Effect of protein on abomasal secretion of acid in sheep

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1. The relationship between protein content of abomasal digesta and abomasal secretion of acid was studied in sheep fitted with re-entrant cannulas in the proximal duodenum.

2. A close relationship was found between the rate of passage of protein through the abomasum and acid secretion, both after varying the protein content of the rations supplied, and after direct introduction of proteins into the abomasum (infusion tube).

3. It is concluded that proteins stimulate abomasal secretion of acid.

Functionally, the ruminant abomasum is comparable to the simple stomach of single-stomached animals. As in the simple stomach, hydrochloric acid and pepsinogen are secreted by the fundic tubular glands. Nevertheless, some remarkable differences exist. In comparison with the simple stomach, the abomasum secretes much more regularly, also when food is not continuously available (Hill, 1955). The composition of the abomasal contents differs considerably from that of the contents of the stomach. In single-stomached animals, material entering the stomach differs little from the material ingested, but in ruminants dietary components are modified substantially by the forestomach fermentation process. In consequence, protein leaving the forestomachs consists mainly of microbial protein. Moreover, in digesta entering the abomasum, volatile fatty acids are present, even though their concentration is reduced considerably in the omasum (Von Engelhardt & Hauffe, 1975). The fairly continuous secretory activity of the abomasum is believed to be induced by the rather continuous flow of digesta from the forestomachs into the abomasum. The volume of abomasal contents has been demonstrated to be of importance for the regulation of abomasal secretory activity. In addition, the secretory activity of the abomasum is affected by the composition of the digesta entering the abomasum. In this context, volatile fatty acids, particularly, have been considered as active stimulators of abomasal secretory activity (Hill, 1960; Ash, 1961). The aim of the present experiments is to show that proteins are also involved.

MATERIALS AND METHODS

Texel wethers, weighing 45–60 kg, were fitted with hard plastic re-entrant cannulas into the proximal part of the duodenum, a short distance behind the pylorus. Care was taken that the experiments were started after the sheep had recovered and had been well accustomed to the experimental procedure.

Rations consisted of hay or grass, or of ryegrass straw and semi-synthetic components (cellulose, maize starch and soya-bean protein). Dry matter intake varied from 589 to 1047 g/d; crude protein (nitrogen × 6·25) intake from 28 to 218 g/d. Rations were supplied according to varying feeding regimens. They were offered in two, four or six equal portions

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during a period of 24 h. The types of experimental design used, according to which the various rations and feeding regimens were allocated to the sheep, are described in detail by Van Bruchem (1977). Sheep received the rations at least 10 d before sampling of duodenal digesta was started.

On sampling days, the re-entrant cannulas were disconnected. Digesta flowing from the proximal cannula were collected in vials kept at body temperature. After each 10 min period, vial contents were weighed, a 10 % sample was withdrawn, and the remainder was returned through the distal cannula. In this way duodenal digesta were sampled for 164 periods of 12 h. In total ten sheep were used. The samples obtained during each sampling period were pooled and kept at $-20°$.

A later series of experiments was carried out with sheep fitted with re-entrant cannulas in the proximal duodenum and with a silastic infusion tube into the abomasal fundus. They were maintained on rations of hay (600 g/d) and concentrates (300 g/d), supplied in six equal portions during a period of 24 h. Through the abomasal infusion tube suspensions of protein in saline (9 g sodium chloride/l) were introduced continuously into the abomasum. Different proteins were used: soya-bean protein, casein, gelatin and gluten. Infusion rates ranged from 2·12 to 5·29 g crude protein/h. Intra-abomasal infusions were started approximately 40 h in advance of the duodenal sampling period. Duodenal digesta were sampled for 142 periods of 8 h. In total seven sheep were used. Samples obtained during each sampling period were pooled and kept at $-20°$.

During the sampling periods the rate of passage of digesta in the duodenum was determined by weighing. In the digesta samples, total acid concentration was determined by titration to pH 7 with sodium hydroxide (0·1 mol/l). The N content of the digesta leaving the abomasum was estimated by the Kjehldahl method using mercuric oxide and potassium sulphate as catalysts, crude protein being calculated as $N \times 6{\cdot}25$. The rates of passage of total acid and of crude protein in the duodenum were calculated as: contents in duodenal digesta × rate of passage of digesta in the duodenum.

