REGULATION OF FEED INTAKE IN SHEEP

THE ROLE OF HORMONES AND METABOLITES

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REGULATION OF FEED INTAKE IN SHEEP

THE ROLE OF HORMONES AND METABOLITES

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Proefschrift

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STELLINGEN

1. Ook herkauwers vertonen snelle post-prandiale hormonale responsen.
   (Dit proefschrift)

2. Bij het interpreteren van insuline infusie experimenten dient er rekening gehouden te worden met het rantsoen dat verstrekt wordt.
   (Dit proefschrift)

3. Een combinatie van milde (verzadigende) signalen kan tot daadwerkelijke verzadiging leiden in herkauwers.
   (Dit proefschrift)

4. Exogene toediening van CCK beïnvloedt de endogene afgifte van dit hormoon.
   (Dit proefschrift)

5. Marginale jodiumdeficiëntie tijdens de zwangerschap kan de neurale ontwikkeling van de foetus negatief beïnvloeden.
   (Proefschrift P. Versloot, 1997, Wageningen)

6. Tijdens zwangerschap is er niet alleen sprake van een ongevoeligheid voor insuline maar ook voor leptine.
   (Proefschrift A. Nieuwenhuizen, 1998, Groningen)

7. Geen enkel model is perfect, behalve een fotomodel; en zelfs die niet als je van heel dicht bij kijkt.

8. Wie achter de kudde aanloopt, stapt snel in de stront van anderen.

9. Het verstrekken van anticonceptie middelen aan AIO's zal de produktiviteit ten goede komen.

10. Vele gebruikers van het World Wide Web zijn eerder prooi dan spin.

11. Door de grote inperking van de mogelijkheden om binnen te roken blijven alleen de die-hards over.

12. De nummerherkenning van de PTT reduceert de mogelijkheden van (schoon)moeders om tijdens een sportuitzending onverwacht te bellen.

13. Het toenemend gebruik van kleurenpresentaties maakt het er voor kleurenblinden niet duidelijker op.

14. Er is geen betere stelling dan een IKEA stelling.
(Esther)

15. Het vinden van een gat in de markt is een kwestie van er op en er af komen.

16. Het zou goed zijn wanneer er op het werk evenredig veel aan God gedacht zou worden als aan het werk tijdens een kerkdienst.

Henri Leuvenink
Regulation of feed intake in sheep, the role of hormones and metabolites
Wageningen, 27 oktober 1998

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Regulation of voluntary intake

As all other animals, ruminants consume their feed in discrete meals (59). In the search for factors affecting feed intake several theories were developed. Generally these theories can be divided into two groups: those focusing on physical regulation and those focusing on physiological regulation. Theories focusing on physical regulation suggest that the capacity of the digestive tract is an important limiting factor in feeding (51, 62, 111, 114, 139). Especially when feeding bulky feed this may be true although several papers report a large flexibility in filling grade and capacity (12, 13, 150).
Since ruminants are capable of meeting their energy requirements under a wide range of circumstances and feed stuffs the concept of physiological regulation was introduced. Physiological regulation (or metabolic regulation) can be defined as feed-back signals arising from sensors (receptors) in the periphery which inform the central nervous system about the metabolic status of the individual. In the brain, presumably in the hypothalamus, these signals are integrated and decisions are made whether or not to eat (8, 57, 63, 152).
A simplified schematically overview of process of feeding and the feed back signals arising from the process of feeding and digestion is given in figure 1.
Feed that is consumed will first pass the mouth of the animal. Receptors in the

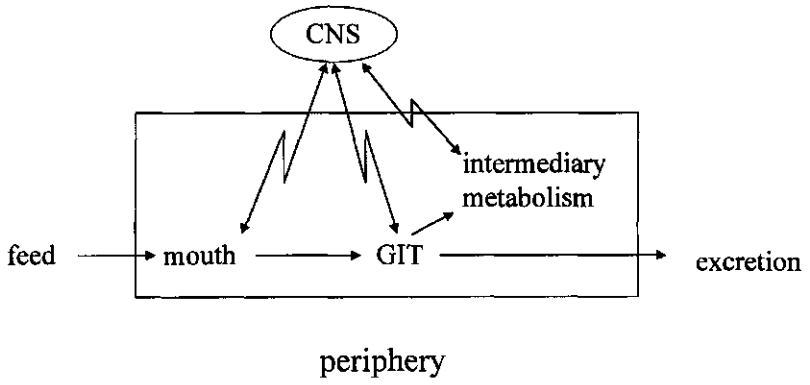

Figure 1: Schematically representation of feed intake regulation

mouth of the animal send signals about, taste, texture, temperature etc. of the feed to the CNS. A decisive action of the brain will follow whether or not to proceed with eating. Once the feed has passed the mouth, it will reach the gastrointestinal tract (GIT). Again, receptors will translate physical parameters (like pH, osmolarity) into nervous signals, which are transported to the brain. The feed is digested in the GIT and the absorbed nutrients enters the intermediary metabolism. Signals arising from digestion and metabolisation of nutrients like metabolites or hormones involved in digestion and metabolism may be transported to the brain by nerves but also by the blood circulation.

1.2 Anatomic overview of the blood circulation

Blood volume of a sheep of 75 kg approximately amounts to 6 litres. In order to ensure an adequate distribution of nutrients and other blood borne components, blood is circulating. Main propulsive organ is the heart which pumps blood towards the head of the animals and the periphery by arteries (Fig. 2). Once it has passed the peripheral tissues, blood is collected by veins and transported again to the heart. The blood enters the lung-circulation (not shown in the figure) where is oxygenated.

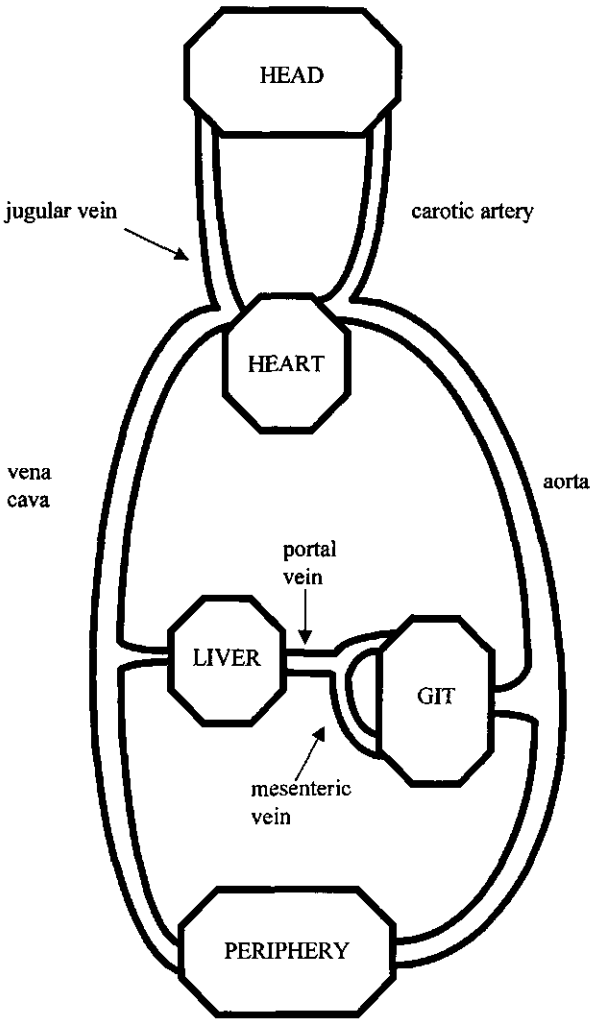

Figure 2: Schematic overview of the blood circulation

After this short loop, the blood is pumped towards the periphery again.
The GIT is also provided with blood through arteries. Veins arising from the GIT (for example mesenteric veins), do not transport the blood towards the heart directly, but

