

## Kinetics of ethanol metabolism in sheep

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(Received 23 October 1991; accepted 15 January 1992)

**Summary** — Kinetic aspects of ethanol metabolism were studied in sheep after intravenous or intraruminal infusion of ethanol.  $V_{max}$  and  $K_m$  in fed animals were respectively  $295 \pm 10 \text{ mg}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$  (l = litre of body water) and  $32.1 \pm 2.4 \text{ mg}\cdot\text{l}^{-1}$ . Elimination half-life was  $1.47 \pm 0.26 \text{ h}$ . The corresponding values in the fasted animal were not significantly different. During venous infusion an increase in plasma acetate, inversely correlated to plasma ethanol, was observed. No modification in glycemia occurred. Intraruminal infusion of ethanol increased the concentration of all SCFA in the rumen juice, the largest part of this modification being relative to acetate. Repetition of the infusion over a period of 11 consecutive days increased the number of SCFA in the rumen, indicating microflora adaptation to ethanol utilization. Taking into account the range of ethanol concentrations found in silage ( $10\text{--}50 \text{ g}\cdot\text{kg}^{-1} \text{ BW}$ ) we can consider that ethanol is readily metabolized simultaneously by the rumen microflora and the enzymatic system of the host. With a corresponding daily intake of ethanol ( $0.2\text{--}1 \text{ g}\cdot\text{kg}^{-1} \text{ BW}$ ) both systems are not saturated and plasma ethanol level always remains below  $0.25 \text{ g}\cdot\text{l}^{-1}$ .

ethanol / kinetics metabolism / sheep / ruminants

**Résumé** — **Cinétique du métabolisme de l'éthanol chez le mouton.** On a étudié chez le mouton les aspects cinétiques du métabolisme de l'éthanol soit en administrant des bolus intraveineux d'éthanol soit en réalisant des perfusions intraruminales. Les paramètres cinétiques,  $V_{max}$ ,  $K_m$  et demi-vie de l'éthanol sont respectivement de  $295 \pm 10 \text{ mg}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$  (l = litre de liquide corporel),  $32,1 \text{ mg}\cdot\text{l}^{-1} \pm 2,4 \text{ mg}\cdot\text{l}^{-1}$  et  $1,47 \pm 0,19 \text{ h}$  chez le mouton alimenté. Ils ne diffèrent pas significativement chez le mouton à jeun. L'acétate plasmatique est inversement corrélé à l'alcoolémie après administration d'un bolus d'éthanol. La glycémie n'est pas modifiée. Des perfusions intraruminales d'éthanol augmentent le taux de tous les acides gras à chaîne courte dans le jus de rumen, la variation maximale portant sur l'acétate. La répétition des perfusions pendant 11 jours consécutifs induit une adaptation de la microflore se traduisant par une augmentation de la transformation intraruminaire d'éthanol. L'éthanol contenu dans les ensilages dans la limite des concentrations observées ( $10\text{--}50 \text{ g}\cdot\text{kg}^{-1} \text{ MS}$ ) est métabolisé à la fois par la microflore du rumen et le système enzymatique du mouton. Les ingestions d'éthanol correspondantes ( $0,2\text{--}1 \text{ g}\cdot\text{kg}^{-1} \text{ PV}$ ) ne saturent pas les deux systèmes. Les concentrations maximales d'éthanol dans le plasma restent dans tous les cas inférieures à  $0,25 \text{ g}\cdot\text{l}^{-1}$ .

éthanol / cinétique / métabolisme / mouton / ruminant

## INTRODUCTION

Ethanol is often produced in significant amounts during conservation by fermentation of forages or by-products. A wide range of concentrations from 0 to 50 g·kg<sup>-1</sup> DM can be found in grass and maize silages. Therefore a lactating ewe ingesting daily 1.5 kg DM of silage could ingest up to 75 g of ethanol·d<sup>-1</sup>. A similar estimate for a 600 kg lactating cow would result in daily ethanol ingestion of up to 600 g.

In ruminants, contrary to man and monogastric animals, a significant proportion of any ingested ethanol can be transformed into short chain fatty acids (SCFA) by rumen bacteria (Pradhan and Hemken, 1970; Czerkawski and Breckenridge, 1972; Durix *et al*, 1991), but this transformation is limited, and as the amount of ethanol in the diet increases, the proportion of ethanol metabolized by the tissues of the ruminant becomes dominant.

Ethanol can contribute up to 10% of the energy requirements, but may be a detrimental factor for the liver, especially in high-yield dairy cows during the peripartum period.

