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DISAPPEARANCE OF ETHANOL FROM ISOLATED SHEEP RUMEN

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The absorption of ethanol from the rumen was studied in three British Milk sheep equipped with a rumen cannula. After removal of the rumen content and washing the forestomachs several times the reticulo-omasal orifice was closed and through the cannula 20 or 60 ml ethanol and 2 ml Cr-EDTA were infused in physiological saline. The entire fluid volume was 3000 ml. At the start of the experiment (0 min) and subsequently in the 5th, 15th, 30th, 45th, 60th and 75th minutes samples were taken from the fluid present in the forestomachs. During the 75-min experiment the amount of ethanol gradually decreased in the rumen. The rate of disappearance varied according to concentration. The graph depicting the change of ruminal ethanol concentration shows a curve typical of passive transport. The equation describing the disappearance of ethanol was $y = -0.0474x^2 + 5.6544x + 10.869$ after the administration of 20 ml ethanol, and $y = -0.1377x^2 + 19.541x - 24.606$ after the infusion of 60 ml ethanol. It was established that ethanol was absorbed through the rumen wall by a passive transport process.

Key words: Rumen, ethanol absorption

Ethanol is usually not regarded as an important substance for domestic animals, although the amount of ethanol that can be taken up by animals, especially ruminants, is not negligible. Especially the fermented feeds are rich in ethanol. According to our earlier measurements, the ethanol concentration of corn silage amounted to 0.1 to 1.6% of the dry matter (Veresegyházy et al., 1995). Other authors (Weinberg et al., 1991) reported even higher ethanol concentrations in corn silage, corresponding to 1–4% of the dry matter content. Calculating from the ethanol concentration of fermented feeds, the daily ration of a high-producing dairy cow may contain as much as 200 to 500 g of ethanol (Durix et al., 1991). This amount may be even higher if the ration of cows is supplemented with distillery by-products (brewer's grains, molasses), which also may contain 3–3.3% ethanol on dry matter basis (Hibbs et al., 1986).

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Alcohol may also be formed *in situ* in the rumen, as an end-product of symbiontic metabolism. Ethanol is produced by certain species of rumen bacteria (*Ruminococcus albus;* Czerkawski, 1986) or fungi such as *Saccharomyces cerevisiae* (Kung et al., 1997), *Orpinomyces joyonii* (Kovar et al., 2000) and *Pyromyces communis* (Julliand et al., 1998) during their carbohydrate metabolism. The amount of ethanol formed in this way is variable and greatly depends on the composition of microflora and carbohydrate content of the ration.

A certain part of ethanol consumed by the animal will be metabolised in the rumen while the remaining part is absorbed from the intestine. Absorbed ethanol is degraded by the alcohol dehydrogenase enzyme of the liver into acetaldehyde which is then transformed into acetic acid. The ethanol involved in such transformation processes primarily supplies energy. However, numerous systemic effects of ethanol have also been described. In mature ovine fetuses ethanol diminished cerebral blood flow as well as oxygen uptake and glucose utilisation by the brain (Gleason and Hotchkiss, 1992). The intravenous infusion of ethanol was found to impair placental blood flow (Falconer, 1990). Others have reported that ethanol exerts an influence on immunosuppression (Jayasinghe et al., 1992). *In vitro* experiments have demonstrated that ethanol enhances gluconeogenesis in liver and kidney cortex slices (Ghosh and De Sarkar, 1996).

Ethanol also influences rumen fermentation: after the direct infusion of 800 ml of 47.5% ethanol the volatile fatty acid concentration in the rumen increased. Not only the concentration of the ketogenic volatile fatty acid but also that of propionic acid, a gluconeogenic volatile fatty acid, rose (from 14.3 mmol/l to 16.6 mmol/l; Emery et al., 1959). Similar observations have been made by Japanese researchers who reported that ethanol enhanced the production of volatile fatty acids and rumen gases, but among the volatile fatty acids the ratio of acetate increased at the expense of propionate (Myazaki et al., 1989).