fuse to form the portal vein. This vein transports the blood through the liver to the vena cava.
Major functions of the liver include detoxification and gluconeogenesis (particularly in ruminants). The liver may act as a gatekeeper and a strategic organ for monitoring the entrance of components from the GIT. It is therefore not surprising that the liver is highly innervated with both efferent and afferent nerve fibres, which may be involved in the regulation of feed intake (2).
From a scientific point of view the portal vein is of particular interest. The portal vein collects the blood arising from the GIT containing nutrients absorbed from the GIT, and hormones and other components secreted by the pancreas, spleen and GIT. With the use of multicathetherized animals in combination with measurement of portal blood flow, it is possible to quantify net portal release of substrates or hormones. The arterio-venous difference technique is most commonly used (87). This technique involves implantation of chronic catheters in an artery and portal (or mesenteric) vein. Portal release, as an indicator for production of components by the portal-drained viscera (PDV), is calculated as the portal vein - artery difference multiplied by the portal flow (87). Although much progress is made in the techniques for measuring (portal) flow, there are still many pitfalls in measuring portal flow (79). In this thesis measurement of blood flow was not included since long-term measurement of blood flow was technically not possible. Even without the difficulties expected with the blood flow measurements, the main problem working with multicatheterized animals is catheter patency. This problem may be addressed as the major practical and possibly economical problem when experimenting with catheterised animals (74, 79). Use of inert soft material and aseptically handling will improve catheter patency and application of streptokinase can be useful too (84).

1.3 Nutrients

In the search for blood borne factors involved in feed intake regulation it seems logical to consider the possibility that the body measured the energy content of the ingested food by means of the blood concentration of the absorbed digestion products. Several theories concerning the satiating effects of digestion products were developed, amongst them the well known glucostatic theory introduced by Mayer in 1963 (100). Although these theories proved too simple, satiating effects may be observed of infusion of digestion products

1.3.1 Volatile fatty acids

1.3.1.1 Origin and utilisation

Volatile fatty acids (VFA), also called short-chain fatty acids, are the major fermentation products of the rumen flora. Ruminants are almost exclusively dependent of VFA for meeting their energy requirements (15, 144) VFA's however are also produced in the lower digestive tract of humans and all animal species (15, 25, 39, 90, 113). Intestinal fermentation in non-ruminants largely resembles fermentation in the ruminant fore-stomach (15). In the rumen, acetate (50-70%), propionate (15-25%) and butyrate (10-15%) are predominantly formed as a result of

anaerobic breakdown of carbohydrates (60). Protein degradation leads to formation of branched-chain fatty acids like iso-butyrate and iso-valerate (113).
Generally, a high concentrate diet (or fresh grass) results in high amounts of VFA in the rumen (60). Under most conditions, acetate is the predominant VFA, but propionate and butyrate are always formed in substantial amounts. Diets rich in starch favour propionate production (15, 144). Slowly fermentable feed such as low quality roughages promotes acetate production (15, 60).
Produced VFA's are rapidly absorbed from their site of production and transported by the portal vein towards the liver (15). Large quantities of VFA's (especially butyrate) are metabolised by the ruminal and intestinal epithelium resulting in production of beta-hydroxy-butyrate (15). The major part of the remaining VFA's is removed from the circulation by the liver (22). The liver removes propionate for production of glucose (22). Acetate is mainly used as a source of energy (15).
As a consequence of the metabolisation of absorbed VFA's by the rumen/intestinal wall, portal ratio of acetate: propionate: butyrate: other VFA's, differs from the ratio found in the rumen. Peripheral VFA's on their turn show a different ratio and lower concentrations as compared to portal levels due to the removal of VFA's by the liver (3, 22).
Levels of VFA's in the blood depend on the amount absorbed, the amount taken up by the portal drained viscera, the clearance by the liver and the uptake by the peripheral tissue.

1.3.1.2 Effects of feeding on blood concentrations

Since the amount of VFA formed after ingestion of a meal is dependent on the type of feed consumed, VFA levels in the blood can differ between feed. Ratios of VFA's found in the blood do not resemble those in the rumen. Absorption from the rumen increases with increasing chain length, but the amounts appearing in the venous effluent (i.e. portal vein) are in the reverse order (15, 22, 144). Portal ratios found are 80-95% (acetate), 10-25% (propionate), 1-5% (butyrate) (15, 107, 118, 120, 121). As a consequence of the removal of VFA's (especially butyrate and propionate), peripheral concentrations are much lower as compared to portal concentrations.
Feeding has been reported to increase VFA concentrations in both portal (43, 44, 46, 124) and jugular veins (43, 44, 46). Meal fed animals usually show larger fluctuations as compared to ad libitum fed animals (33, 43). It is rather surprising that most studies focused on long-term effects (hours), taken into account that a meal usually does not exceed a period of 20 minutes.

1.3.1.3 Effects on feed intake

Many studies reported feed intake reduction after infusion of VFA's in the blood stream (1, 2, 55, 56, 109) or in the rumen (5, 6, 43, 44). The majority of the studies were performed with relatively high dosages or over prolonged periods. One should therefore be careful in assigning a role as physiological regulators to VFA's. Lower dosages of VFA's infused, showed minor or no effect (43, 44, 46, 112). Factors interfering with the potential satiating effect of VFA's may include pH, osmolality and induction of hormone release (44, 46, 61, 70). Although one could argue about the validity of some studies, it is very likely that ruminal acetate concentration is involved in satiety (61).

As for the intravenous infusion of VFA's some studies provided evidence for a role in inducing satiety (1, 2, 7, 58) while others did not (43, 44, 109). Intraportal infusions of various VFA's showed that propionate is probably the only VFA which might be effective in reducing intake at a physiological range (46, 58, 112).