RESULTS

The first series of experiments, in which the composition of the ration was varied, showed that the rate of passage of total acid in the duodenum was significantly positively correlated with that of crude protein. Since both factors were calculated as the product of the content in duodenal digesta and the duodenal flow rate of digesta this relationship is partly mathematical. For this reason, the partial correlation coefficient, corrected for the flow-rate effect, was calculated: r 0·707 ($P < 0{\cdot}001$).

Fig. 1 shows the rate of passage of total acid in the duodenum *v.* rate of passage of crude protein. Despite the significant relationship, the variability was quite high not only as a consequence of differences between sheep, but probably also because the proteins supplied in the different rations varied both in amount and nature. Besides, variability in protein content of the rations also implies variability of the remaining components of the ration, which is of special interest in ruminants, since they affect the fermentation process in the forestomachs. Despite this variability, the rate of passage of total acid in the duodenum shows a clear relationship with that of crude protein. A straight line proved to fit the values best.

The results of the second series of experiments, in which the amount of protein entering the abomasum was varied by the infusion of differing amounts of different proteins into the abomasum, showed a comparable relationship. Previously (Van Bruchem, 1977), it has been found that the stimulative effect of proteins introduced directly into the abomasum on abomasal acid secretion was not determined by the buffering capacity of the protein alone.

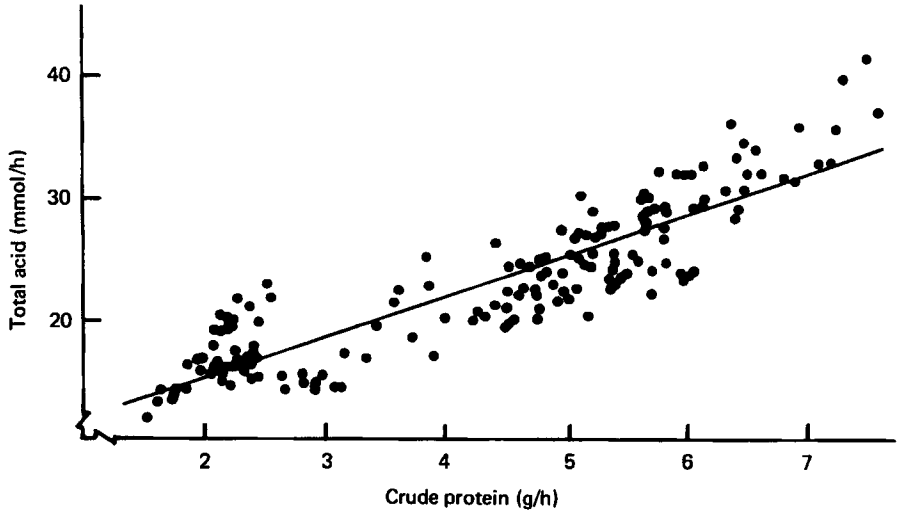

Fig. 1. Relationship between the rate of passage of total acid in the duodenum and that of crude protein after supply of different amounts of protein with the ration. Regression equation: $y = 8{\cdot}13 + 3{\cdot}42\,x$.

