

## The effect of supplemental biotin in dairy cow diets on fibre fermentation patterns as measured by *in vitro* gas production

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### Abstract

Six ruminally-cannulated cows were assigned to a 2 x 3 changeover experiment with two periods to determine the effect of supplemental biotin on *in vitro* fibre fermentation kinetics. All the cows received the same basal diet but with different biotin concentrations. Treatments were no biotin supplementation (Control) or biotin mixed into the feed to provide 40 mg biotin/cow/day (Biotin). In each period, three cows received the Control and three cows the Biotin treatment. After an adaptation period of 21 days, rumen liquor was extracted and used as inoculum in gas production trials with lucerne hay, oat hay and wheat straw as substrates. Gas production was measured after 12, 18, 24, 30 and 48 hours fermentation. For all three substrates, biotin supplementation resulted in a significant increase in gas production rate during the early hours of fermentation (0-12 hours), but not at the subsequent times. It was concluded that biotin supplementation appears to increase initial fermentation rate, which may suggest an increased rate of NDF degradation in biotin-supplemented cows.

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**Key words:** Dairy cows, nutrition, biotin, fibre fermentation, gas production

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### Introduction

Biotin is renowned in animal nutrition for its role in maintaining and improving hoof health. In recent years, biotin supplementation to dairy cows at levels of 10 to 20 mg/day has also been associated with an improvement in milk production (Bergsten *et al.*, 1999; Zimmerly & Weiss, 2001). An increase in milk production resulting from increased feed intake may be related to improved hoof health. However, Zimmerly & Weiss (2001) observed linear milk production responses to biotin supplementation within the first week post-calving which were ascribed to metabolic effects of biotin supplementation. As far back as 1967, Milligan *et al.* found that the omission of biotin from the medium resulted in less cellulose digestion in an *in vitro* trial. However, no documented results could be found where the relationship between biotin supplementation and fibre digestion in ruminants has been rigorously investigated. A milk production study is currently being planned at the Stellenbosch University to determine whether the effect of a higher level of biotin supplementation than was used in the mentioned studies, *viz.* 40 mg/day, would still be linear or not. As a preliminary study, it was decided to choose the highest level of biotin supplementation of the anticipated milk production study to investigate the effect of biotin supplementation on ruminal fermentation kinetics of different fibrous substrates.

### Materials and Methods

Six non-lactating ruminally cannulated Holstein cows were randomly assigned to two groups in a 2 x 3 changeover experiment. All cows received oat hay *ad libitum*, and one of two concentrate feeds at a level of 4 kg/day to provide either 0 or 40 mg supplemental biotin/cow/day (Biotin supplied by DSM Nutritional Products South Africa Pty. Ltd., Johannesburg). The concentrate was provided twice daily as a top dressing at 2 kg/feeding and all the cows consumed the total quantity of concentrate fed daily. Cows received the respective treatments for 28 days before being changed over to the other treatment. All cows therefore received both treatments.

After an adaptation period of 21 days in each period, rumen liquor was sampled from each cow and transported to the laboratory in six 1.5 L thermos flasks under anaerobic conditions to be used in an *in vitro* gas production trial. Lucerne hay (205 g/kg CP, 420 g/kg NDF), oat hay (92 g/kg CP, 660 g/kg NDF) and wheat straw (57 g/kg CP, 780 g/kg NDF), representing high, medium and low quality forages, were used as substrates. An amount of  $0.5 \pm 0.01$  g of substrate was accurately weighed into a series of glass vials of known volume (116 – 120 mL). A 20 mm stirrer magnet of known volume was also added to each vial. Six

vials were used per substrate per treatment and per cow. Thirty six blank bottles (six per treatment per cow) containing no substrate were included to correct for gas production resulting from sources other than the substrate.