Not only symbionts producing ethanol but also those utilising ethanol live in the rumen. In our earlier *in vitro* experiment the rumen microflora was found to be able to utilise ethanol (Veresegyházy et al., 1995). Other authors suggested that, in addition to being utilised by rumen microorganisms as an energy source, ethanol had substantial direct effects on lipid metabolism taking place in the rumen (Myazaki et al., 1989).

Mortality due to acute ethanol toxicosis has been reported in cows and calves (Hibbs et al., 1986). The animals died within 1–3 days after the consumption of distillery by-products in both cases. This suggested that ethanol was absorbed from the rumen. As no relevant data could be found in the literature, the objective of the present study was to investigate whether ethanol can be absorbed from the rumen. Another objective of the work was to study the rate of disappearance from the rumen and the type of transport.

Materials and methods

Three British Milk ewes (average body weight: 85 kg) were used in this experiment. At first a rumen fistula was prepared surgically in each animal, then the opening of the fistula was closed with a Bar Diamond 8C type cannula (P.O. Box 60, Bar Diamond Lane, Parma, ID 83660-0060). Animal care was performed according to the Hungarian Law for Care and Use of Animals, and all animal procedures were approved by the Institutional Animal Care and Use Committee at Szent István University, Faculty of Veterinary Science. The experiment was started at the 6th week after the operation, after full recovery of the animals.

At the beginning of the experiment the animals were put in a chute, and the rumen content was collected into a container through the opening of the fistula. The rumen content was kept under anaerobic conditions at 39 °C throughout the experiment. The rumen was washed with body-warm tap-water until the effluent became clear. This usually required 5–6 cycles of washing. Subsequently, the modified version of the apparatus developed by Engelhardt and Sallmann (1972) for the isolation of rumen was put in place. The principle of the modification was that the apparatus was fitted up with two inflatable rubber balloons instead of one, to hold the closing device to be placed into the reticulo-omasal orifice in position; thus, the closing device could not move in any direction during the experiment. The diameter of the closing cylinder was increased to 35 mm from the original 20 mm to ensure more stable fixation. As the part of the apparatus serving for draining off saliva could not fixed in the oesophagus, a known amount of Cr-EDTA[**] was added to the ethanol solution in order to measure liquid volume changes due to salivary secretion and fluid absorption during the experiment.

After isolation of the rumen the opening of the fistula was closed. A sampling tube was placed at the middle part of the cannula applied for the time of the experiment, next to a plastic tube that served for inflating the rubber balloon of the closing device. With a 400-ml syringe, 2800 ml physiological NaCl solution then either 20 or 60 ml absolute ethanol in 200 ml volume was infused into the rumen. This was followed by the injection of 2 ml Cr-EDTA. Theoretically the ethanol concentrations were 115.3 and 346.4 mmol × L^{-1}, respectively. Compounding of the liquid required approximately 5 min. This was followed by a sampling from the rumen (0-min sample) intended for the determination of the actual initial ethanol and Cr-EDTA concentrations. Further samples were taken at 5, 15, 30, 45, 60 and 75 min after taking the 0-min sample. The samples were

[**]Preparation of Cr-EDTA: 28.4 g $CrCl_3 \times 6\ H_2O$ + 40 g Na_2EDTA in 0.5 l volume of water was kept in water bath at 100 °C for 1 h. Excess EDTA was removed by 8 ml 1 mol/l $CaCl_2$ solution. The pH was adjusted to a value between 6 and 7 by the addition of NaOH, and the solution was made up to 1 litre.

immediately frozen to –20 °C and stored at that temperature until processed. After the last sampling, the liquid content of the rumen was removed, the rumen content replaced and the cannula closed. This process was repeated with both ethanol amounts in each animal.

The Cr content of the samples was determined by an atomic absorption method immediately after filtration (Atomic Absorption Spectrophotometer, AA-670 l F Shimadzu), using acetylene-air gas mixture with flame atomisation, at 357.9 nm (L233-24NB Hollow-Cathode lamp).

The concentration of ethanol was determined by a gas-chromatographic method (Apparatus Sigma 3B gas chromatograph; column: 4% Carbowax 20M on Carbopack B-DA (2 m); temperatures: column thermostat 58 °C, injector 200 °C and detector 260 °C).

