RUMEN FLUID PH AND PLASMA FATTY ACID PROFILE CHANGES IN SHEEP FED DIFFERENT LEVELS OF CONCENTRATE FEEDS AND OIL PALM FROND PELLETS

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Summary

A study was carried out to examine the relationship between rumen fluid pH and changes in the plasma fatty acid contents of sheep as a result of dietary manipulation. Thirty-three 7-month-old Malin x Barbados Blackbelly male lambs were randomly assigned into three groups of 11 animals each, and fed either 80 % commercial sheep/goat pellet + 20 % (% w/w) oil palm frond (OPF) pellet (C80 group), 50 % commercial sheep/goat pellet + 50 % OPF pellet (% w/w) (C50 group), or 80 % OPF pellet + 20 % (% w/w) commercial sheep/goat pellets (C20 group) for 12 weeks. At the end of the trial, plasma fatty acid profiles and rumen fluid pH of the animals were determined upon slaughter. The C80 group which had the lowest rumen fluid pH at 0, 4 and 6 hours post-feeding also showed the highest ($P<0.01$) levels of plasma n-6 polyunsaturated fatty acids and the highest ($P<0.01$) total unsaturated : total saturated fatty acid ratios. The C20 group however, had the highest ($P<0.05$) amount of plasma n-3 polyunsaturated fatty acids and the highest ($P<0.05$) rumen fluid pH at 0, 4 and 6 hours post-feeding. These results showed trends of increasing contents of plasma unsaturated fatty acids with the reduction in rumen fluid pH. It was concluded that the dietary manipulation resulted in the alteration of ruminal activities to an extent that favoured accumulation of plasma unsaturated fatty acids in the plasma of the C80 group.

Keywords : Dietary manipulation, Plasma Fatty Acids, Rumen pH.

INTRODUCTION

The rumen fluid pH is a good indicator of overall rumen functions (Kay, 1983) and the plasma fatty acid profiles reflect the dietary fatty acid uptake patterns from feedstuffs in the ruminant (Jenkins and Thies, 1997). The absorption of the dietary fatty acids in the ruminant's alimentary tract is linked intimately with the rumen functions (Doreau and Ferlay, 1994). This supports the fact that tissues from herbivores are relatively rich in unsaturated fatty acids, but not ruminants due to the extensive biohydrogenation of the unsaturated fatty acids in the rumen (Gurr and Harwood, 1991). Studies in sheep (Goh *et al.,* 2000) and goats (Rajion et al., 1996) have shown that the fatty acid content of the plasma and meat was affected by the level of concentrate and fibrous feeds (oil palm frond pellets and grass) included in the treatment diets. It was known that animals eating low amounts of concentrate but high roughage have rumen fluid pH ranging from 6.0 to 7.0 while those on high concentrate have a pH of 5.0 to 6.0 (Orskov and Ryle, 1990). This paper aimed to determine any possible link(s) between rumen fluid pH (used as an indicator of rumen function status) and plasma fatty acid profiles in sheep fed different levels of concentrate feed and oil palm frond pellets.

MATERIALS AND METHODS

Animals and Blood Collection

Thirty-three individually housed, 7-month-old Malin x Barbados Blackbelly male lambs were allotted randomly into three groups of 11 animals each. The C80 group was fed a mixture of 80 % commercial sheep/goat pellet + 20 % (% w/w) oil palm frond (OPF) pellet. The C50 animals were fed a diet of equal amounts of commercial sheep/goat pellet + OPF pellet (% w/w), and the C20 animals were fed 80 % OPF pellet + 20 % (% w/w) commercial sheep/goat pellets. The animals were fed twice daily at 3.5 % bodyweight dry matter intake. Water was provided *ad libitum* and a salt lick was provided as required. The trial lasted 12 weeks excluding a two-week adjusting period. Blood collections were carried out on all animals (n=33) by jugular venipuncture into ethylenediamino tetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson, New Jersey, USA). Animals were bled at the end of the trial 3 days before slaughter. The total fatty acids were then extracted individually from these samples.