Forty millilitres of buffer (Goering & Van Soest, 1970), containing tryptose and a cysteine based reducing agent, was added to each vial. Vials were transferred to an incubator set at 39°C to allow the buffer to reduce. During this time rumen fluid was collected and blended individually while continually purging with CO<sub>2</sub>. Vials containing the substrate and buffer were inoculated with 10 mL of rumen fluid from the respective cows then sealed with stoppers and crimp caps. Vials were then transferred to the 39°C incubator and placed on magnetic stirring plates set to stir the vial contents for a minute every 1.5 hours. Pressure readings were taken from each bottle after 12, 18, 24, 30 and 48 hours incubation using a digital pressure gauge (SenSym ICT, Honeywell Inc., Morris N.J., USA) fitted with a 21 gauge needle. Pressure readings were converted to gas volumes and corrected for respective blank values.

Data were subjected to a one-way ANOVA with the aid of Statistica 6.1 (2003). Least square means were determined and significance was declared at  $P < 0.05$ .

## Results and Discussion

The effect of biotin supplementation to rumen liquor donor cows on hourly *in vitro* gas production rate of the different substrates is presented in Table 1.

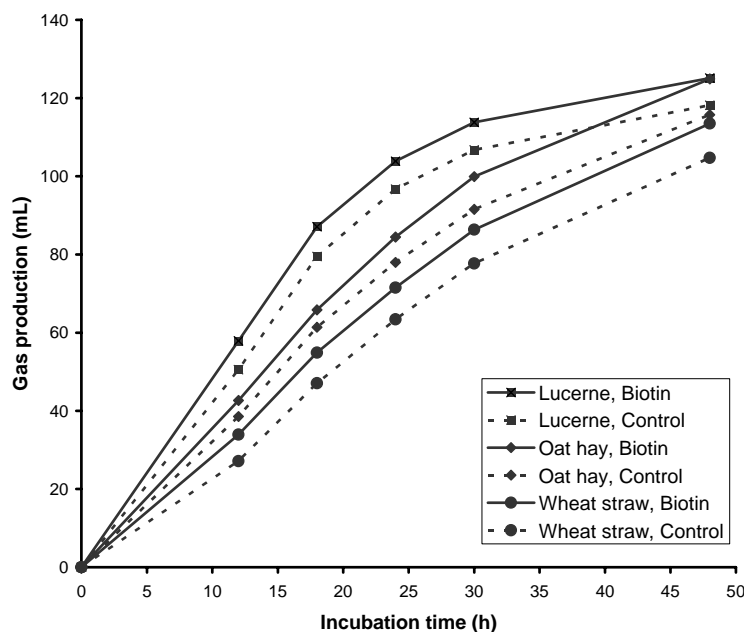
**Table 1** Rate of *in vitro* gas production (mL/h) resulting from the incubation of different forage substrates with ruminal inoculum from cows receiving either 0 mg (Control) or 40 mg (Biotin) supplemental biotin per day

Incubation time	Lucerne			Oat Hay			Wheat straw		
	Control	Biotin	P	Control	Biotin	P	Control	Biotin	P
0 - 12 h	4.21	4.83	<0.001	3.21	3.56	0.006	2.26	2.83	<0.0001
12 - 18 h	4.83	4.86	0.76	3.80	3.86	0.54	3.32	3.49	0.09
18 - 24 h	2.87	2.80	0.25	2.77	3.11	0.03	2.72	2.78	0.49
24 - 30 h	1.67	1.66	0.91	2.26	2.58	0.03	2.39	2.47	0.16
30 - 48 h	0.63	0.63	0.88	1.34	1.39	0.09	1.50	1.51	0.72

Biotin supplementation to donor cows resulted in a higher ( $P < 0.01$ ) rate of gas production in all the substrates during the early hours of fermentation compared to no supplementation. From 12 hours onwards, fermentation rate of lucerne and wheat straw appeared to be similar for the two treatments, while the rate of oat hay fermentation was higher ( $P < 0.05$ ) for the biotin treatment from 18-30 h. It is not clear why oat hay behaved differently. Biotin supplementation probably stimulated cellulolytic bacteria as they depend on other microbes for their metabolic biotin requirements (Baldwin & Allison, 1983). For all the substrates, fermentation rate decreased after 18 hours and was much reduced after 30 hours, especially for the higher quality forage (lucerne), which showed the highest initial gas production rates.