To estimate the above possibility, it is important to have sufficient knowledge of the kinetics of alcohol metabolism in ruminants. With the exception of the data of Ørskov *et al* (1967) who reported blood concentrations of ethanol in the range of 0.13–0.27 g·l<sup>-1</sup> after a continuous rumen infusion of a daily quantity of 875 g of ethanol in lactating cows, no other data are available on the subject.

In the present study, the kinetics of ethanol metabolism was first studied by using an intravenous administration of an aliquot of ethanol to circumvent the microbial metabolism in the rumen, then by continuous infusions of ethanol into the rumen to determine the total transformation of ethanol by the digestive tract and by the tissue of sheep.

## MATERIALS AND METHODS

### *Animal and experimental design*

#### **Intravenous ethanol injection**

Three sheep weighing respectively 40, 50 and 55 kg were used. They were fitted with a permanent intravascular catheter. An aliquot of ethanol (0.4 g·k<sup>-1</sup> BW) diluted in a 0.9% sodium chloride was rapidly injected (1 min) into a jugular vein. Blood samples were collected over a 6-h period after ethanol administration, every 5 min during the first hour then every 15 min during the 90 following min, and finally every 30 min, for determination of ethanol, acetate, lactate and glucose. For each animal, intravenous infusion of ethanol was repeated 4 times, twice with sheep fasted for 24 h and twice with sheep fed good hay (31.87% crude fiber, 12.87% crude protein, 11.60% ash, 0.55 kg digestible organic matter per kg), 150 g per h for 8 consecutive hours. Intravenous infusions were repeated every 7 days; in fed animals, they were performed between the 2 first meals. Blood samples were collected on heparin, kept in ice, centrifuged at 1 500 g and plasma was stored at -30 °C until assayed.

#### **Continuous ruminal infusions**

Two other sheep weighing 50 and 37 kg were equipped with permanent rumen cannula. In the first experiment 1 sheep (50 kg BW) received a 4-h intraruminal infusion of 20 g of ethanol (0.4 g·kg<sup>-1</sup> BW). The amount of ethanol and the duration of infusion were chosen to correspond respectively to the average quantity of ethanol ingested with ethanol containing silages and the average time of ingestion of this silage (4 h). Rumen samples were collected every hour from the beginning of the infusion, over an 8-h period and stored at -30 °C for ethanol and SCFA determination. This protocol was repeated on 11 consecutive days to test a possible adaptation of rumen microflora. A control period without ethanol was performed using the same animal under the same experimental conditions.

In the second experiment, the other sheep (37 kg) received various intraruminal ethanol in-

fusions (total doses administered 0.4 g, 0.9 g, 1.8 g·kg<sup>-1</sup> BW in order to test the dose-response effect on rumen and plasma ethanol; the first 2 doses corresponded respectively to the average and the upper value of ethanol ingestion with a silage diet, the third dose to twice this last value.

### Analytical procedures

Plasma and rumen juice were analyzed for acetate and other SCFA by gas chromatography (Jouany, 1982), and for ethanol, lactic acid and glucose by enzymatic procedures (Trinder, 1969; Bernt and Gutmann, 1974; Gutmann and Wahlefeld, 1974) respectively.

### Modeling of kinetic curves and statistical analysis

Individual time curves of plasma ethanol after an ethanol injection were analyzed with the SIPHAR computer programme (Gomeni and Gomeni, 1987). Double modelling of the non-linear parts of the curves was used, first according to Michaelis-Menten kinetics to determine  $V_{max}$  and  $K_m$  and then according to classical exponential kinetics to determine the half-life.

For ethanol and acetate, areas under the curves (AUC) were determined and a linear regression between both groups of AUC was established with the SIPHAR programme. Data concerning SCFAs in the rumen during the control and experimental periods were submitted to a 2-factor variance analysis (time of sampling after the beginning of the perfusion and days of perfusion).

## RESULTS

### Kinetics of intravenous administration of ethanol

Experimental results are shown in figure 1. There was no significant difference be-

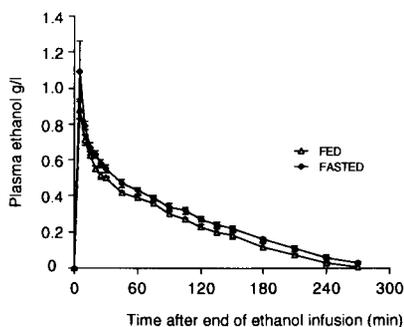


Fig 1. Plasma ethanol concentration time curves after an intravenous injection of ethanol (0.40 g·kg<sup>-1</sup> BW) in fed ( $\Delta$ ) or fasted ( $\blacklozenge$ ) sheep (mean  $\pm$  SEM,  $n = 6$ ).