Plasma fatty acid determinations

The total fatty acids were extracted from the plasma samples (n=33) using a chloroform-methanol (2:1, v/v) solvent system according to a modified Folch method (Rajion, 1985). The fatty acids were transmethylated to fatty acid methyl esters (FAME) using 14 % methanolic boron trifluoride, and separated on a Supelco SP™-2330 fused silica capillary column (30m, 0.25mm ID, 0.20 mm film thickness, Supelco Inc., Bellefonte, PA, USA) in a 5890 Hewlett-Packard Gas-Liquid Chromatograph (Hewlett-Packard Co., Avondale, PA, USA). The injector temperature was programmed at 220 °C and the detector at 220 °C. The column temperature was set at the range of

100-190 °C with temperature programming at the rate of 5 °C/ minute increment to facilitate optimal separation. Identification of the fatty acid methyl esters was based on the comparison of the sample retention times to those of a known fatty acid methyl ester standard (Sigma Chemical Co., St Louis, MO, USA). An internal standardisation method was used to quantify the various fatty acids in the plasma, where a known concentration of heneicosanoic acid (21:0) (Sigma Chemical Co., St Louis, MO, USA) was added to each sample prior to transmethylation.

Rumen fluid pH determinations

Rumen fluid pH was determined at slaughter from all 33 animals. Prior to the slaughter, the animals were allotted randomly into 3 groups within one treatment diet based on the number of hours slaughtered after feeding. Restrictions in the daily slaughtering capacity resulted in the uneven subject numbers. Four animals from each group were slaughtered immediately (0 hours) and at 4 hours post feeding (4 hours). The remaining 3 animals from each treatment group were slaughtered 6 hours post feeding (6 hours). All animals that were fed at 4 and 6 hours before slaughter finished their regular amount of the treatment rations within 15 minutes. Collection of rumen liquor samples were done upon evisceration. Two hundred millilitres of rumen liquor were taken from each animal and strained through 4 layers of cheese cloth to remove particulate feed particles. The rumen fluid pH was then measured within 5 minutes with a Mettler-Toledo pH meter (Mettler-Toledo Ltd., England).

The plasma fatty acid data sets were analysed using a one way Analysis of Variance (ANOVA). This was to compare the effect of the three treatment diets on the animals' plasma fatty acid levels after 12 weeks of treatment (SPSS, 1998). The Duncan multiple comparison test were performed in the event of significant ANOVA findings. A non-parametric one way ANOVA (Kruskal Wallis test) was performed on the ratios of unsaturated : saturated fatty acid, polyunsaturated : saturated fatty acids and n-6 : n-3 fatty acids to evaluate the effects of treatment diets on these ratios in the sheep plasma. In the event of a significant finding, Q-statistics test will be used as a multiple comparison test (Heath, 1995).

The rumen fluid pH data sets were analysed using the two-way ANOVA procedure to elucidate the effect of treatment diets, hours post feeding and the interaction effect of treatment diets * hours post feeding on the rumen fluid pH changes. The Duncan multiple comparison procedures were then used for significant ANOVA findings (SPSS, 1998).

RESULTS

After 12 weeks of treatments, it was found that oleic acid (18:1, $P<0.05$), and linoleic acid (18:2 n-6, $P<0.01$) were significantly higher in the C80 group (Table 1). However, the plasma linolenic acid concentration was highest ($P<0.01$) in the C20 groups. Apart from erucic acid (22:1) which was shown to be significantly lower ($P<0.01$) in the C50 group, all the other fatty acid levels in the comparison were quite similar in terms of amount across the three treatment groups. Summarising these results, all three treatment groups showed significantly different levels of plasma polyunsaturated fatty acids (PUFA) n-6 ($P<0.01$) and PUFA n-3 ($P<0.01$). The C80 group had the highest circulating PUFA n-6 levels at 30.5 ± 1.5 mg/100mL. This value was also significantly different ($P<0.01$) from the plasma levels of PUFA n-6 in both the C50 and the C20 groups. The plasma PUFA n-3 levels of the C20 group were highest at 1.9 ± 0.1 mg/100mL. This was significantly higher ($P<0.01$) than the C80 group. The fatty acid profile of the HAF group remained between that of the C20 and C80 groups for both PUFA n-6 and PUFA n-3 values. The ratio of total unsaturated fatty acid (monoenes, PUFA n-3 and PUFA n-6) to total saturated fatty acid contents in the plasma (1.70) for animals in the C80 group was significantly the highest ($P<0.01$) when compared to the other groups. This was supported by the fact that the C80 group also had the highest ($P<0.01$) amount of total plasma unsaturated fatty acids in contrast to the C50 and C20 groups. However, total plasma saturated fatty acids were similar across all treatment groups. The polyunsaturated : saturated (P:S) ratio was also the highest ($P<0.05$) in the C80 group, while the values for both the C50 and C20 groups were similar.