Biotin supplemented at 0, 10 or 20 mg/cow/day has resulted in a linear response in milk production (Zimmerly & Weiss, 2001). Increased initial fermentation rates due to biotin supplementation observed in the current study may be indicative of faster NDF degradation which, if true, could impact on passage rate from the rumen, DM intake and milk production. This effect could be more apparent in high yielding dairy cows on high concentrate diets as Da Costa Gomez *et al.* (1998) reported that biotin synthesis was decreased by approximately 50% when the concentrate to forage ratio increased from 17:83 to 50:50. In the current study, the rumen liquor donor cows were non-lactating and the concentrate to forage ratio was approximately 25:75.

The effect of biotin supplementation on cumulative *in vitro* gas production is presented in Figure 1. The higher initial fermentation rate of the biotin treatments is apparent. The effect of early hour differences was maintained until 48 hours because subsequent fermentation rates were either similar (lucerne and wheat straw) or higher (oat hay). Where biotin was omitted from an *in vitro* medium, Milligan *et al.* (1967) observed a reduction in fibre digestion. They postulated that the biotin deficiency caused a blockage of one or more steps in the propionate production pathway resulting in a depletion of vital intermediates, which in turn resulted in reduced rates of cellulose digestion.



**Figure 1** Cumulative gas production (mL/h) resulting from the incubation of different forage substrates with ruminal inoculum from cows receiving either 0 mg (Control) or 40 mg supplemental biotin (Biotin) per day

## Conclusion

Rumen liquor obtained from non-lactating cows supplemented with 40 mg biotin/day resulted in increased *in vitro* gas production from fermentation of lucerne hay, oat hay and wheat straw substrates. Early hour fermentation (0-12 hours) was increased, resulting in cumulative gas production effects over 48 hours. The increased initial fermentation rates due to biotin supplementation may be indicative of faster NDF degradation, which, if true, could increase passage rate from the rumen, dry matter intake and subsequently milk production. Further research is required to confirm this hypothesis.

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## References

- Baldwin, R.L. & Allison, M.J., 1983. Rumen metabolism. *J. Anim. Sci.* 57 (Suppl), 461 – 477.
- Bergsten, C., Greenough, P.R., Gay, J.M., Dobson, R.C. & Gay, C.C., 1999. A controlled field trial of the effects of biotin supplementation on milk production and hoof lesions. *J. Dairy Sci.* 82 (Suppl 1), 34.
- Da Costa Gomez, C., Masri, W., Steinberg, W. & Abel, H., 1998. Effect of varying hay/barley proportions on microbial biotin metabolism in the rumen simulating fermenter Rusitec. *Proc. Soc. Nutr. Physiol.* 7, 30 - 36.
- Goering, H.K. & Van Soest, P.J., 1970. Forage fiber analyses (Apparatus, reagents, procedures and some applications). *Agric. Handbook No. 379.* ARS-USDA, Washington, DC. USA.
- Milligan, L.P., Asplund, J.M. & Robblee, A.R., 1967. *In vitro* studies on the role of biotin in the metabolism of rumen microorganisms. *Can. J. Anim. Sci.* 47, 57–64.
- Statistica 6.1, 2003. StatSoft, Inc. 2300 East 14<sup>th</sup> Street, Tulsa, OK 74104, USA.
- Zimmerly, C.A. & Weiss, W.P., 2001. Effects of supplemental dietary biotin on performance of Holstein cows during early lactation. *J. Dairy Sci.* 84, 498–506.