

ATP AND DNA AS MICROBIAL PARAMETERS IN THE ALIMENTARY TRACT

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The aim of the present work was to investigate the use of ATP and DNA as parameters for microbial activity and biomass in the alimentary tract of cows and pigs. ATP was selected because of the promising results obtained in studies of other ecosystems such as the deep sea (Holm-Hansen and Booth, 1966) and activated sludge, and DNA was used because the concentration of DNA in the biomass is known to be rather constant. These parameters have been applied in five experiments performed as described below. Some of the results were published previously (Wolstrup and Jensen, 1978).

Material and Methods

ATP was determined using the luciferin-luciferase method and DNA was measured colorimetrically using the reaction with diphenylamin. The VFA production rate was measured by the *in vitro* zero time method by incubation of digesta directly in the rumen.

Experiment 1

The influence of nutrient supply on the ATP concentration in rumen microorganisms was studied in heifers fed a high level of nutrients continuously, and after 12-24 h starvation.

Experiment II :

The influence of feeding frequency on the ATP concentration in microorganisms and on VFA production rate was studied by feeding heifers 2 or 12 times per day.

Experiment III :

The influence of protein sources on the concentration of ATP and DNA was studied in heifers fed a basal diet of barley, supplemented with isonitrogenous amounts of urea, casein, soybean-protein or feather meal.

Experiment IV :

The influence of carbohydrate sources on the concentration of ATP, DNA and production rate of VFA was studied in heifers fed a basal diet of casein and barley straw, supplemented with sucrose, oat starch, citrus pulp + oat starch (1:1) or wood pulp + sucrose (1:1), amounting in all to 70 % of the total ration.

Experiment V :

The concentrations of ATP and DNA in ileum, caecum and faeces were studied in pigs fed rations with different concentrations of crude protein, crude fat, crude fibre and cellulose.

Results and Discussion

In experiment I the concentration of ATP was 55.3 ± 5.29 mg ATP/l in rumen samples from animals fed at 2 hour intervals and 26.8 ± 4.18 mg ATP/l from animals starved 12-24 h. These values were significantly different ($P < 0.001$).

The biomass was determined by total cell counts and the ration biomass/ATP was calculated. This ratio was between 1 000 and 2 000, and this correlates with results found on the basis of pure cultures. The ratios were different in the two treatments, showing that the concentration of ATP in the cells depends on the nutrient supply to the animals and hence to the microorganisms.

In experiment II a concentration of 27.7 ± 5.42 mg ATP/l was found in rumen samples from cows fed at two hour intervals and 11.7 ± 1.61 mg ATP/l in rumen samples from cows fed twice daily. These results were significantly different ($P < 0.001$). The average ratios biomass/ATP were 960 and 1975 for 12 and 2 times feeding per day, respectively. As in experiment I, the biomass was calculated on the basis of total cell counts.

The correlation coefficient between ATP concentration and the VFA production rate was 0.89 ($P < 0.001$). Both the correlation and the level of ATP in the microorganisms clearly illustrates the influence of the frequency of nutrient supply.

In the next experiment, the DNA content was also used as a marker for biomass, because the previous two experiments had shown that ATP varied according to treatment, although difficulties with extracellular DNA and with differences between the DNA concentration in bacteria and protozoa have not been solved.

The results in experiment III showed that the biomass determined by means of DNA (732-942 mg/l) or total cell counts was independent of nitrogen sources, but the concentration of ATP was significantly higher in samples from cows fed casein (62.5 mg/l), compared with urea (23.6 mg/l), soybean protein (12.3 mg/l) or feather meal (20.6 mg/l). Consequently, the ATP/DNA ratio was also significantly higher in rumen samples from cows fed casein (table 1). ATP and DNA were also determined in faeces. The concentration of ATP was between 0.64 and 0.89 mg/kg DM and the concentration of DNA between 338 and 457 mg/kg DM, giving an ATP/DNA \times

Table 1.— The ratio ATP/DNA $\times 10^{-3}$ obtained in experiments III and IV.