The fluid volume and ethanol content in the rumen were calculated according to the dilution rate of Cr-EDTA and the ethanol concentration in the samples. Absorbed ethanol was considered to be the difference of the ethanol content of the sample taken at 0 min and at the other sampling times.

Results and discussion

The results are expressed as a percentage of the ethanol concentrations measured in the rumen fluid of the different animals at the first sampling. The reason for this is that widely varying ethanol concentrations were recorded even after the infusion of identical amounts of ethanol. Trying to find the reasons for this phenomenon, it has been concluded that a substantial part of ethanol evaporates from the fluid even at body temperature. However, in a closed system a dynamic balance develops between the fluid and the gas space within a few minutes after infusion. The development of this equilibrium was facilitated by the repeated mixing of the fluid with a syringe. The volume of fluid infused into the rumen was identical, but the volume of the rumen, and thus the volume of the gas space, varied markedly by animal. The difference found in the initial ethanol concentrations is attributed to this phenomenon. The difference in the amount of ethanol absorbed between the time of ethanol infusion and the first sampling also contributes to the development of dissimilar ethanol concentrations.

During the experiment, the change of fluid space until the next sampling was calculated from the dilution rate of Cr-EDTA co-administered with ethanol. In the 75-min experimental period, saliva production varied widely even in the same animal on different days of the experiment. The volume of saliva production varied between 0.215 and 0.832 litre/75 min. Naturally, this also caused a minor change in alcohol concentration during the incubation period but did not influence the amount of ethanol.

The graphic curve of ethanol absorption was of similar character irrespective of whether 20 cm^3 (Fig. 1) or 60 cm^3 (Fig. 2) absolute ethanol was used in the experiment. The absorption rate expressed in percentage was higher if the lower amount of ethanol was infused. After the administration of 20 cm^3 ethanol 81%, while after the administration of 60 cm^3 ethanol 64.2% of the initial ethanol amount disappeared from the rumen fluid. The amounts of absorbed ethanol, however, showed the opposite trend. From the lower volume of infused ethanol 16.2 cm^3, while from the higher volume 38.5 cm^3 was absorbed during 75 min. This means that after the infusion of a threefold concentration the absorbed amount increased 2.38-fold. The equations calculated from the absorption results were as follow:

$y = -0.0474x^2 + 5.6544x + 10.869$ and
$y = -0.1377x^2 + 19.541x - 24.606$

The fact that ethanol is absorbed from the closed rumen was proved by an error committed during one of the experiments. In that experiment 200 cm^3 ethanol was infused instead of 60 cm^3 into the rumen of two animals. By the 40th–45th minute of this experiment both animals started to exhibit the characteristic nervous signs induced by alcohol. These signs developed completely by the 75th minute, despite the fact that fluid flow from the rumen was prevented. The animals showed staggering gait and were unsteady on their hind legs. After they had been taken back into their cage, they fell into a deep sleep. This error made it clear that the ethanol which disappeared from the rumen fluid during the experiments became absorbed through the rumen wall.

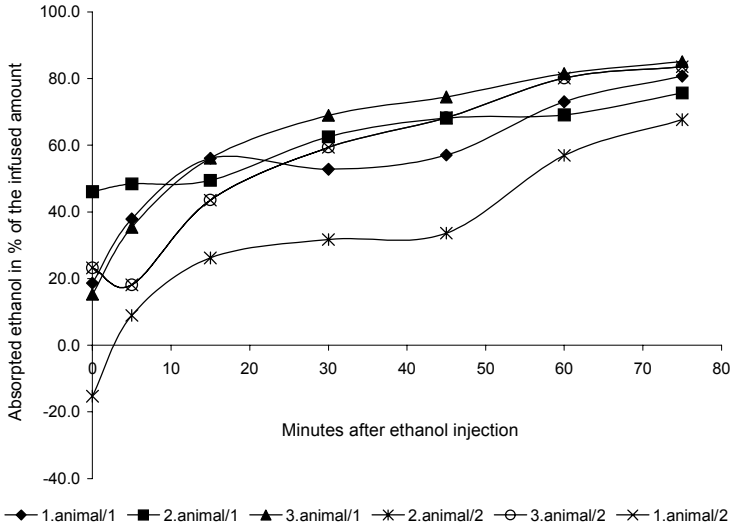

Fig. 1. Ethanol absorption from washed, isolated sheep rumen after infusing 20 cm^3 concentrated ethanol