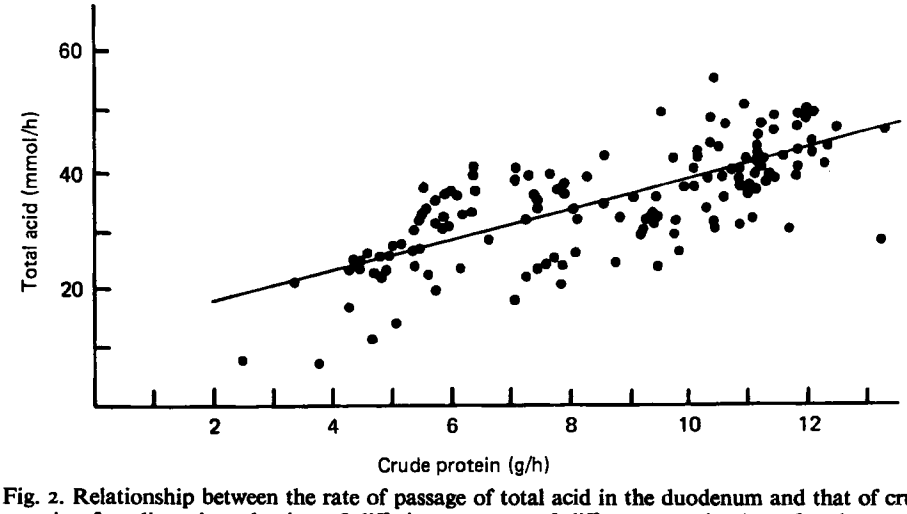

Fig. 2. Relationship between the rate of passage of total acid in the duodenum and that of crude protein after direct introduction of differing amounts of different proteins into the abomasum. Regression equation: $y = 12{\cdot}88 + 2{\cdot}49\,x$.

Hence, the nature of the protein (e.g. amino acid composition) was also thought to be involved. In this series of experiments various proteins were used, with the variability affecting more directly (no modification in the rumen) acid secretion. Probably for this reason, the results showed an even higher variability (Fig. 2), than those shown in Fig. 1. Nevertheless, a positive correlation existed between the rate of passage of total acid and that of crude protein in the duodenum. As in the previous series of experiments, the partial correlation coefficient was calculated, being corrected for the duodenal flow rate: r 0·251 ($P < 0{\cdot}005$). A straight line proved to fit the values best.

DISCUSSION

For single-stomached animals different mechanisms have been proposed to be involved in the regulation of gastric acid secretion. Regarding the nervous system, acid secretion can be stimulated via the vagus nerve, e.g. by sham feeding, or inhibited by sympathetic stimulation. Also reflexes, mediated intramurally or centrally are involved, initiated by mechano- or chemoreceptors located in the fundic or antral region. Moreover, secretion of acid is regulated by the gastrointestinal tract hormones: e.g. stimulation by gastrin released by cells present in the antral mucosa, while inhibition is by hormones released by the mucosa of the small intestine, such as secretin and cholecystokinin.

It is likely that acid secretion in the abomasum in ruminants is regulated by comparable mechanisms. That acid secretion of the abomasum is affected neurally was demonstrated, for example by McLeay & Titchen (1970), who showed that showing food to sheep causes an increase in acid secretion mediated by the vagus nerve and by Hill (1960) and Ash (1961), who found an increased abomasal secretion of acid after distension of the abomasal fundus, probably initiated by tension receptors present in the abomasal wall.

Gastrin is also involved in the regulation of abomasal acid secretion. McLeay & Titchen (1977) demonstrated that perfusion of an antral pouch with cholinergic stimulants caused an increased secretion of acid, obviously mediated by a cholinergic stimulation of gastrin release. It has also been demonstrated that an increased gastrin concentration in blood plasma coincides with an increased secretion of acid caused by infusion of a protein suspension or an inorganic buffer solution into the abomasum (Van Bruchem, 1977). Finally, Reynolds *et al.* (1978) showed that increased abomasal acid secretion in sheep postprandially was preceded by a rise in blood plasma gastrin level.

With respect to the effect of digesta composition on gastric acid secretion, special attention has been given to proteins and their degradation products. In dogs, a close positive relationship was found between gastric secretion of acid and the protein content of the diet (Saint Hilaire *et al.* 1960). This stimulative effect on acid secretion is probably mediated both neurally and hormonally. Protein breakdown products are known as active gastrin releasers. Konturek *et al.* (1976) showed that perfusion of a vagally-denervated fundic pouch in dogs with L-amino acid solutions stimulated acid secretion, probably mediated by receptors present in the fundic mucosa. This stimulative effect was found to be pH-dependent, as is the release of gastrin.

It has been proposed that in ruminants volatile fatty acids synthesized in the forestomachs are mainly responsible for the induction of abomasal secretion of acid. Volatile fatty acids are implicated because the pH of abomasal digesta is one of the determinants of acid secretion; they are buffering constituents of the digesta leaving the forestomachs and in this respect they affect the amount of acid to be secreted in order to attain the normal pH of abomasal digesta. However, irrespective of their buffering capacity a specific stimulative effect of volatile fatty acids on abomasal secretion of acid, was shown to be of minor importance after direct introduction of considerable amounts into the abomasum (Van Bruchem, 1977). This discrepancy can possibly be explained as follows. Volatile fatty acids are quite important determinants of the buffering capacity of the digesta entering the abomasum. They are not in the digesta leaving the abomasum, since they are absorbed to a considerable extent from the abomasal contents. Van Bruchem (1977) showed that of volatile fatty acids introduced into the abomasum directly, 60% or more was absorbed from the abomasal contents. In the abomasal contents proton concentration is high. Consequently absorption of the volatile fatty acids takes place easily, because they are hardly dissociated. Von Engelhardt *et al.* (1968) found that volatile fatty acids diffused easily from isolated goat abomasum. At lower pH values fractional clearance constants