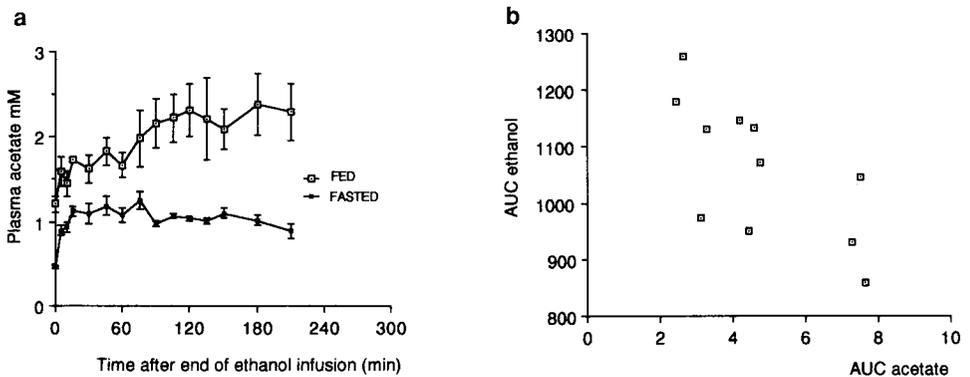
tween concentration time curves of fed and fasted animals. The non-linear portions of the curves could be related to Michaelis-Menten kinetics with a correlation coefficient of 0.99. Table I indicates the  $V_{max}$  and  $K_m$  values for fasted and fed animals. No significant differences could be seen between fed and fasted animals. From the experimental  $V_{max}$  values, an upper and lower estimation of  $V_{max}$  in relation to the body weight was calculated on the basis that the empty body of sheep contains 65–70% water, and the volume of the digestive tract constitutes 10–20% of the body weight. This estimation was in agreement with the volume of distribution calculated by the computer programme. No difference between half life of ethanol in fed or fasted sheep was shown.

### Evolution of plasma parameters after an ethanol injection

Plasma acetate increased in both fasted and fed sheep after the intravenous infusion of ethanol (fig 2a) and its level was inversely correlated to ethanol plasma concentration (fig 2b) ( $r = -0.69$ ). Plasma

**Table 1.** Kinetic parameters of ethanol metabolism in sheep after an intravenous ethanol infusion (0.40 g•kg<sup>-1</sup> BW).

	<i>Fed sheep</i>	<i>Fasted sheep</i>
$K_m$ (mg•l <sup>-1</sup> ) (l: litre of body water)	32.1 ± 1.9	31.9 ± 2.4
$V_{max}$ (mg•h <sup>-1</sup> •l <sup>-1</sup> )	295 ± 10	292 ± 15
$V_{max}$ estimated (mg•h <sup>-1</sup> •kg <sup>-1</sup> BW)		
Upper limit	186	184
Lower limit	153	152
Half-life	1.47 ± 0.26	1.48 ± 0.19

**Fig 2.** Plasma acetate concentration time curves after an intravenous injection of ethanol (0.40 g•kg<sup>-1</sup> BW) in fed and fasted sheep (a) (mean ± SEM, *n* = 6,3 sheep, 2 duplicates), and correlation between plasma acetate and ethanol AUC (area under the curve, cm<sup>2</sup>•h<sup>-1</sup>) (b).

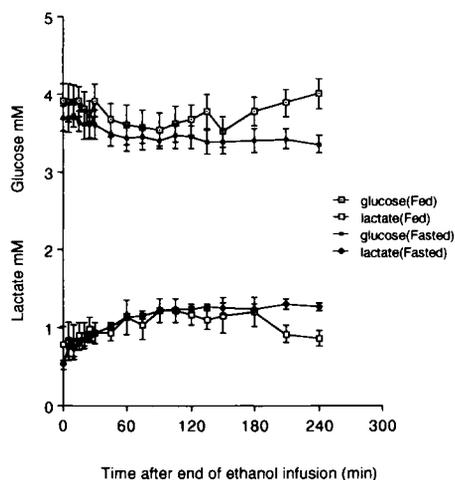
lactate increased and glucose decreased slightly after the ethanol injection (fig 3).