1.3.2 Glucose

1.3.2.1 Origin and utilisation

Due to the breakdown of carbohydrates in the rumen, only a small amount of glucose is taken up from the small intestine (22, 119, 122). As a consequence, ruminants depend on their supply of glucose mainly on gluconeogenesis from propionate and glucogenic amino acids by the liver but also the kidney may produce some glucose (22 , 122).
As in non-ruminants, glucose is the main fuel for the brain, uterus and mammary gland (22). Also other organs, which do not rely to a large extent on glucose, utilise glucose. Muscle, may account up to 20-40% of the glucose uptake. Glucose uptake by muscle is subjected to hormonal regulation and may be decreased in fasted animals or increased during exercise (21, 24, 71).

1.3.2.2 Effects of feeding on blood concentrations

Since there is little or no absorption of glucose from the GIT, one could assume that feeding does not influence glucose levels. On the other hand, large quantities of VFA's are entering the blood circulation after feeding. Reported effects of feeding on glucose levels are variable. Some researchers found a post-prandial increase (9, 11, 16, 112) while others found a decrease in glucose concentration, especially when concentrates were fed (33, 43), or no effect (93, 98).

1.3.2.3 Effects on feed intake

Intravenous infusion of glucose on feed intake is usually not effective in depressing feed intake (60). Even in non-ruminants, there is still debate on the biological value of the observed intake reducing effects of glucose (60, 134, 135).

1.4 Hormones

As a consequence of the absorption of nutrients and/or changed metabolism, blood concentrations of metabolic hormones are changing. Furthermore, the process of digestion induces changes in gastrointestinal hormones, due to mechanical or chemical stimulation of receptors in the digestive tract. Both the metabolic hormones and the gastrointestinal hormones may play a decisive role in the regulation of feed intake since they are reported to change following a meal and often a satiating effect can be observed when they are infused at relatively high rates.

1.4.1 Metabolic hormones insulin, glucagon and growth hormone

1.4.1.1 Origin and biological action

Insulin

Insulin is synthesised in the Beta cells of the Islets from Langerhans. The Islets are located in the pancreas. Main action of insulin is the regulation of the blood glucose levels in human and animals. It is therefore not surprising that in non-ruminants increased blood glucose generally evokes increased insulin release from the pancreas. Many other hormones (like glucagon, CCK, gastrin) or nutrients (like amino acids) may influence insulin secretion. Rapid changes in insulin release are mediated by the nervous system.
In ruminants, VFA's are the most important stimuli for insulin release (42, 109). Intraruminal and intravenous infusion of propionate and butyrate were shown to increase insulin levels (95, 97). Although clear effects of supra-physiological dosages were observed, the physiological relevance is doubtful. It was suggested that relative changes in VFA concentration (especially propionate) might trigger insulin release (46). As in non-ruminants, the autonomic nervous system is involved in the regulation of insulin secretion. Vagal stimulation resulted in insulin increases (23). Stimulation of sympathetic nerves resulted in lowered insulin release, possibly through α receptors (102).

Glucagon

As insulin, glucagon is produced in the pancreas. A-cells, also found in the Islets of Langerhans, produce glucagon. Stimuli of glucagon secretion in ruminants are propionate and butyrate (42, 73, 95, 125, 126, 128), but also gut hormones (96, 97) and non-branched amino acids (80). Sympathetic stimulation enhances glucagon secretion possibly through $\alpha 2$ receptors (102, 103).

Growth Hormone

As in other mammals, Growth Hormone (GH) is secreted episodically from the anterior pituitary in ruminants (130, 133, 143). Secretion of GH is regulated by two hypothalamic hormones, GH releasing hormone (GRH) which stimulates GH secretion, and somatostatin which inhibits GH secretion (27, 65, 130, 133). In sheep, GRH probably plays a primary role for dictating the pulsatile secretory pattern of GH (65). Mean pulse interval of GH may be 60 minutes. Interestingly, the reported GH pulses are not observed in peripheral plasma levels in ruminants (32, 68).
Other factors influencing GH secretion are sex steroids (19, 20, 105), CCK (50, 131), opioid peptides (143), amino acids (40, 80, 88) and glucose (37).

1.4.1.2 Effects of feeding on blood concentrations

Insulin

As in non-ruminants (135), a small increase in insulin levels is often observed immediately after meal start in ruminants (10, 45, 129, 147). This initial surge is often followed by a prolonged, nutrient induced secretion (38, 98). There is some evidence that highly degradable feeds may result in higher insulin levels as compared to less degradable feeds (38, 72, 106, 112, 145, 146, 148). Also plane of nutrition was shown to correlate with insulin levels (72, 148). Probably the increased insulin levels are rather due to prolonged half-life time of insulin than increased insulin release (141).

Glucagon

In ruminants, the effect of feeding on glucagon levels is less investigated compared to the effect of feeding on insulin levels. Enhanced glucagon levels following a meal were reported in sheep (11, 98, 127), but less evident increases were reported in goats (45, 46). There is some evidence that sheep fed at a low energy level show higher glucagon levels compared to sheep fed at a high energy level, possibly due to increased secretion rates (106).

Growth hormone

Since GH is involved in partitioning of nutrients for selected processes like growth or milk production (19, 20, 28) it is not surprising that attention was paid to the effects of feeding on GH levels. Especially the effect of plane of nutrition on GH levels is well documented. Generally, animals fed at a lower rate or with low quality feed show enhanced GH levels (35, 36, 38, 92, 137, 140). Fasting leads to even higher GH levels (38, 64, 92).
Feeding is reported to decrease GH levels in sheep (9, 10, 60, 147) but not in goats (45). Interestingly, spontaneous meals were reported to be preceded by a small peak of GH in sheep (60).

1.4.1.3 Effects on feed intake

Insulin

The possible effects of insulin on feed intake have been tested by exogenous supply of insulin. The intake response to insulin is rather complex. It was postulated that the effects of insulin on intake resemble one cycle of a sine wave (70). Insulin administration at a low rate would induce satiety while intermediate administration, resulting in insulin levels increased 10-15 times, might be ineffective. However, higher insulin levels would induce hyperphagia due to hypoglycaemia while even higher (pharmacological) levels would induce CNS disturbances.
Although attractive, the hypothesis is based on little experimental data. The observation that in sheep, low dose infusions in jugular and portal veins were effective in reducing intake (47, 48) is important.

Glucagon

Exogenous supply of glucagon resulted in reduced intake in sheep, but levels may be supra-physiological (48). While in non-ruminants glucagon may be involved in meal-size regulation, evidence in ruminants is very limited (44).

Growth Hormone

Despite the large number of papers concerning the effect of exogenous supply of GH on animal metabolism, very few experiments were performed investigating the effect of GH administration on feed intake. An attempt to evoke eating by mimicking the GH peak preceding a meal was not successful (60).

