat, crude fibre and higher amounts of some vitamins and minerals on a DM basis (Chavan and Kadam, 1989; Lorenz, 1980).

The nutrients present in the fresh barley sprouts were released as a result of mastication by the animal. Mastication during ingestion of herbage has been reported to release >50% of soluble carbohydrates and 30% of intracellular nitrogen (Boudon and Peyraud, 2001; Mangan *et al.*, 1976) and facilitate colonisation of plant tissue by micro-organisms (Pond *et al.*, 1984). The release of soluble carbohydrates and nitrogen from the sprouts likely favoured proliferation of micro-organisms for a more efficient degradation of the low protein basal diet that gave the higher intake values relative to the treatments without sprout supplementation. Intake of low-quality forage often increases with protein supplementation (Beatty *et al.*, 1994; Krehbiel *et al.*, 1998; McCollum and Galyean, 1985) but that may not be the case when the forage is adequate for maintaining rumen fermentation (Swanson *et al.*, 2000) as was the case in this study, i.e., T1.

The freeze drying performed for the second sprout treatment may have served as a means of rupture of the cells for the release of the moisture. The cells would have

ruptured with freezing because of the usual increase in volume of water with freezing. It is believed that these rupture points in addition to mastication created a source of entry for microbes for a relatively faster degradation of the supplement relative to the treatments without sprouts supplementation. The voluntary intake by the sheep in this study when considered on the basis of metabolic weight did give a change in trend as shown in Table 2. The difference was that only the freeze-dried barley sprouts treatment differed from the treatments without sprouts supplementation. The fresh hydroponic barley sprouts treatment did not produce a difference ( $p>0.05$ ) though it tended to be higher in DMI. The DM and OM digestibility did not differ ( $p>0.05$ ) among the four treatments in this study. It is likely that sufficient nutrients were supplied by the basal diet to provide for adequate microbial fermentation. The microbial population is known to have an effect on digestibility of substrate in the rumen (Bach *et al.*, 1999, 2005; Dehority, 2003; Stern *et al.*, 2006).

The nitrogen balance results showed no difference due to supplementation type given. The values of nitrogen balance in the current study varied from  $2.2 \text{ g day}^{-1}$  for the barley grain supplementation to  $4.1 \text{ g day}^{-1}$  for the barley sprouts supplementation. Reports with values for nitrogen balance close to the current study based on low quality grass hay were given by Salisbury *et al.* (2004) or slightly higher for sheep on pasture by Maas *et al.* (2001).

**Total VFA concentrations, VFA proportions and pH in rumen fluid:** The mean values of VFA in the current study are similar to those reported by Mathis *et al.* (2000) on medium to low quality forages (Osakwe and Steingass, 2006) on grass hay diet supplemented with *Leucaena* leaves. In the current study, there were differences among means in total VFA due to supplementation but time x treatment interaction was not observed. There was also a noticeable difference in VFA concentration due to time presumably reflecting the increased rate of fermentation after the day's ration.

The mean VFA concentrations were higher ( $p<0.01$ ) in the sheep supplemented with sprouts (T3 and T4) than the control or the barley grain supplementation animals. The control treatment (oat chaff) which was a low quality fibrous diet might have taken a longer time to release nutrients by fermentation while the supplemented treatments would have provided readily soluble nutrients for faster microbial growth and fermentation. Annison and Lewis (1959) reported that sugar was fermented almost

instantaneously when it entered the rumen whereas the fermentation rate of fibre reached a peak at about 4-6 h post ingestion. There was no difference in total VFA between the control and barley grain supplementation treatments most likely due to source of the oaten chaff used-from a failed crop which contained some grains in the barley chaff. There were no differences ( $p>0.05$ ) in response in acetic and propionic acid proportions due to supplementation. The common intermediate in the rumen degradation of all feed carbohydrates is pyruvate (Friggens *et al.*, 1998). The mechanism that controls the partition of pyruvate among the VFA appears to be a function of the balance of microbes species present in the rumen which in turn is a function of the composition and form of feeds ingested (Friggens *et al.*, 1998). Studies have linked the proportions of VFA to the chemistry of feeds (Murphy, 1984; Murphy *et al.*, 1982).