### ***Intraruminal infusion of ethanol***

#### **Rumen modifications**

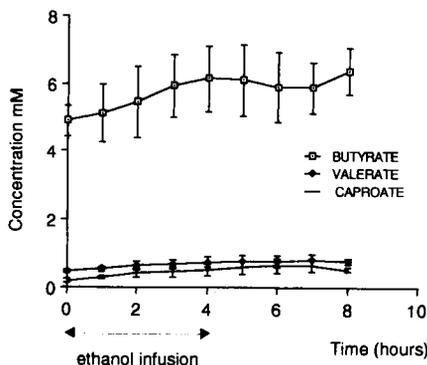
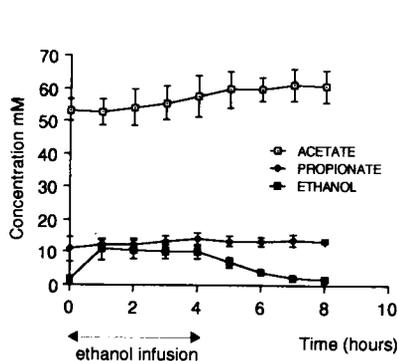
Figure 4a and 4b shows the mean daily variations of ethanol and SCFA concentra-

tions in the rumen juice following a 4-h intraruminal infusion (0.1 g•h<sup>-1</sup>•kg<sup>-1</sup> BW) of ethanol, repeated on 11 consecutive days. Concentrations of all SCAFs increased in the rumen during ethanol infusion, and for acetate during a 2-h period after the end of the infusion. These variations were highly significant (*P* < 0.001). In comparison, non-significant variations were observed during the control period.



**Fig 3.** Plasma glucose and lactate concentration time curves after an intravenous injection of ethanol ( $0.40 \text{ g}\cdot\text{kg}^{-1} \text{ BW}$ ) in fed and fasted sheep (mean  $\pm$  SEM,  $n = 6,3$  sheep, 2 duplicates).

Moreover, the average daily concentration of SCFAs during the experimental period was modified from the first to the 11th day of experimentation (fig 5a, 5b).



**Fig 4.** Rumen SCFA and ethanol concentration time curves during an intraruminal 4 h ethanol infusion ( $0.1 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ ) administered over 11 consecutive days in a fed sheep (mean  $\pm$  SEM,  $n = 11$ ).

All the SCFA concentrations increased with the exception of butyrate which decreased slightly ( $P < 0.001$ ). In the same manner, variations observed during the control period were not significant.

During this experiment ethanol was detected in plasma only at the 4th h of infusion ( $0.07 \pm 0.02 \text{ g}\cdot\text{l}^{-1}$ )

### Dose-response effect of rumen infusion of ethanol

Results are presented in figure 6a and b. The 4-h infusion period ( $0.1 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ ) without priming dose was not long enough for a plateau to be reached; plasma ethanol levels observed with this dose remained very low ( $< 0.1 \text{ g}\cdot\text{l}^{-1}$ ). A plateau was approximately reached with the second rate of infusion ( $0.135 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ ). The infusion of the upper dose ( $0.27 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ ) with a priming dose, led to ethanol accumulation in plasma and in rumen juice, indicating that microbial and tissue enzymatic systems utilizing ethanol were overloaded.

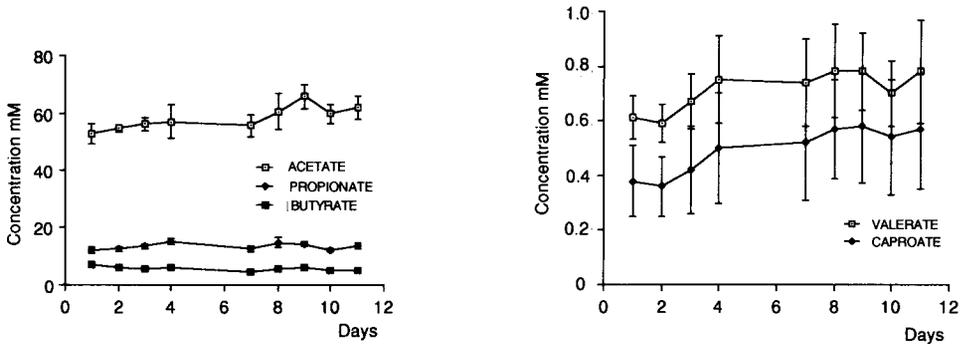


Fig 5. Effect of a 4-h intraruminal ethanol infusion ( $0.1 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ ), administered over a period of 11 consecutive days on SCFA concentration in the rumen (mean  $\pm$  SEM,  $n = 8$ ).

