

Methionine hydroxy-analogue : an efficient mean to reduce intestinal microflora activity

Y. Mercier¹, P.M. Becker², J.D. van der Klis² and P.A. Geraert^{1*}

¹ ADISSEO France SAS, 03600 Commentry, ² Wageningen UR, Animal Sciences Group, 8200AB Lelystad, E-mail: Pierre-andre.geraert@adisseo.com

Abstract

With the coming Antibiotic Growth Promoter ban scheduled next year in Europe, all possible alternatives have to be studied. The use of organic acids has frequently been considered as a possible mean to control the gut microflora. The present studies demonstrate that the methionine hydroxy-analogue (HMTBA), which can be considered as an organic acid, allows to lower the whole gut microbial activity compared to powder methionine. Moreover, HMTBA appears to induce specific metabolic pathways, which can support the antimicrobial potential.

Introduction

Antibiotic Growth Promoters (AGP) have been widely used for many decades in animal production. In Europe, AGP ban will occur in 2006 and such a ban could expand worldwide in the next 10 years. One of the key actions of antibiotics is to reduce the gut microflora, and hence, lower the competition between bacteria and host for nutrients (Dibner & Richards, 2005). Since the mid 90's, many alternative strategies have been tested in poultry (Engberg, 2004) as well as in swine (Kjeldsen, 2004). Addition of organic acids to the feeds appeared to have specific effects on some bacteria such as *Campylobacter jejuni* and *Campylobacter coli* (Chaveerach & al. 2002). Moreover, organic acid mix used as feed additive lowers poult enteritis and mortality syndrome in turkeys by decreasing the bacterial content of the gut (Roy & al. 2002). The pKa of most organic acids used as feed acidifiers is in the range of 3.5 to 4.8. The liquid methionine hydroxy-analogue (2-hydroxy-4-methylthio butanoic acid, HMTBA) has a pKa of 3.8 (Dibner, 2002). Moreover, a specific bactericidal effect of HMTBA on *Campylobacter jejuni* has already been demonstrated (Geraert & al. 2005).

The objective of this paper is to determine the effect of the source of methionine (Methionine vs HMTBA) on the global microflora activity *in vivo*.

Material and Methods

Trial 1: Male Ross broilers were fed diets deficient in methionine (control) or supplemented with 0.09 % of different additional sources (DL-methionine, DLM or DL-HMTBA, HMTBA) from 0 to 28 days of age. At the end of the growing period, birds were sacrificed and ileal and caecal digestive tract contents were collected under a CO₂ atmosphere to protect the anaerobic microflora. The microbial activities were estimated by the quantification of gas production using an *in vitro* test adapted from the "Hohenheimer Futterwerten Test" classically used to study rumen juice activity. The volume of gas produced was monitored for 24 hours under anaerobic conditions with four replicates per sample. For each intestinal medium incubated, the dry matter content was determined and used to correct the gas volume monitored.

Trial 2: Ross Broilers were fed a corn/soybean diet supplemented with DLM as additional methionine source from 0 to 21-day old. At 21 days of age, birds were killed and the total jejuno-ileal chyme was collected and 10 times diluted with buffer used for rumen fluid incubation studies. 100 ml fermentation bottles were filled with 30 ml of suspension and flushed with N₂/CO₂ (80/20). The 37°C incubation time was started by addition of 1 ml of DLM or HMTBA solution corresponding to 5 mM for DLM and 8.6 mM of HMTBA, respectively, at the start of incubation. Gas chromatography was used to identify and quantify the volatile sulphur compounds (H₂S and CH₃-SH) as described by Derikx & al. (1990).

Statistical treatments have been performed using Statview™ (abacus Concept, 1998).

Results and Discussion

Trial 1: Figure 1 shows the gas volumes obtained with ileal (Fig 1a) and caecal (Fig 1 b) media incubated for 24 hours. The gas production differs between the two media in terms of the final volume and the kinetics obtained. A lag phase is observed for all treatments in the ileal media from 0 to 4 hours.

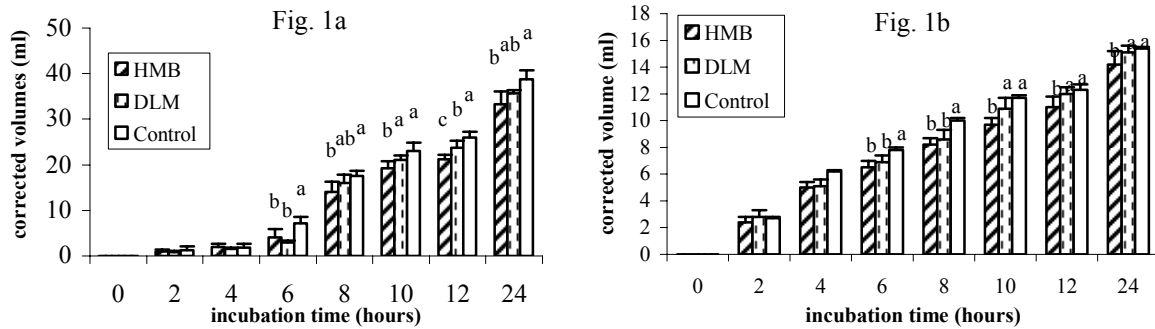


Figure 1 : Corrected gas volume (ml) production during the 24 hours incubation at 37 °C under agitation for ileal media (1a) and caecal media (1b). Bars marked with different letters at the same incubation time differ significantly (p<0.05).