The composition and form of feeds in this study would not have differed much to the extent that fermentation patterns giving rise to differences in VFA proportions of acetate and propionate. The rate of fermentation of substrate also has an effect on product of fermentation. Rapid fermentation associated with grain diets is associated with increased production of organic acids, increased production of microbial protein, decreased fibre digestion, decreased ammonia concentrations and decreased acetate: propionate ratio (Huntington *et al.*, 2006; Oba and Allen, 2003a-c). The rate of fermentation in the four treatments might not have varied in the current study which is likely why there was no difference in propionate.

There was however, a difference ( $p<0.001$ ) in butyric acid and in minor VFA concentration ( $p<0.05$ ) among the different supplement treatments. It has been established that marked changes in molar proportions of the concentrations of VFA in the rumen can be induced in response to dietary manipulations (Sutton *et al.*, 2003). Factors such as type and physical form of diet, level of intake, frequency of feeding, microbial profile in the rumen, outflow/dilution rates affect the molar proportions of the main VFA in the rumen (France and Siddons, 1993). Butyric acid synthesis in the rumen may occur from acetate or from compounds giving rise to Acetyl-CoA such as pyruvate (Barker, 1961).

It is believed that the inter-conversion of acetate and butyrate is pH dependent with an alkaline pH favouring formation of butyrate and an acid pH for acetate (Greville and Tubbs, 1968). The reverse of this however was reported (Satter and Esdale, 1968) and this suggests that for the current study the interplay of factors mentioned

earlier would have given a higher concentration of butyrate due to fresh barley sprouts supplementation, than pH alone. The minor VFA concentrations in the rumen are usually relatively high with high levels of protein in the diet and energy is limiting to incorporate the peptides or amino acids into microbial protein (Bach *et al.*, 1999). Low concentrations of minor VFA in the rumen could also be as a result of reduced proteolysis. In the current study, the supplementation of oaten chaff with barley grain gave a higher minor VFA concentration than the control most likely due to reduced proteolysis in the control. The two treatments that used sprouts supplements did not however, differ significantly from the control despite being numerically greater.

No differences were observed among treatments in the concentration of glucogenic VFA, lipogenic VFA and glucogenic: lipogenic ratio due to supplementation of the low protein oaten chaff. The pH values tended to follow the production of total VFA with the lower VFA concentration in the rumen fluid giving a higher pH value and vice versa. This can be attributed to the fact that the VFA are acidic so a higher concentration would tend to lower the pH value relatively.

**Ammonia concentration in rumen fluid:** Time x treatment interactions were not observed for ammonia concentration in this study but differences in concentration due to time were observed ( $p<0.001$ ) which was likely as a result of an increase in fermentation rate following the morning feeding. The mean rumen ammonia concentration differed ( $p<0.001$ ) among the treatments due to supplementation of the poor quality roughage. The results in this study confirmed that differences in ruminal degradability of supplements occurred.

From Table 1, there was an indication of the difference in CP content of the supplements. Similarly, other researchers reported an increase in rumen ammonia N with increase in CP supplementation (Bohnert *et al.*, 2002; Salisbury *et al.*, 2004). The highest value for total mean rumen ammonia concentrations in the current study was from fresh barley sprout supplementation. Following the release of ammonia in the rumen, there needs to be a synchrony in supply of the required energy among other nutrients to effectively incorporate it into microbial protein (Hoover and Stokes, 1991). When rate of protein degradation exceeds the rate of carbohydrate fermentation large quantities of N can be lost as ammonia and conversely when the rate of carbohydrate fermentation exceeds protein degradation rate, microbial protein synthesis can decrease (Nocek and Russell, 1988). From

the current study, the treatment with the highest rumen ammonia (numerically) also maintained a consistently higher VFA concentration relatively, indicating there might have been a good synchrony of the energy and N for increased microbial activities.

The un-ionised ammonia concentration did not differ among treatments in the current study, though the control treatment had the numerically highest value. This could be due to its higher pH of 6.16 relative to the other treatments. It has been established that total rumen ammonia exists in both ionised and un-ionised forms and these are pH dependent (Siddons *et al.*, 1985). The transfer of the un-ionised form across membranes is believed to be by passive diffusion (Lewis *et al.*, 1957). The unionised form of ammonia is believed to diffuse through membranes more freely than the ionised form. A feed treatment that gives a relatively higher pH value, therefore is more likely to offer more of the un-ionised form of ammonia, the absorption of which is more efficient.