1.4.2 Gastrointestinal hormones Cholecystokinin, Pancreatic Polypeptide, Gastrin

1.4.2.1 Origin and utilisation

Cholecystokinin

Cholecystokinin (CCK) is a peptide hormone produced by two distinct cell types, namely, endocrine cells and nerve cells. Circulating CCK is mainly derived from endocrine cells (I-cells) located in the mucosa of the upper gastrointestinal tract (30). CCK consists of several different molecular forms containing the bioactive C-terminus (77). Different forms are found in all animals, but length of the CCK forms differs between species. In humans, CCK molecules containing 4, 8, 22, 33, 39 and 58 amino acids are reported (30). In ruminants, CCK-8, CCK-33, CCK-39 and CCK-58 may be present (54, 67). Biological activity of sulphated CCK is higher compared to non-sulphated CCK.
Two different subtypes of CCK-receptors can be distinguished, qualified as CCK-A and CCK-B receptors. The CCK-A receptor is characterised by its high affinity for sulphated CCK-8 compared to non-sulphated CCK or gastrin. Difference in affinity for sulphated CCK versus non-sulphated CCK is much smaller for the CCK-B receptor (76, 77, 85). In contrast to non-ruminants, CCK-B receptors may be abundant in the vagal nerve endings of the ruminant (83). One should therefore be cautious extrapolating findings in non-ruminants to ruminants.
Main biological actions of CCK are its modulatory effects on gastrointestinal secretions and motility (49, 78, 94). CCK also stimulates the secretion of enzymes and bicarbonate from the exocrine pancreas (136, 153) and insulin, glucagon and Pancreatic Polypeptide (PP) from the endocrine pancreas (96).

Pancreatic Polypeptide

PP is a 36-amino acid residue peptide, with an amidated tyrosine residue at the carboxyl terminus (91). PP cells have been demonstrated in the pancreas of a great variety of species including ruminants (91, 138). Release of PP has been demonstrated following vagal stimulation in calves and sheep (138). CCK may also stimulate PP in man (89, 101) and dogs (138). PP may be involved in the regulation of gastric and pancreatic secretion. Generally, PP inhibits exocrine secretion and relaxes the gallbladder through vagal regulation (91).

Gastrin

Gastrin is a hormone produced by G cells in the gastric antrum and duodenum (26, 123, 138, 149). G-cells respond to the presence of substances (especially peptides and amino acids) in the lumen of the part of the gut in which they are located (26, 108, 123, 138, 149). Gastrin release is promoted by adrenaline and gastrin releasing peptide, while somatostatin decreases gastrin release (26, 123, 138, 149). Vagal control of gastrin release was shown in man (149) and ruminants (26, 34). Although there is limited evidence in ruminants, main physiological function of gastrin is the regulation of gastric acid secretion (26, 34, 115, 123, 149). It also may be involved in the regulation of rumen motility (14, 78, 94).

1.4.2.2 Effects of feeding on blood concentrations

Cholecystokinin

Little is known about the effect of feeding on CCK levels in ruminants. Studies in goats (66) and dairy cows (67) revealed no effect of feeding on jugular CCK levels. In non-ruminants, laboratory animals and humans, CCK levels are increased following a meal (30, 110, 117, 151),

Pancreatic Polypeptide

In non-ruminants, PP levels are normally increased following a meal (41, 81, 104). There is limited information concerning PP levels following a meal in ruminants. A transient lowering was found in sheep while in calves a postprandial increase during 30 minutes was observed (138). In one study, no effect on postprandial PP levels was observed but PP levels were higher in sheep fed at higher rates (31).

Gastrin

Increased levels due to feeding were also reported in calves and lambs (138) and monogastrics (34, 149). In adult sheep, the increase was usually not observed (31, 138). Pre-ruminating sheep and calves showed similar gastrin release patterns following a meal as non-ruminants while ruminating sheep and calves revealed no effect of feeding (82).

1.4.2.3 Effects on feed intake

Cholecystokinin

Many studies show that infusion of several forms of CCK result in meal termination in humans (86, 116), laboratory animals (4, 29) and less evident in ruminants (55, 132, 142). Most studies used the sulphated octapeptide form of CCK (CCK-8) which is thought to be the biologically active form. The physiological significance of the effect on feed intake is uncertain because often some malaise is induced probably by altered gastric contractions (17, 30, 62, 78). Infusion of CCK is reported to increase cortisol and prolactin concentrations in sheep, which can be considered as a stress response (53).

In ruminants, CCK-8 and CCK-33 inhibit feeding in sheep when infused peripherally by various routes (55, 69). It was shown that effects of CCK-8 are probably mediated through CCK-B receptors (55). In the same study, evidence for both anterograde and retrograde axonal transport of CCK-8 through vagal fibres is provided.

Pancreatic Polypeptide

No reports are known concerning the effect of PP on intake in ruminants. In non-ruminants, central administration of PP led to increased feed intake (99) while peripheral infusion decreased feed intake (18).

Gastrin

Many infusion studies were performed using the gastrin analogue pentagastrin. Pentagastrin was shown to decrease intake in pigs (75, 114) and sheep (69). The effects of these infusions were probably mediated by peripheral receptors since ICV administration of pentagastrin did not decrease intake in sheep (52).
The physiological significance of the infusion studies may be limited since dosages used are relatively high and probably induce disturbed rumen motility which can explain the observed reduction in intake (69, 78, 94).

1.5 Outline of the thesis

This thesis focuses on blood-borne factors related to feed intake in sheep. This includes the major energy providing components, metabolic hormones and gastrointestinal hormones.
The aim of this thesis is:

1. To gain insight in the changes in nutrient and hormone concentrations following meals of different feed qualities.
2. To investigate blood borne nutrients/hormones which may be involved in the regulation of feed intake.

To study this, experiments were performed in wether sheep provided with mesenteric, portal and jugular catheters.
To address the effect of feeding and feed quality on nutrients and hormones, animals were fed during 90 minutes. Before, during and after feeding, blood was withdrawn from jugular and portal catheters in order to select candidates for meal size regulation (Chapters 2 and 3). After this selection, mesenteric infusions of propionate as a regulating nutrient were performed (Chapters 4 and 5). The effect of infusion of insulin and CCK, as regulating hormones is discussed in Chapter 6 and Chapters 7 & 8. In Chapter 9, an attempt is made to model the experimentally obtained results in order to explain changes in blood concentration of hormones and metabolites following a meal and/or infusion.

