The effect of silver nano-particles (Nanosilver) on *in vitro* gas production of barley grain and lucerne hay

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Introduction It is generally believed that heavy metals react with proteins by combining the thiol (-SH) groups, which leads to the inactivation of the proteins (Cho *et al.*, 2005). Silver nano-particles (Ag-NPs) are being used vastly as a strong anti-germ product. In general, Ag ions, which have antimicrobial activity, are used as an antibacterial agent. The antibacterial activity of Ag ions is inhibits intracellular enzyme activity (Cho *et al.*, 2005). Therefore, the other possibility can be considered that remaining Ag ions in Ag-NPs solution or dissolved Ag ions might affect bacterial growth. Recently, dairy farmers have tended to apply this anti-bacterial for sanitization of the environment (as a disinfectant material) of animals. However, the environmental residual effect of this anti-bacterial on ruminant microbial activity is still questionable. In the present study, a gas production technique was applied in order to determine the effect of silver nano-particles on *in vitro* gas production of barley grain and lucerne hay.

Materials and mthods The gas production technique was conducted according to Menke and Steingass (1988) procedure. Rumen fluid was taken from three sheep before the morning feeding and was passed through 4 layers of cheese cloth. Adding of CO_2 gas was continued after the rumen fluid was poured in the syringes. The applied solutions were: Main element solution (5.7 g Na_2HpO_4 + 6.2 g $KHpO_4$ + 0.6 g $MgSO_4 \times 7H_2O$) made up to 1 L with distilled water. Trace element solution (13.2 g $CaCl_2 \times 2H_2O$ + 10.0 g $MnCl_2 \times 4H_2O$ + 1g $CoCl_2 \times 6\ H_2O$ + 0.8 g $FeCl_2 \times 6H_2O$) made up to 100 ml with distilled water. Buffer solution (35 g $NaHCO_3$ + 4 g $(NH_4)HCO_3$) made up to 1 L with distilled water. Resazurin solution (100 mg resazurin) was made up to 100 ml with distilled water. Reduction solution: First 2 ml 1N-NaOH and then 285 mg $Na_2S \times 7H_2O$ were added to 47.5 ml distilled water. Solutions were mixed up in the following order: 474 ml distilled water, 0.12 ml trace element solution, 237 ml buffer solution, 237 ml main element solution and 1.22 ml resazurin solution. Samples (barley grain or lucerne hay) were milled to pass a 2 mm screen. 0.3 g of each sample was placed in gas production syringes. Then, the nanosilver solutions which were prepared in three different dilutes (40,100 and 160 ppm) were added into the syringes (1 ml per each syringe). The treatments were: lucerne hay, lucerne hay + 40 ppm nanosilver, lucerne hay + 100 ppm nanosilver, lucerne hay + 160 ppm nanosilver, barley grain and barley grain + 100 ppm nanosilver. Four replications were run per each treatment. The ratio of rumen fluid to buffer solution was 1:2 (10 ml rumen fluid and 20 ml buffer solution per each syringe). Then, the syringes were incubated in a 39 ˚C pre-heated water bath. The amount of produced gas was determined at 2, 4, 6, 8, 12, 24, 48 and 72 hours after the incubation. Data were analyzed using feed-plot program based on the model of $P= b\ (1 - e^{-ct})$, where P = amount of produced gas in time, b = gas production from fermentable fraction and c = fractional constant rate of gas production.

Results Gas production coefficients are indicated in Table 1. Adding nanosilver to the syringes had no significant effect on the amount of produced gas for either lucerne hay and barley grain.

Table1 The effect of nanosilver on gas production coefficients of lucerne hay and barley grain (mean ± SE)

Item	Gas production coefficients		
	Gas production from fermentable fraction	Fractional constant rate of gas production	Coefficient of determination (R^2)
Lucerne hay	43.6 ± 2.07	0.12 ± 0.010	0.96
Lucerne hay + nanosilver (40 ppm)	45.8 ± 5.10	0.10 ± 0.020	0.83
Lucerne hay + nanosilver (100 ppm)	43.0 ± 2.80	0.10 ± 0.010	0.95
Lucerne hay + nanosilver (160 ppm)	46.4 ± 3.60	0.10 ± 0.010	0.91
Barley grain	59.2 ± 6.60	0.05 ± 0.020	0.84
Barley grain + nanosilver (100 ppm)	77.7 ± 2.10	0.05 ± 0.005	0.98

Conclusions Results of the present study indicated that there was no inhibitory effect of nanosilver on *in vitro* gas production for either lucerne hay or barley grain. Therefore, it seems there was no effect of the nanosilver on *in vitro* microbial activity as observed from the gas production coefficients. However, further research is needed to evaluate the impact of this anti-bacterial product on *in vitro* microbial growth rate and *in vivo* conditions.

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References

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