were found to exceed 1/h. However, when they are absorbed in the associated form, hydrogen ions are absorbed as well. Therefore a considerable proportion of the hydrogen ions secreted by the abomasum as a result of the stimulative effect of the volatile fatty acids entering the abomasum will not reach the duodenum and are not measured by the present experimental procedure.

According to the present results, proteins are the main determinants of the amount of acid reaching the duodenum. The stimulative effect of proteins on acid secretion may vary considerably, not only because of differences in buffering capacity but also as a consequence of more specific effects (Van Bruchem, 1977). Possibly, the amino acid composition is involved. These differences can also be deduced from the variability of the values, after introduction of different proteins into the abomasum (Fig. 2). However, under physiological conditions, variability in the composition of the proteins entering the abomasum is less, since they consist mainly of microbial protein synthesized in the forestomachs. Fig. 1 shows that this microbial protein is closely related to acid secretion; for when different amounts of protein are supplied orally, different amounts of microbial protein will enter the abomasum.

With the experimental procedure adopted, it is not possible to estimate the secretion of acid in the abomasum quantitatively, partly, because the hydrogen ions will recombine with bicarbonate ions present in the digesta leaving the omasum. Assuming a flow of digesta from the omasum of 300 g/h (Ash, 1962) and a bicarbonate content of these digesta of 20 mmol/l (Von Engelhardt & Hauffe, 1975), 6 mmol hydrogen ions/h would be needed to neutralize these bicarbonate ions. Assuming a content of volatile fatty acids in these digesta of 60 mmol/l (Von Engelhardt & Hauffe, 1975) and an absorption of these volatile fatty acids from the abomasum of 0·70 (Van Bruchem, 1977), an additional 12·6 mmol hydrogen ions/h would disappear from the abomasum, because volatile fatty acids are absorbed from the abomasum in the associated form.

Some hydrogen ions may leak back from the abomasal contents into the blood stream. According to the results of Davenport (1967), a decrease of hydrogen ion concentration in unstimulated vagally denervated canine fundic pouches from 100 mmol/l to approximately 94 mmol/l was found after 30 min. In the sheep abomasum, total acid concentration is approximately 50 mmol/l (Van Bruchem, 1977). Therefore, the efflux of hydrogen ions through the abomasal wall might be less. On the other hand, mean retention time of the fluid phase of the digesta in the abomasum exceeds 30 min. Van Bruchem *et al.* (1979) found mean retention times ranging from 25 to 56 min.

It is also possible that the present experimental procedure affects the flow of digesta from the abomasum. It has been found that short-term collections of up to 24 h duration may depress intestinal flow-rate (Van 't Klooster *et al.* 1972; Ulyatt & MacRae, 1974). Our collection periods were even shorter. Therefore such flow-rate depressions were certainly involved. On the contrary, however, our collection procedure may have affected the duodenal flow-rate positively as well. Samples of 10 % were withdrawn and not replaced by donor digesta; under such conditions a slight increase in duodenal flow-rate is to be expected.

In summary, with the experimental technique applied, it is not possible to determine the amount of acid secreted in the abomasum quantitatively. Nevertheless, a strong relationship exists between the amount of acid secreted in the abomasum and the amount of acid flowing into the duodenum. And therefore, based on rates of passage of acid in the duodenum, it is still possible to draw conclusions on abomasal secretion of acid under different conditions, since our experiments were comparative.

In conclusion, the ruminant abomasum exhibits a rather continuous secretory activity of acid, as induced by the continuous flow of digesta from the forestomachs. Acid secretion is related to the buffering capacity of these digesta, and in this way volatile fatty acids are determinants of the secretion of acid. It appears that proteins are also active stimulants.

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