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CHAPTER 2

Effect of feeding on metabolic blood parameters in meal-fed sheep

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ABSTRACT

Short-term effects of feed intake on jugular and portal concentration of Volatile Fatty Acids (VFA's), Beta-hydroxy-butyrate (BHB) and glucose were studied in sheep. Two experimental pelleted grass diets, qualified as High Quality (HQ) and Low Quality (LQ), were fed in a cross-over design.
Rapid changes were observed in both jugular and portal veins due to feeding. Portal vein (PV) concentrations of acetate, propionate, butyrate, iso-butyrate and BHB were increased post-prandially in HQ-fed sheep. Due to consumption of LQ feed, bi-phasic patterns were found in acetate, propionate and butyrate levels, measured in the jugular vein (JV).
The effect of feeding on nutrient concentration may largely differ as a result of feed quality. The PV-JV difference is used as an estimation of the release of nutrients in the portal vein. Differences in peripheral concentration of a blood component do not necessarily result from a changed production of the component but can also be a result of changed uptake by peripheral tissues. In most cases, the observed early changes (until 30 minutes past meal start) were probably due to changes in uptake rather than alterations in release. The changes in metabolite concentrations, observed later then 30 minutes were likely due to changes in release.

keywords: volatile fatty acids, glucose, beta-hydroxy butyrate, ruminants, feed intake

INTRODUCTION

To cover their energy requirements, ruminants are largely depending on volatile fatty acids (VFA's) (5). Due to the fermentative nature of their digestion, ruminants normally absorb little or no dietary carbohydrate as hexose sugar (7) VFA's and other nutrients are absorbed and transported by the blood. Blood coming from the digestive system, and several associated endocrine organs like pancreas, is collected in the portal vein and transported to the liver. Here, part of the nutrients is converted to other metabolites or cleared from the blood circulation (19, 31).
As all other animals, ruminants are meeting their nutritional requirements by eating several discrete meals a day (14). Meals are controlled by a variety of peripheral signals (11, 12, 15). These signals are transferred to the central nervous system, especially the hypothalamus. Several studies show that VFA's are candidates for acting as a signal substance (16, 18). It has been postulated that the liver plays an important role in induction of satiety through VFA's (1, 20). It is also likely that effects of other metabolites or hormones on feed intake are mediated by the liver (15). Infusion of satiety inducing hormones/metabolites often result in reduced feed intake (16). The observed effects could partly be explained by their specific biological function, but it could also be an indirect effect, inducing (metabolic) stress (11, 12). If a hormone or metabolite is involved in meal size regulation, feeding should induce a change in concentration of the hormone or metabolite. In order to induce satiation, the change in concentration should occur before the end of a meal.
Although ruminants consume their feed in meals, it is surprising that there are relatively few studies concerning the effects of a meal on short-term hormone and metabolite fluctuations in the blood.

The present study was therefore designed to investigate the effect of feeding and feed quality on VFA, BHB and glucose profiles in the jugular and portal veins of meal-fed sheep.

MATERIALS AND METHODS

Animals
Eight Swifter wether sheep (1.8 ± 0.01 years old, 75 ± 3 kg LW) were housed indoors in ground pens and were kept at room temperature (18 ± 2 °C). Lights were on from 6.00 - 21.00 hour.

Feed
Animals were fed two pelleted grass diets. Diets were based on grass harvested from adjacent pastures, at two different growth stages (early spring and late summer). After harvesting, grass was dried and pelleted. Diets were qualified as High Quality (HQ) and Low Quality (LQ) based on crude protein and fibre contents. Dry matter of the pellets was determined by drying at 103 °C, ash in an oven at 550 °C, N content according to Kjeldahl and cell wall constituents according to Goering & Van Soest (17). Cellulose was calculated as ADF-ADL, hemicellulose as NDF-ADF, and lignin as ADL. Composition of the feed is shown in Table 1.

Table 1. Chemical composition of the experimental feed (g/kg)

	HQ	LQ
Dry Matter	965	961
In DM		
OM	844	864
CP	241	141
Cellulose	210	253
Hemi-cellulose	284	263
Lignin	23	41

abbreviations: HQ, High Quality; LQ, Low Quality; DM, dry matter; OM, organic matter; CP, Crude Protein.

Feed (45 g/kg$^{0.75}$) was offered three times daily with an eight- hour interval. Feed was offered for 1.5 hour and the refusals were automatically discarded and weighed. Water and salt lick were available ad libitum.

Surgery
Animals were provided with silastic catheters in the portal and jugular veins as described previously (35). Animals were routinely treated post-surgically with analgesics and antibiotics. Catheter patency was maintained by weekly flushing with physiological saline containing heparin (5000 IU/l). If a catheter was blocked, it was treated with streptokinase (21). Experiments were started six weeks after surgery when feed intake and body weight were normal again for at least two weeks, and animals had well adapted to the experimental procedures.

Blood sampling
Blood samples were withdrawn through polyethylene tubes that were connected to the animals' catheters at least 30 minutes before start of the experiment. Blood sampling occurred without handling the animal. In this way, stress related to blood sampling was minimised. Before and after the experiments, sampling lines were cold sterilised with 70% ethanol.
Blood samples were collected in chilled tubes containing EDTA. Tubes were centrifuged (4 °C, 1800 G) and plasma was stored in small aliquots at -20 °C before analysis.

Analyses
Plasma glucose was determined spectrophotometrically by the GOD/PAP method (Boehringer Mannheim nr 166391). Plasma beta-hydroxy-butyrate (BHB) was determined spectrophotometrically (Boehringer Mannheim nr 907979).
Plasma VFA's were determined in deproteinised plasma by gas chromatography.

Experiments
Animals were fed either a LQ diet or a HQ diet according to a cross-over design. Four sheep were fed a LQ diet and four sheep a HQ diet. Animals were allowed to adapt to the experimental feed for 3 weeks.

Calculations and statistics
For each sheep, portal vein - jugular vein (PV-JV) differences were calculated for each time point as an indicator for portal appearance of nutrients produced in the portal drained viscera. As an indicator for total production during the postprandial period, difference between the area under the curve of the PV and JV curves (dAUC), was calculated over the postprandial period for each individual sheep.
All results are expressed as means. If applicable, pooled SE is shown at the first data point. The data were analysed using analysis of variance (GLM procedure, SAS) (32).
For comparison between diets the following model was used:
$y = \mu + sheep + diet + period + error$.
For comparison between veins:
$y = \mu + sheep + diet + error$
For determining differences from basal level (t=-30 and -15):
$y = \mu + diet + error$

RESULTS

Intake during the test period did not significantly differ between diets (LQ: 800±65 g vs. HQ 900±100 g).

Blood parameters
Acetate levels of sheep fed either a HQ or a LQ diet are shown in figure 1.
Portal acetate concentrations were consistently higher than jugular concentrations irrespective of feed quality. Portal as well as jugular levels of HQ-fed sheep increased postprandially. Portal and jugular levels of LQ-fed sheep were increased rapidly and remained elevated until t=300 min. No effect of feed quality was found in