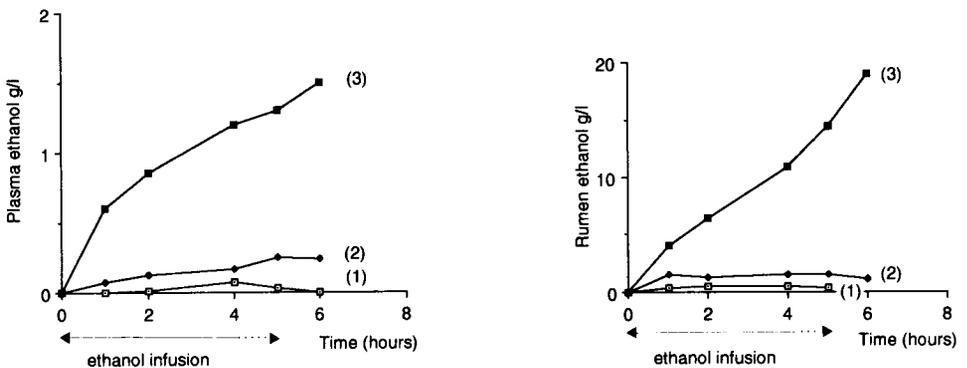


Fig 6. Concentration time curves after different intraruminal infusions of ethanol in a fed sheep: 1),  $0.1 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ ; 2), priming dose ( $0.18 \text{ g}\cdot\text{kg}^{-1} \text{ BW}$ ) + infusion  $0.135 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ ; 3), priming dose ( $0.36 \text{ g}\cdot\text{kg}^{-1} \text{ BW}$ ) + infusion  $0.270 \text{ g}\cdot\text{h}^{-1}\cdot\text{kg}^{-1} \text{ BW}$ , in plasma (a) and rumen (b), ( $n = 1$ ).

## DISCUSSION

The sheep can readily oxidize ethanol with its own endogenous alcohol dehydrogenase at a rate of  $\approx 150\text{--}180 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$ . Mean  $V_{\text{max}}$  and  $K_m$  are similar to those found in man by Wilkinson *et al* (1976) and Bruno *et al* (1983). They are slightly lower than those found by Makar and Mannering

(1970) in the rat and monkey. The plasma ethanol concentration corresponding to 95% of the  $V_{\text{max}}$  calculated from the Michaelis-Menten equation is  $\approx 0.6 \text{ g}\cdot\text{l}^{-1}$ . As with an uptake of ethanol corresponding to the highest value found in silages, plasma ethanol concentration is  $\approx 0.3 \text{ g}\cdot\text{l}^{-1}$ , it can be considered that, in practical conditions, the endogenous alcohol dehydrogenase system is not saturated.

The increase in acetate level in plasma after the intravenous administration of an ethanol dose confirms the conversion of ethanol into acetate. The release of acetate by the liver after ethanol administration is well documented in other species (Hawkins and Kalant, 1972). In ruminants, acetate issued from ethanol can increase the acetate pool produced by the microbial digestion of carbohydrates and can either be oxidized by peripheral tissues or utilized to synthesize body or milk fat (Pradhan and Hemken, 1970).

Effects of ethanol on glucose metabolism have been investigated in man and in laboratory animals. In most cases, the administration of an ethanol dose induces hyperglycemia in the post-absorptive state, and hypoglycemia in the fasted state, and gluconeogenic substrates such as lactate increase (Hawkins and Kalant, 1972). In sheep, only a slight increase in plasma lactate and a slight decrease in glucose was noted in the fed and in the fasted animals, but no hypoglycemia occurred. In ruminants fed only with hay, glucose utilized by tissues other than splanchnic tissues is totally produced by gluconeogenesis (Reynolds and Huntington, 1988). We can consider that gluconeogenesis is not affected by ethanol.