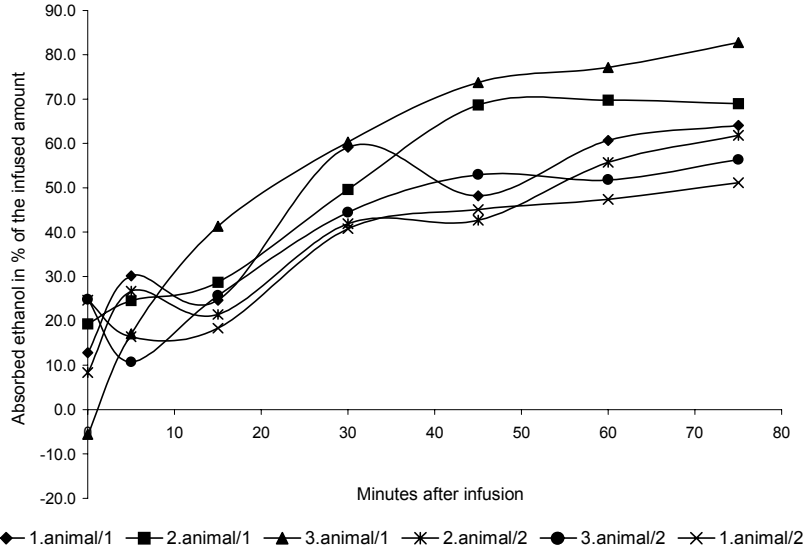

Fig. 2. Ethanol absorption from washed, isolated sheep rumen after infusing 60 cm^3 concentrated ethanol

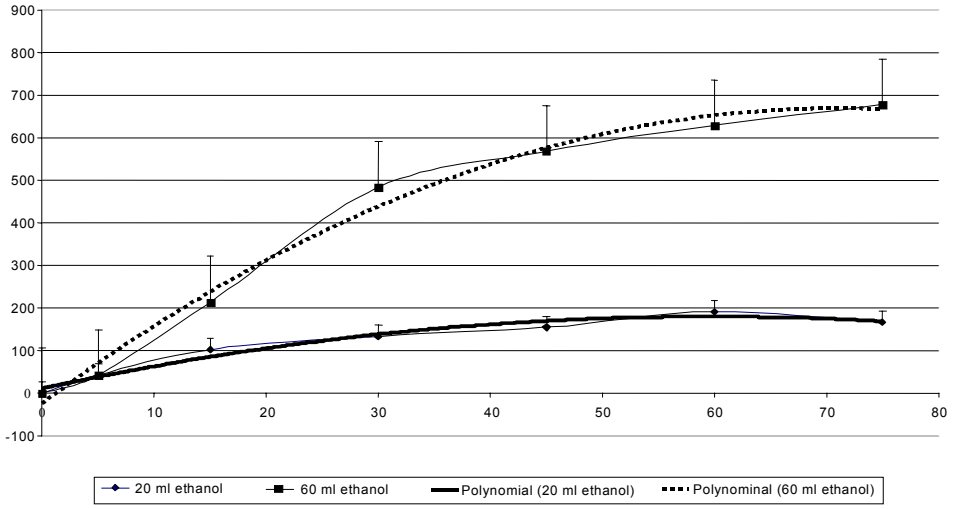

Fig. 3. Average ethanol absorption (mmol) after infusing 20 or 60 cm^3 absolute ethanol into the washed, isolated sheep rumen

The curve depicting this absorption process levels out, indicating that the rate of absorption decreases parallel to the fall of ethanol concentration.

Conclusions

From the results of the present experiments it can be concluded that ethanol is absorbed from the rumen. The rate of absorption is directly proportional to the ethanol concentration in the rumen. This fact and the type of the absorption curve suggest that ethanol is probably absorbed from the rumen by a passive transport process.

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