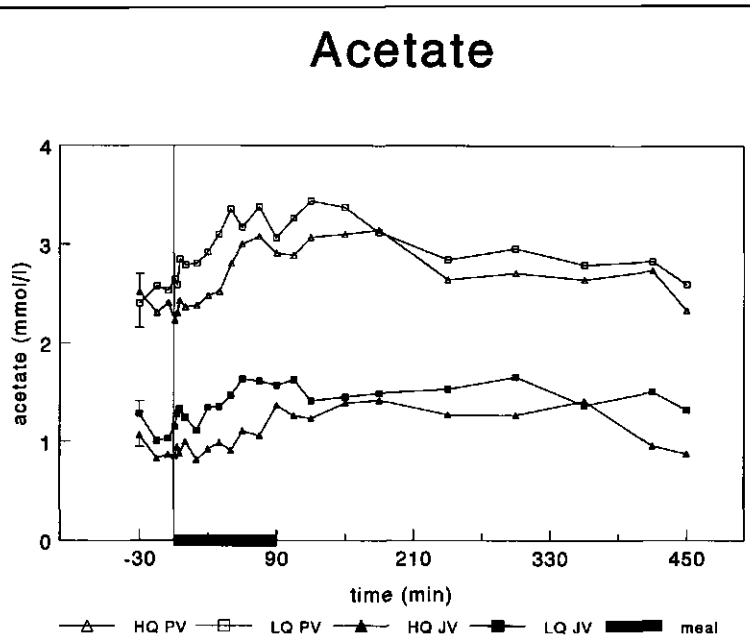

Figure 1: Portal (PV) and jugular (JV) acetate concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

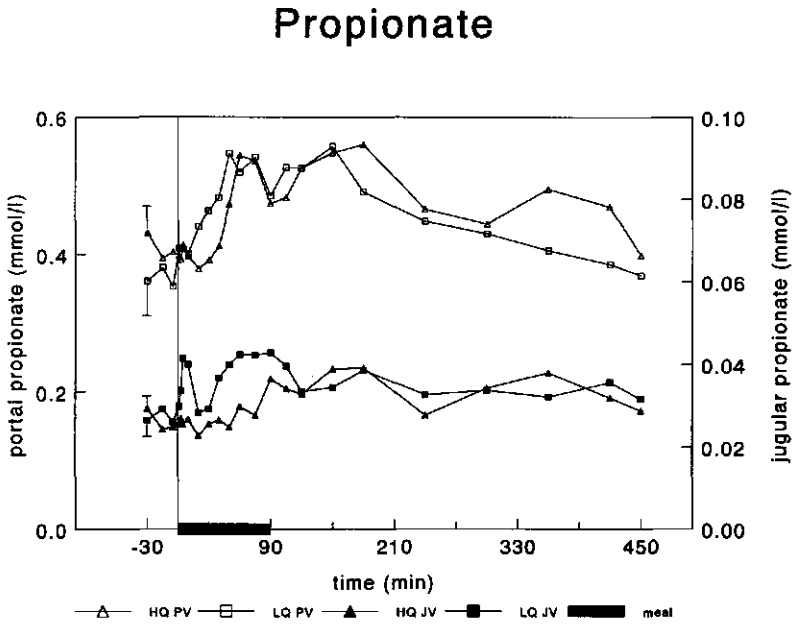

Figure 2: Portal (PV) and jugular (JV) propionate concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

the portal vein. Jugular acetate levels of HQ-fed sheep were significantly lower from meal start until t=120, as compared to LQ-fed sheep.
As with acetate, portal propionate levels were significantly higher than jugular levels (Fig. 2). Portal propionate levels of HQ-fed sheep were increased postprandially while jugular levels of HQ-fed sheep were not significantly influenced. The observed increase in portal propionate concentrations of LQ-fed sheep lasted from t= 60 until t=240. Jugular concentrations of LQ-fed sheep showed a biphasic pattern. A rapid peak was followed by a more gradual increase. No feed quality effect was found in the portal vein. Jugular propionate levels of animals fed a LQ feed showed significantly higher levels than HQ-fed animals from t=30 until t=90. Both HQ-fed and LQ-fed sheep showed postprandially increased PV-JV differences compared to pre-feeding levels (Fig. 3).
Portal butyrate concentrations of HQ-fed sheep were usually lower than jugular concentrations except during the meal period (Fig 4). Portal concentrations of LQ-fed sheep remained lower compared to jugular levels. HQ-fed sheep showed an initial decrease in portal and jugular butyrate levels, followed by a gradual increase leading to significantly elevated levels. Portal levels of LQ-fed sheep were enhanced postprandially. A rapid peak followed by a marked increase was shown in the jugular vein of LQ-fed sheep. No effect of feed quality was found in the portal vein while jugular concentrations of sheep fed a HQ diet were generally lower than butyrate levels of LQ-fed animals. Figure 5 shows that PV-JV differences were decreased after meal start in LQ fed animals, but not in HQ fed sheep.
A significant difference between portal and jugular levels of iso-butyrate was seen in both dietary groups (Fig. 6). Portal iso-butyrate levels of HQ-fed animals were increased postprandially but jugular levels remained at basal level. LQ-fed animals showed decreased portal and jugular levels after 2 hours. An effect of feed quality on iso-butyrate levels was observed only from t=300 until t=450 in the portal vein, where HQ-fed sheep showed higher levels than LQ-fed sheep.
Iso-valerate levels (Fig. 7) were significantly higher in the portal vein as compared to the jugular vein in the HQ as well as the LQ group. Iso-valerate levels did not change dramatically due to feeding. Feeding a HQ diet resulted in increased portal iso-valerate levels from t=180 until t=450 as compared to sheep fed a LQ diet.
Beta-Hydroxy-Butyrate levels (Fig. 8) were significantly higher in the portal vein as compared to the jugular vein in both dietary groups. BHB concentrations in the portal and jugular veins of HQ-fed sheep were decreased immediately after meal start followed by a gradual increase. Portal as well as jugular BHB concentrations of LQ-fed sheep showed a slow postprandial increase. BHB levels of HQ-fed sheep were lower as compared to LQ-fed sheep, only during the initial period after feeding in both veins. PV-JV differences of BHB decreased compared to pre-feeding levels in HQ fed sheep immediately after meal start (Fig. 9).
Glucose levels were neither changed dramatically due to feeding nor influenced by sampling site (Fig. 10). The only exception was the small but significant increase in the jugular vein of LQ-fed sheep during the first 10 minutes after feed start. In this period, jugular levels of the LQ-fed sheep were significantly higher as compared to portal levels. HQ-fed sheep showed slightly higher glucose levels as compared to LQ-fed sheep in both jugular and portal veins.