The rumen fluid pH in the C20 group was significantly higher ($P<0.01$) than both C50 and C80 groups at all hours post-feeding (Table 2). When sampling time was taken into account, rumen fluid pH at 0 hours was significantly higher ($P<0.01$) than both 4 and 6 hours post-feeding for all treatment groups.

DISCUSSION

Diets containing a large proportion of concentrate are generally given to ruminants when high performances are expected. The inherent problem with the feeding of concentrate is that the rumen pH may be depressed to values below 6 which is known to inhibit the cellulolytic microflora, thus reducing the digestion and intake of cellulosic feeds (Istasse *et al.*, 1986). The rumen fluid pH values obtained were in agreement with those reported by Islam (1999). It was noted that the C80 group which had the highest amount of total plasma unsaturated fatty acids also had the lowest rumen fluid pH. Taking the critical pH for effective fibre digestion to be between pH 6.0 – 7.0 (Orskov and Ryle, 1990), it appears that cellulolysis was severely impaired in the rumen of the animals in the C80 group. This may explain to some extent why the plasma concentration of PUFA n-3 in these animals was low as these fatty acids are found predominantly in the leaves of plants (Gurr and Harwood, 1991). In addition, only 20 % of the diets of the C80 group were made up of oil palm frond pellets.

Table 1. Sheep plasma fatty acid concentration (mg/100mL) after 12 weeks of treatment (Mean ± Standard Error of Mean)

Fatty acids	Treatment diets		
	C20 (n=11)	C50 (n=11)	C80 (n=11)
Palmitic (16:0)^{ns}	19.6 ± 1.4	18.1 ± 1.1	20.9 ± 1.2
Palmitoleic (16:1)^{ns}	8.0 ± 0.4	7.1 ± 0.4	7.6 ± 0.7
Stearic (18:0)^{ns}	23.3 ± 1.8	25.0 ± 1.4	21.7 ± 1.7
Oleic (18:1) *	32.8 ± 2.3^{a}	31.7 ± 1.2^{a}	37.7 ± 1.4^{b}
Linoleic (18:2 n-6) **	22.5 ± 1.3^{a}	24.0 ± 1.5^{a}	30.5 ± 1.5^{b}
Linolenic (18:3 n-3) **	1.9 ± 0.1^{a}	1.6 ± 0.1^{a}	0.80 ± 0.03^{b}
Arachidic (20:0)^{ns}	3.5 ± 0.6	3.3 ± 0.2	4.0 ± 0.3
Eicosaenoic (20:1)^{ns}	5.5 ± 0.3	5.2 ± 0.3	6.0 ± 0.4
Behenic (22:0)^{ns}	3.9 ± 0.2	3.8 ± 0.3	4.3 ± 0.4
Erucic (22:1) **	6.3 ± 0.4^{a}	2.5 ± 0.3^{b}	4.8 ± 0.4^{c}
Total Saturated^{ns}	50.3 ± 3.9	50.1 ± 2.8	50.4 ± 2.9
Total Unsaturated **	71.3 ± 2.8^{a}	69.4 ± 3.7^{a}	87.4 ± 3.1^{b}
Total PUFA n-3 **	1.9 ± 0.1^{a}	1.6 ± 0.1^{a}	0.80 ± 0.03^{b}
Total PUFA n-6 **	22.5 ± 1.3^{a}	24.0 ± 1.5^{a}	30.5 ± 1.5^{b}
Ratio P : S *	0.51^{a}	0.52^{a}	0.68^{b}
Ratio U : S **	1.42^{a}	1.40^{a}	1.70^{b}

Ratio P:S is the ratio of total polyunsaturated fatty acids to total saturated fatty acids
Ratio U:S is the ratio of total unsaturated fatty acids to total saturated fatty acids
^{ns} Not significantly different
** Values with different superscripts in a row differ significantly at P<0.01
* Values with different superscripts in a row differ significantly at P<0.05

Table 2. Rumen pH changes in sheep designated hours post-feeding (Mean ± Standard Error of Mean)