After 6 hours of incubation, the fermentation started and the gas volume increased till the end of incubation. Conversely, in the caecal media no lag phase was observed and the gas production appeared linear from 2 to 24 hours of incubation. At the end of incubation time, the gas volume was higher in the ileal samples compared to caecal samples. The lag phase observed in the ileal samples could partly be explained by the lower proportion of anaerobic bacteria, compared to caecal samples (Salanitro, 1978) which needed to multiply before the gas production became visible. The differences in final gas volumes between ileal and caecal samples could be attributed to the presence of more nutrients in ileal media compared to caecal.

The *in vivo* treatments significantly affected the *in vitro* gas production. Indeed, from 8 h till the end of incubation, the amount of gas produced by the samples obtained from DL-HMTBA-fed broilers appeared significantly lower than the one obtained with broilers fed the control diet without additional methionine source. The gas production in the samples that issued from DL-methionine-fed broilers was intermediate between control and HMTBA. Similar results were obtained using the caecal samples. After 12 hours of incubation, the gas production in the samples in the DL-HMTBA fed broilers appeared significantly lower from those obtained from control or DLM fed chickens. These results indicated that the microflora activity was lower in the samples from HMTBA-fed broilers compared to DLM- or control-fed ones. The rather small numerical differences observed might be explained by the low dietary methionine supply (0.09%) and by the age of the animals (28 d).

Trial 2: Figures 2a and 2b show H₂S and methanethiol (CH₃SH) productions, respectively, in the jejuno-ileal samples supplied *in vitro* with DL-methionine or DL-HMTBA or no additional source of methionine (control). The H₂S production showed no differences between treatments, which indicated similar microbial activities in the different samples and the lack of an effect of the treatments on this gas production. In contrast to the findings with H₂S, the methanethiol production appeared significantly different between treatments. The samples supplemented with DL-methionine exhibited a higher methanethiol production: at 12 μmol DL-HMTBA/bottle, samples showed a slight methanethiol production between 20 and 28 hours of incubation, which did not exceed 2 μmol/bottle and control samples showed only a marginal CH₃SH production. These results demonstrated that methionine sources supplied in

the media led to significant differences in the production of methanethiol. The higher methanethiol production observed after addition of DLM, when compared to HMTBA (Fig. 2b), could be explained by an adaptation of the intestinal microflora to DL-methionine because of the feeding regimen, or a specific inhibiting effect of HMTBA on a part of the gut microflora, because no adverse effect of HMTBA was seen on the production of H₂S (Fig. 2a). The possible specific effect of HMTBA is in accordance with the findings of Chavez et al. (2004) who have shown, in an *in vivo* trial, a lowering effect of liquid DL HMTBA, compared to powder DL methionine, on methyl sulfur volatile compound production in excreta.

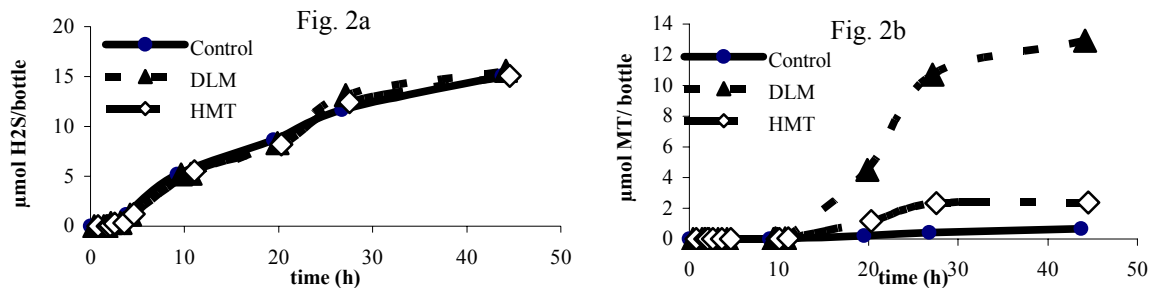


Figure 2 : Evolution of H₂S (Fig.2a) and Methanethiol (CH₃SH) (Fig.2b) concentrations in the gas phase during 37 °C incubation.

Conclusion

The results of this *in vitro* study indicate that HMTBA, compared to DL methionine, has a potential to lower a part of bacterial activity through *in vivo* or *in vitro* administration. Concluding from the results presented, it can be stated that HMTBA has the potential to contribute to the solution of the problems imposed by the AGP ban in the future in poultry.

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