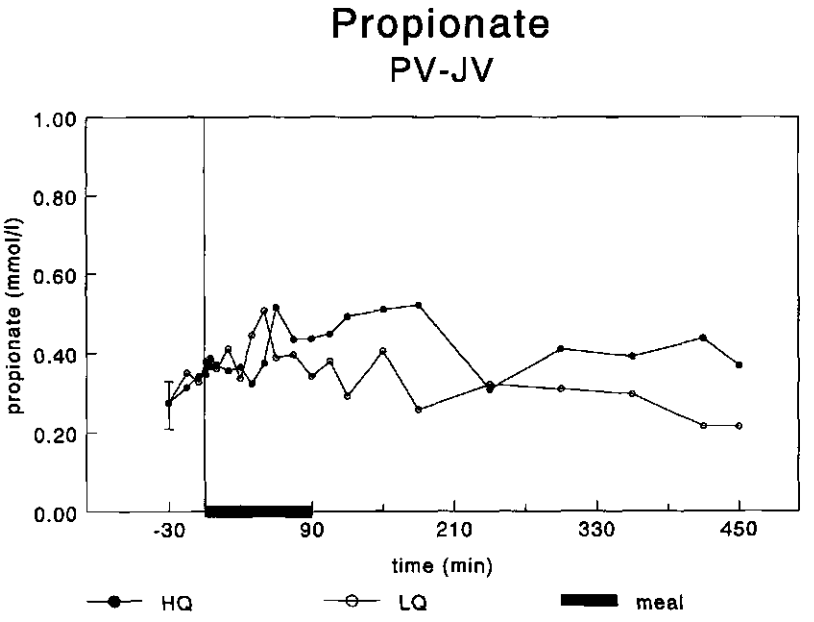

Figure 3: Portal-jugular vein difference of propionate levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

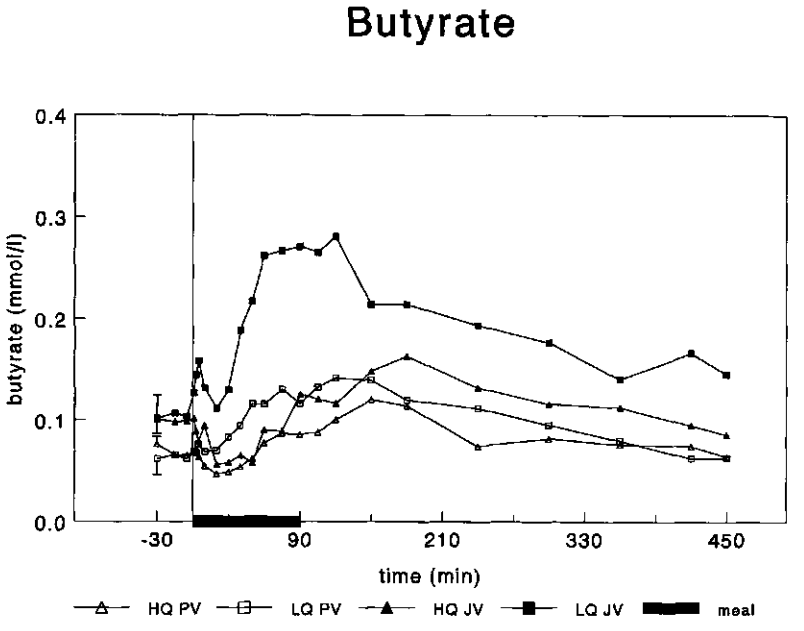

Figure 4: Portal (PV) and jugular (JV) butyrate concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

dAUC's of butyrate, iso-butyrate and iso-valerate were significantly higher in the HQ-fed group as compared to the LQ-fed group (Table 2).

Table 2. Postprandial AUC (area under curve) of portal vein minus AUC jugular vein of sheep fed either a HQ or a LQ diet.

	HQ		LQ		P
Acetate	707.4	± 64.2	669.1	± 72.6	0.48
Propionate	201.0	± 11.8	187.7	± 21.4	0.35
Butyrate	-14.6	± 3.3	-31.1	± 5.1	0.01
iso-butyrate	9.6	± 0.4	7.2	± 0.9	0.01
iso-valerate	6.5	± 0.4	5.3	± 0.4	0.01
BHB	43.6	± 10.5	53.5	± 16.4	0.40

values are expressed as means ± SE (mmol.min/l).

DISCUSSION

Volatile Fatty Acid (VFA) appearance in the blood circulation has been a field of interest for many researchers (6, 9, 10, 12). Surprisingly, only a few papers deal with the rapid changes related to feeding (9, 12). Some studies describe the effect of feeding, measuring portal or jugular levels of nutrients starting at a later time point (hours after feeding) (3, 6, 34). Rapid changes may seem unexpected since production of VFA in the rumen often shows a time lag (36), and rumen concentrations may even become lower in dairy cows shortly after a meal (Chilibroste, Personal Communication).

The increments in plasma VFA found in the present study may suggest an enhanced uptake of VFA from the rumen. This does not necessarily mean that the production in the rumen is enhanced. Alternatively, it could also be explained by a higher and/or more effective blood flow through the rumen papillae and enhanced rumen motility, since transport across the rumen wall is primarily a passive permeation process (28, 33). Furthermore, it should be noticed that decreased uptake of nutrients by the (peripheral) tissues or the liver may also lead to increased plasma concentrations. To quantify the net portal release of substrates or hormones the arterio-venous difference technique is often used (22). Portal release, as an indicator for production of components by the portal-drained viscera (PDV), is calculated as the portal vein - artery difference multiplied by the portal flow (22). Long-term measuring of portal flow is technically difficult. For this reason we chose not to measure portal flow.

Postprandial increases of acetate in both veins on both diets were observed. Increased levels of total VFA after a meal are well described (3, 23, 25, 29). Acetate levels of both LQ and HQ-fed sheep were increased after start of a meal in both portal and jugular veins. This was probably not due to increased release of acetate in the portal vein since PV-JV differences were not changed dramatically. Possibly, uptake of acetate by the liver or peripheral tissues was lowered leading to increased acetate concentrations.

dAUC-acetate was not significantly higher in HQ-fed sheep as compared to LQ-fed sheep. This implies that the increased jugular concentrations of LQ-fed sheep as

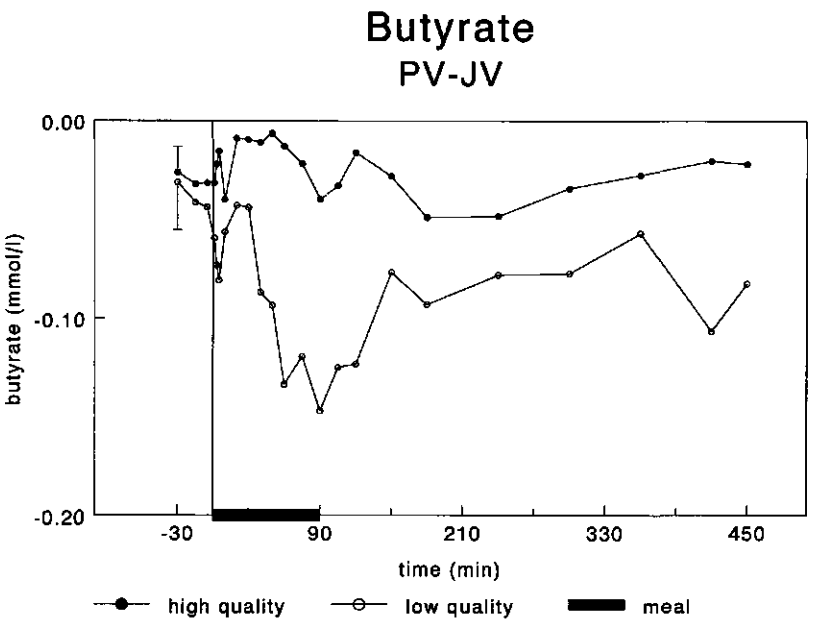

Figure 5: Portal-jugular vein difference of butyrate levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