Treatment diets	Hours post-feeding		
	0 hours^{ns}	4 hours	6 hours
C20	6.80 ± 0.06^{a} (n=4)	6.25 ± 0.01^{b} (n=4) x	6.50 ± 0.07^{c} (n=3) x
C50	6.65 ± 0.01^{a} (n=4)	5.91 ± 0.05^{b} (n=4) y	5.82 ± 0.03^{b} (n=3) y
C80	6.44 ± 0.18^{a} (n=4)	5.77 ± 0.05^{b} (n=4) z	5.69 ± 0.02^{b} (n=3) y

^{a,b,c} Means with different superscripts within row differed significantly at P<0.05 due to sampling time
^{x,y,z} Means with different superscripts within column differed significantly at P<0.05 due to treatment diets
^{ns}Not significantly different at P<0.05
Interaction effect (treatment diets * hours post-feeding) was not significant.

The significantly higher plasma PUFA n-6 and oleic acid (18:1) in these animals suggest that more of these fatty acids were made available in the hind gut for uptake. Generally it is believed that the reduced rumen microbial biohydrogenation of these unsaturated fatty acids contributed to their availability (Gurr and Harwood, 1991; Jenkins and Thies, 1997). It is known that in the rumen, hydrogenation is almost complete for free linolenic acid (18:3 n-3), and between 60 - 95 % for free linoleic acid (18:2 n-6). This proportion decreases when the level of concentrates increase in the diet (Doreau and Ferlay, 1994). The PUFA n-6 is known to be abundant in concentrate feedstuffs which comprised mainly of soya bean meal, maize and various other oil seeds (Gurr and Harwood, 1991). The extremely low rumen fluid pH in the C80 animals may have altered the rumen microbial flora (Orskov and Ryle, 1990) and effectively limiting biohydrogenation of these dietary unsaturated fatty acids (Goh *et al.*, 1999). Changes in plasma stearic acid (18:0) and oleic acid (18:1) are more difficult to interpret since they are also regulated by the action of the animals' tissue desaturases (Jenkins and Thies, 1997).

These results clearly provided the evidence of the increased levels of plasma unsaturated fatty acids due to the decreased rumen fluid pH. The decreased rumen fluid pH could have reduced the ruminal activities including biohydrogenation in the rumen. Therefore, this approach through dietary manipulation represents a useful tool to produce mutton with a higher unsaturated fatty acid content.

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RINGKASAN

PERUBAHAN PROFIL PH BENDALIR RUMEN DAN ASID LEMAK PLASMA PADA BEBIRI YANG DIBERI MAKAN KONSENTRAT DAN PELET DEDAUN KELAPA SAWIT PADA ARAS YANG BERBEZA

Satu kajian telah dijalankan untuk menganalisis perkaitan di antara pH cecair rumen dan perubahan dalam kandungan asid lemak bebas dalam plasma bebiri akibat manipulasi pemakanan. Tiga puluh tiga ekor bebiri jantan Malin x Barbados Black Belly diberi makan sama ada, 20 % pelet dedaun kelapa sawit + 80 % konsentrat (kumpulan C80), 50 % pelet dedaun kelapa sawit + 50 % konsentrat (kumpulan C50) atau 80 % pelet dedaun kelapa sawit + 20 % konsentrat (kumpulan C20) selama 12 minggu. Kandungan asid lemak dalam plasma bebiri ditentukan di akhir tempoh kajian seiring dengan waktu pH cecair rumen diukur dalam bebiri yang sama. Kumpulan C80 yang menunjukkan pH cecair rumen yang terendah ($P<0.01$) pada 0, 4 dan 6 jam selepas makan, juga menunjukkan nilai tertinggi bagi kandungan asid lemak poliunsaturat n-6 dalam plasma ($P<0.01$) serta nisbah jumlah asid lemak tak tepu : jumlah asid lemak tepu ($P<0.01$). Bagaimanapun, kumpulan C20 mempunyai kandungan asid lemak poliunsaturat n-3 yang tertinggi ($P<0.05$) dan pH cecair rumen yang tertinggi ($P<0.01$) pada 0, 4 dan 6 jam selepas makan. Keputusan ini menunjukkan kecenderungan peningkatan kandungan asid lemak tak tepu dalam plasma akibat perubahan pH cecair rumen. Adalah disimpulkan bahawa manipulasi pemakanan telah mencetuskan perubahan aktiviti rumen ke tahap yang menggalakkan pengayaan asid lemak tak tepu dalam plasma kumpulan C80.