**Rumen bacterial outflow:** The rumen microbial outflow did not differ ( $p>0.05$ ) among the means of all the treatment groups. These results suggest the supply of microbial nutrients in the form of energy and nitrogen needed for synthesis of microbial protein did not differ between treatments.

### CONCLUSION

This study was designed to test the hypotheses that hydroponic sprouts give higher livestock performance than the original grain and also that the presence of a grass juice factor in the sprouts will increase livestock performance. Results of this study suggest that supplementation of poor quality chaff with hydroponic barley sprouts led to an increase in DM intake by sheep. This was shown by the higher intake figures of the two forms of hydroponic barley sprouts (fresh and freeze-dried) supplementation offered the animals. The increased intake, however did not translate to better digestibility, microbial outflow or nitrogen retention. This suggests there might not have been a special factor (grass juice) in the sprouts which gives rise to increased performance as indicated by some researchers.

The rumen fluid parameters tended to be higher for the hydroponic barley sprouts supplementation when compared to the control or barley grain supplementation. Total ammonia concentration was higher for the fresh barley sprouts than for the barley grain and control suggesting that poor quality roughage yields more rumen

ammonia when supplemented with fresh hydroponic barley sprouts. The total ammonia concentration did not however, differ between the fresh or freeze-dried hydroponic barley sprouts. The total VFA concentrations were higher for the freeze-dried and fresh hydroponic barley sprouts than the control but not different from the barley grain supplementation in the current study. This suggests that sprouting did not give rise to a higher VFA concentration when poor quality roughage was supplemented. It can be concluded from this study that supplementing poor quality roughage (oaten hay) with hydroponic barley sprouts increased DMI and total rumen ammonia concentration. However, there was no confirmation of the presence of a grass juice factor purported to be present in sprouts which gives increased performance. From literature, study reported on rumen fluid parameters with regards to feeding of sprouts is very scarce; the common reports being those on weight changes and milk production parameters. More research needs to be done in that regard.

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### REFERENCES

- AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., pp: 200-210.
- Annison, E.F. and D. Lewis, 1959. Metabolism in the Rumen. Methuen, London.
- Bach, A., I.K. Yoon, M.D. Stern, H.G. Jung and H. Chester-Jones, 1999. Effect of type of carbohydrate supplementation to lush pasture on microbial fermentation in continuous culture. *J. Dairy Sci.*, 82: 153-160.
- Bach, A., S. Calsamiglia and M.D. Stern, 2005. Nitrogen metabolism in the rumen. *J. Dairy Sci.*, 88: E9-E21.
- Barcelos, M. de F.P., E.V. de B.V. Boas and M.A.C. Lima, 2002. Nutritional aspects of combined sprouts of soybean and corn. *Ciencia e Agrotecnologia*, 26: 817-825.
- Barker, H.A., 1961. *The Bacteria*. Academic Press, New York.