Feed complement	Rumen Content	Faeces
Urea	28	2
Casein	68	2
Soybean protein	15	2
Feather meal	30	2
Sucrose	8	3
Starch	16	5
Citrus pulp + starch	22	2
Wood pulp + sucrose	12	3

Table 2.— The ratio ATP/DNA $\times 10^{-3}$ obtained in experiment V and some values of pure cultures of bacteria and protozoa calculated from values in the literature.

Feeding groups	Caecum	Ileum	Faeces
Crude protein	0.9	0.9	0.2
Crude fat	0.3	0.4	0.09
Crude fibre	0.2	0.5	0.04
Cellulose	0.6	0.4	0.09
Bacteria	: 25-50		
Protozoa	: 142-285		
Bacteria + Protozoa	: 43-85		

10^{-3} ratio of 2 (table 1). No significant differences were seen between treatments, but the ATP/DNA ratio was seven to twenty times lower than that found in rumen samples. This may be caused by several factors, including the possibility that the content of less active and dormant cells may be high in faeces. In addition, the concentration of extracellular DNA may be high, indicating that the determination of biomass in faeces by the DNA method may result in an overestimation.

In the IVth experiment the concentration of ATP ranged between 7-17 mg/l and the DNA concentration was 645-876 mg/l rumen fluid. No significant differences were found between the ATP values in samples from cows fed different carbohydrate sources, but the concentration of DNA in samples from cows fed sucrose and from cows fed citrus pulp plus starch were significantly different and consequently the ATP/DNA ratio was significantly different in the same types (table 1). In this experiment, a correlation coefficient of 0.91 was found between ATP concentration and the production rate of VFA, confirming the results from experiment III.

In the Vth experiment, performed with pigs, the concentration of DNA in the caecum varied from 276 to 358 mg/l, in the ileum from 69 to 118 mg/l and in the faeces from 532 to 804 mg/kg.

The concentration of ATP in samples from the caecum was in the range of 0.09-0.26 mg/l. In samples from the ileum and faeces, the concentration of ATP in about one quarter of the samples was below the method's detection limit. In the samples with detectable amounts of ATP, the concentration in the ileum was in the range of 0.04 - 0.06 mg/l and in the faeces 0.04 - 0.16 mg/kg, and the ATP/DNA ratios were calculated on the basis of these results. Significant differences were not seen between the different feeding groups.

The ATP/DNA ratio was between 0.2 and 0.9 in samples from the ileum and caecum, and between 0.04 and 0.2 in faeces (table 2). The results show significantly lower microbial activity in the ileum than the caecum, and the results also show lower activity in the caecum of pigs than in the rumen of cows.

The ATP/DNA ratio is in the range of 25:50 when calculated on the basis of results from analysis of pure cultures of bacteria, and in a mixture of bacteria and protozoa (1:1) the ratio is 40 - 80 (table 2). The values found in rumen samples in experiment III* and IV agree

reasonably well with the theoretical ratio. The values found in samples from pigs and in faeces from cows show large deviations from the theoretical ratio. A low concentration of ATP in the cells and a high concentration of extracellular DNA may both contribute to explain these low ratios.

Conclusion

The experiments have shown, that ATP is a useful parameter for studying microbial conditions in the alimentary tract of cows and pigs, reflecting the microbial activity more than the microbial biomass. High correlations have been found between the VFA production rate and the ATP concentration in digesta from cows. DNA is a valuable parameter for determination of biomass, bearing in mind the problems with extracellular DNA and the differences between the concentration of DNA in bacteria and protozoa. The experiments have further shown that microbial activity is much more sensitive to change in feed supply than microbial biomass is.

A determination of the adenylate energy charge of the cells,

$$\frac{\text{ATP} + 1/2 \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}$$

(Chapman and Atkinson, 1977) is in progress to explain the activity level of microorganisms in the alimentary tract, particularly under extreme growth conditions.

* Previously published values from experiment III (Wolstrup and Jensen, 1978) were incorrect due to an erroneous dilution factor of 2.5.

References

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