Modification of SCFA concentrations during and after ruminal infusion of ethanol indicates that a proportion of the ingested ethanol is metabolized by the rumen microflora. The most prominent modifications are the increase in acetate, valerate (C5) and caproate (C6) concentrations. These modifications have been noted *in vitro* in a previous study (Durix *et al*, 1991). The present *in vivo* assay confirms that the microflora can transform ethanol but that the effect is maximum only after an adaptation period. In non-adapted animals, rumen metabolism of ethanol is low. An important but rather difficult issue is the proportion of ethanol that is converted into SCFAs by ru-

men microflora in adapted animals. This question cannot be answered directly, but it is possible to make an upper evaluation using data from our previous *in vitro* work (Durix *et al*, 1991). In optimal conditions, the conversion of ethanol to acetate can increase SCFA production up to a maximum of 20%. During our *in vivo* assay, sheep were continuously fed with hay (150 g·h<sup>-1</sup> DM, 0.55%). Total production of SCFA from hay can be calculated from the data of Czerkawski (1986) who estimated that 7.5 mol SCFA are produced from 1 kg DM digested: SCFA (mol) = DM<sub>d</sub> (kg) × 7.5 and then 0.55 × 0.15 × 7.5 = 0.62 mol·h<sup>-1</sup>. Therefore, according to these data, the maximal rate of ethanol conversion in the rumen can be roughly estimated as 0.62 × 0.2 = 0.124 mol·h<sup>-1</sup> (5.7 g·h<sup>-1</sup>), as the largest proportion of ethanol is converted mole by mole into acetate by the microflora. This value is an upper limit obtained with ethanol concentrations in the rumen juice much higher than that observed in our *in vivo* assay. From a practical point of view, if the ethanol supply per h is under 0.1 g·h<sup>-1</sup>·kg<sup>-1</sup> BW, a substantial proportion of this alcohol can be utilized by the rumen microflora. Above this value, ethanol is necessarily oxidized by the tissue enzymatic system. Therefore, taking into account the concentrations of ethanol usually found in grass and maize silages, it can be considered that in normal conditions, both microbial and host systems for ethanol metabolism are far from being saturated. Subsequently deleterious effects of ethanol are unlikely to occur.

## REFERENCES

- Bernt E, Gutmann I (1974) Ethanol: determination with alcohol dehydrogenase and NAD. *In: Methods of Enzymatic Analysis* (Bergmeyer HU, ed) Academic Press Inc, New York, 2nd edn, vol 3, 1499-1502

- Bruno R, Lliadis A, Botta A, Mariotti B, Jullien G, Cano JP (1983) Pharmacokinetic study of ethanol after oral administration: a new approach to enzymatic elimination. *Int J Clin Pharmacol Ther Toxicol* 21, 363-369
- Czerkawski JW (1986) *An Introduction to Rumen Studies*. Pergamon Press, Oxford, UK 1st edn, 222 p
- Czerkawski JW, Breckenridge G (1972) Fermentation of various glycolytic intermediates and other compounds by rumen microorganisms with particular reference to methane production. *Br J Nutr* 27, 131-146
- Durix A, Jean-Blain C, Sallman HP, Jouany JP (1991) Use of a semi-continuous culture system (RUSITEC) to study the metabolism of ethanol in the rumen and its effects on ruminal digestion. *Can J Anim Sci* 71, 115-123
- Gomeni C, Gomeni R (1987) Integrated computer system of statistical and pharmacological data analysis. Proc Int Congr Med Informatics, Rome, 507-516
- Gutmann I, Wahlefeld AW (1974) L(+)-Lactate: determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis* (Bergmeyer HU, ed) Academic Press Inc, New York, 2nd edn, vol 3, 1464-1468
- Hawkins RD, Kalant H (1972) The metabolism of ethanol and its metabolic effects. *Pharmacol Rev* 24, 67-157
- Jouany JP (1982) Volatile fatty acids and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermenter contents. *Sci Aliment* 2, 131-144
- Makar AB, Mannering CJ (1970) Kinetics of ethanol metabolism in the intact rat and monkey. *Biochem Pharmacol* 19, 2017-2022
- Ørskov ER, Hemken RW, Moore LA (1967) Effect of ethanol infusion on milk fat composition and on volatile fatty acids in the rumen liquor. *J Dairy Sci* 50, 692-695
- Pradhan K, Hemken RW (1970) Utilization of ethanol and its effects on fatty acids patterns in ruminants. *J Dairy Sci* 53, 1739-1746
- Reynolds CK, Huntington GB (1988) Partition of portal-drained visceral net flux in beef steers. *Br J Nutr* 60, 539-551
- Trinder P (1969) Determination of blood glucose. *Ann Clin Biochem* 6, 24
- Wilkinson PK, Sedman AJ, Sakmar E, Earhart R, Weidler DJ, Wagner JG (1976) Blood ethanol concentration during and following constant rate intravenous infusion of alcohol. *Clin Pharmacol Ther* 19, 213-239