- Beaty, J.L., R.C. Cochran, B.A. Lintzenich, E.S. Vanzant, J.L. Morrill, R.T. Brandt Jr. and D.E. Johnson, 1994. Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. *J. Anim. Sci.*, 72: 2475-2486.
- Beitz, J.A., 1974. Micro-Kjeldahl analysis by an improved automated ammonia determination following manual digestion. *Anal. Chem.*, 46: 1617-1618.
- Bohnert, D.W., C.S. Schauer, S.J. Falck and T. DelCurto, 2002. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics. *J. Anim. Sci.*, 80: 2978-2988.
- Boudon, A. and J.L. Peyraud, 2001. The release of intracellular constituents from fresh ryegrass (*Lolium perenne*) during ingestive mastication in cows: Effect of intracellular constituent, season and stage of maturity. *Anim. Feed Sci. Technol.*, 93: 229-245.
- Cannon, M.D. and C.A. Emerson, 1939. Dietary requirements of the guinea pig with reference to the need for a special factor. *J. Nutr.*, 18: 155-167.
- Chavan, J.K. and S.S. Kadam, 1989. Nutritional improvement of cereals by sprouting. *Critical Rev. Food Sci. Nutr.*, 28: 401-437.
- Chung, T.Y., E.N. Nwokolo and J.S. Sim, 1989. Compositional and digestibility changes in sprouted barley and canola seeds. *Plant Foods Hum. Nutr.*, 39: 267-278.
- Dehority, B.A., 2003. *Rumen Microbiology*. Nottingham University Press, Nottingham.
- Dikshit, M. and M. Ghadle, 2003. Effect of sprouting on nutrients, anti-nutrients and *in vitro* digestibility of MACS-13 soybean variety. *Plant Food Hum. Nutr.*, 58: 1-11.
- Elvehjem, C.A., E.B. Hart, H.C. Jackson and K.G. Weckel, 1934. The nutritional value of milks-Raw vs. pasteurized and summer vs. winter. *J. Dairy Sci.*, 17: 763-770.
- Erwin, E.S., G.J. Marco and E. Emery, 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.*, 44: 1768-1771.
- Fahey, J.W., Y. Zhang and P. Talalay, 1997. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA.*, 94: 10367-10372.
- Finney, P.L., 1982. Effect of germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. *Recent Adv. Phytochem.*, 17: 229-305.
- Flynn, V. and P. O'Kiely, 1986. Input/Output data for the ACRON germination unit. *Organizing the Movement*.
- France, J. and R.C. Siddons, 1993. *Volatile Fatty Acid Production*. CAB International, Willingford, UK.
- Friggens, N.C., J.C. Oldham, R.J. Dewhurst and G. Horgan, 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. *J. Dairy Sci.*, 81: 1331-1334.
- Greville, G.D. and P.K. Tubbs, 1968. *Essays in Biochemistry*. Academic Press, New York.
- Hillier, R.J. and T.W. Perry, 1969. Effect of hydroponically produced oat grass on ration digestibility of cattle. *J. Anim. Sci.*, 29: 783-785.
- Hoover, W.H. and S.R. Stokes, 1991. Balancing carbohydrates and protein for optimum rumen microbial yield. *J. Dairy Sci.*, 74: 3630-3644.
- Huntington, G.B., D.L. Harmon and C.J. Richards, 2006. Sites, rates and limits of starch digestion and glucose metabolism in growing cattle. *J. Anim. Sci.*, 84: E14-E24.
- Kohler, G.C., C.A. Elvehjem and E.B. Hart, 1937. Further studies on the growth promoting factor associated with summer milk. *J. Nutr.*, 14: 131-144.
- Krehbiel, C.R., C.L. Ferrell and H.C. Freetly, 1998. Effects of frequency of supplementation on dry matter intake and net portal and hepatic flux of nutrients in the mature ewes that consume low-quality forage. *J. Anim. Sci.*, 76: 2464-2473.
- Lewis, D., K.J. Hill and E.F. Annison, 1957. Studies on the portal blood of sheep. 2. Absorption of volatile fatty acids from the rumen of the sheep. *Biochem. J.*, 66: 592-599.
- Lorenz, K., 1980. Cereal sprouts: Composition, nutritive value, food applications. *Crit. Rev. Food Sci. Nutr.*, 13: 353-385.
- Maas, J.A., G.F. Wilson, S.N. McCutcheon, G.A. Lynch, D.L. Burnham and J. France, 2001. The effect of season and monensin sodium on the digestive characteristics of autumn and spring pasture fed to sheep. *J. Anim. Sci.*, 79: 1052-1058.
- Mangan, J.L., R.L. Vetter, D.J. Jordan and P.C. Wright, 1976. The effect of condensed tannins of sainfoin (*Onobrychis viciifolia*) on the release of soluble leaf protein into the food bolus of cattle. *Proc. Nutr. Soc.*, 35: 95A-97A.
- Mathis, C.P., R.C. Cochran, J.S. Heldt, B.C. Woods and I.E. Abdelgadir *et al.*, 2000. Effect of supplemental degradable intake protein on utilisation of medium- to low-quality forages. *J. Anim. Sci.*, 78: 224-232.
- McCollum, F.T. and M.L. Galyean, 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. *J. Anim. Sci.*, 60: 570-577.



- Murphy, M.R., 1984. Modelling Production of Volatile Fatty Acids in Ruminants. In: Modelling Ruminant Digestion and Metabolism, Baldwin, R.L. and R.S. Bywater (Eds.). University of California, Davis, California, pp: 59-62.
- Murphy, M.R., R.L. Baldwin and L.J. Koong, 1982. Estimation of *Stoichiometric* parameters for rumen fermentation of roughage and concentrate diets. *J. Anim. Sci.*, 55: 411-421.
- Nocek, J.E. and J.B. Russell, 1988. Protein and energy as an integrated system: Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.*, 71: 2070-2107.
- Oba, M. and M.S. Allen, 2003a. Effect of corn grain conservation method on feeding behaviour and productivity of lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.*, 86: 174-183.
- Oba, M. and M.S. Allen, 2003b. Effect of corn grain conservation method on ruminal diigestion kinetics for lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.*, 86: 184-194.
- Oba, M. and M.S. Allen, 2003c. Effect of diet fermentability of microbial nitrogen production in lactating dairy cows. *J. Dairy Sci.*, 86: 195-207.
- Osakwe, I.I. and H. Steingass, 2006. Ruminal fermentation and nutrient digestion in West African Dwarf (WAD) sheep fed *Leucaena leucocephala* supplemental diets. *Agrofor. Syst.*, 67: 129-133.
- Peer, D.J. and S. Leeson, 1985a. Feeding value of hydroponically produced barley for poultry and pigs. *Anim. Feed Sci. Technol.*, 13: 183-190.
- Peer, D.J. and S. Leeson, 1985b. Nutrient content of hydroponically sprouted barley. *Anim. Feed Sci. Technol.*, 13: 191-202.
- Plaza, L., B. de Ancos and M.P. Cano, 2003. Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycin max*), Wheat, (*Triticum aestivum* L.) and alfalfa (*Medicago sativa*) treated by a new drying method. *Eur. Food Res. Technol.*, 216: 138-144.
- Pond, K.R., W.C. Ellis and D.E. Akin, 1984. Ingestive mastication and fragmentation of forages. *J. Anim. Sci.*, 58: 1567-1574.
- Randle, S.B., H.A. Sober and G.C. Kohler, 1940. The distribution of the grass juice factor in plant and animal materials. *J. Nutr.*, 20: 459-466.
- Salisbury, M.W., C.R. Krehbiel, T.T. Ross, C.L. Schultz and L.L. Melton, 2004. Effects of supplemental protein type on intake, nitrogen balance and site and extent of digestion in white face wethers consuming low-quality grass hay. *J. Anim. Sci.*, 82: 3567-3576.
- Satter, L.D. and W.J. Esdale, 1968. *In vitro* lactate metabolism by rumen ingesta. *Applied Microbiol.*, 16: 680-688.
- Siddons, R.C., J.V. Nolan, D.E. Beever and J.C. Mcrae, 1985. Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of nitrogen. *Br. J. Nutr.*, 54: 175-187.
- Sneath, R. and F. McIntosh, 2003. Review of Hydroponic Fodder Production for Beef Cattle. Queensland Government, Department of Primary Industries, Dalby, Queensland.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. 2nd Edn., McGraw Hill Book Co. Inc., New York, USA., ISBN-13: 9780070610286, pp: 188-189.
- Stern, M.D. and W.H. Hoover, 1979. Methods for determining and factors affecting rumen microbial protein synthesis: A review. *J. Dairy Sci.*, 49: 1590-1603.
- Stern, M.D., A. Bach and S. Calsamiglia, 2006. New concepts in protein nutrition in ruminants. Proceeding of the 21st Annual Southwest Nutrition and Management Conference, Feb. 23-24, Tempe, Arizona, USA., pp: 45-66.
- Stim, F.E., C.A. Elvehjem and E.B. Hart, 1935. Preliminary observations on certain seasonal variations in the physical properties and nutritive value of cows milk serum. *J. Dairy Sci.*, 18: 333-336.
- Sutton, J.D., M.S. Dhanoa, S.V. Morant, J. France, D.J. Napper and E. Schuller, 2003. Rates of production of acetate, propionate and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci.*, 86: 3620-3633.
- Swanson, K.C., J.S. Caton, D.A. Redmer, V.I. Burke and L.P. Reynolds, 2000. Influence of undegraded intake protein on intake, digestion, serum hormones and metabolites and nitrogen balance in sheep. *Small Rumin. Res.*, 35: 225-233.
- Thomas, J.W. and B.S. Reddy, 1962. Sprouted oats as feed for dairy cows. *Quart. Bull. Mich. Agric. Exp. Stn.* 44: 654-665.
- Tudor, G., T. Darcy, P. Smith and F. Shallcross, 2003. The intake and liveweight change of droughtmaster steers fed hydroponically grown, young sprouted barley fodder (Autograss). Department of Agriculture Western Australia.