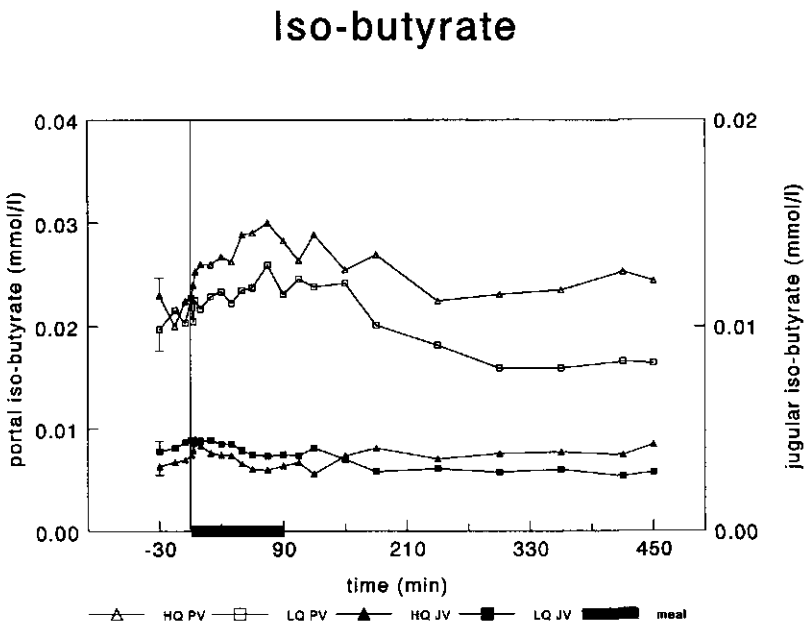

Figure 6: Portal (PV) and jugular (JV) iso-butyrate concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

compared to HQ-fed sheep, due to feed quality were likely caused by a somewhat lower uptake of the peripheral tissue of acetate in LQ-fed sheep.
Propionate levels in the portal vein were about 10 fold higher as compared to the jugular vein. This is due to the very high extraction of propionate by the liver (2, 7). Portal propionate levels of HQ-fed animals were increased post-prandially due to a higher release in the portal vein as shown by the increased PV-JV difference. Jugular vein levels of the HQ-fed animals were not changed dramatically indicating that the liver accurately removed the surplus of propionate produced. As the HQ-fed animals, LQ-fed animals showed a postprandial increase (after t=60) in portal propionate levels. Levels were probably increased due to a higher portal appearance, which was also shown previously (30). The first rapid increase observed in the jugular blood of LQ-fed animals was not caused by a higher release of propionate in the portal vein but rather result from a lower uptake of propionate by the liver. The second increase may be due to the earlier mentioned post-prandial release of propionate. As with acetate, jugular vein levels of propionate in LQ-fed animals were higher than in HQ-fed animals probably due to less accurate removal of produced propionate by the liver since dAUC's did not significantly differ between feeds. Although not found in this study, feed quality was positively correlated with higher production of propionate in a study by Evans (13).
Butyrate levels in both the HQ and the LQ group were significantly higher in the jugular vein as compared to the portal vein. This is remarkable since butyrate is presumed to be absorbed from the rumen (29) leading to a net release of butyrate in the portal vein. In stead of a net release, a net uptake by the portal-drained viscera was observed in the present study. Epithelial cells of the gastrointestinal tract are using butyrate as a major source for covering their energetic needs (29) and may be responsible for the observed net uptake of butyrate.
In the present study, net butyrate production apparently occurred outside the portal-drained region. Explanation may lie in fermentation in the posterior large intestine (5). Blood coming from the posterior part of the colon and the rectum is transported directly to the vena cava.
Another remarkable observation is the effect of feed quality on butyrate concentrations. Portal butyrate levels of the HQ-fed animals showed a decrease during the first 40 minutes followed by a slow increase, while the LQ-fed animals showed an immediate increase. Lowering of the butyrate levels in HQ-fed sheep was probably not due to an enhanced uptake of butyrate by the portal drained viscera (PDV), but more likely due to a decreased net release by the butyrate producing organ(s) outside the PDV since portal-jugular difference did not change during the experimental period.
Jugular butyrate levels of LQ-fed sheep showed a rapid peak followed by a slow increase. As in the HQ-fed group, increased post-prandial levels were not due to decreased PDV uptake but rather to a larger net peripheral release since in the LQ-fed group, PV-JV differences were decreasing, indicating a higher PDV uptake. As with acetate and propionate, jugular vein concentrations of LQ-fed animals were higher than jugular vein levels of HQ-fed animals. In case of butyrate, this was caused by a less accurate uptake, as suggested for acetate and propionate, but due to a larger production of butyrate by organs not drained by the portal vein. It can be postulated that the higher fibre content of the LQ feed promoted fermentation in the anterior colon. dAUC was more negative in LQ fed animals as compared to HQ fed animals. This may indicate that more butyrate is formed in the PDV in the HQ fed group (33), leading to a smaller uptake of butyrate from the blood.

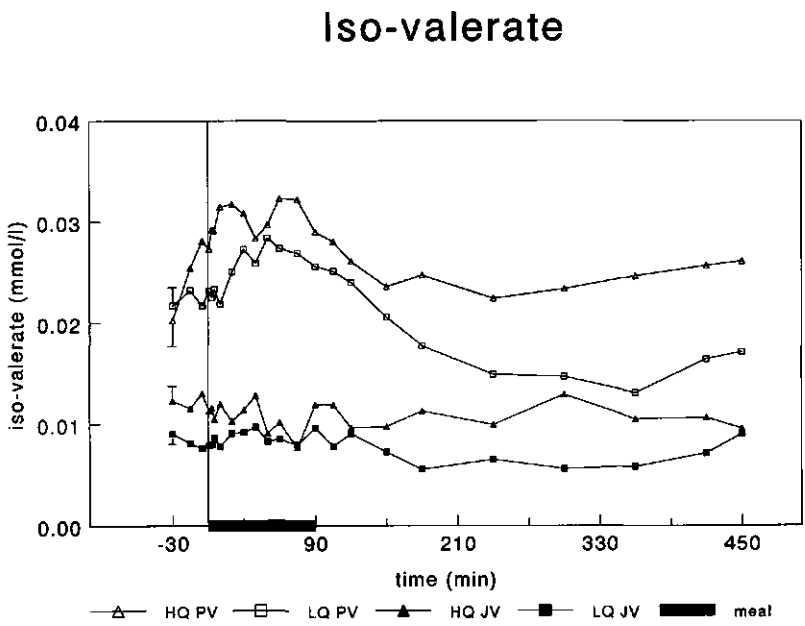

Figure 7: Portal (PV) and jugular (JV) iso-valerate concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

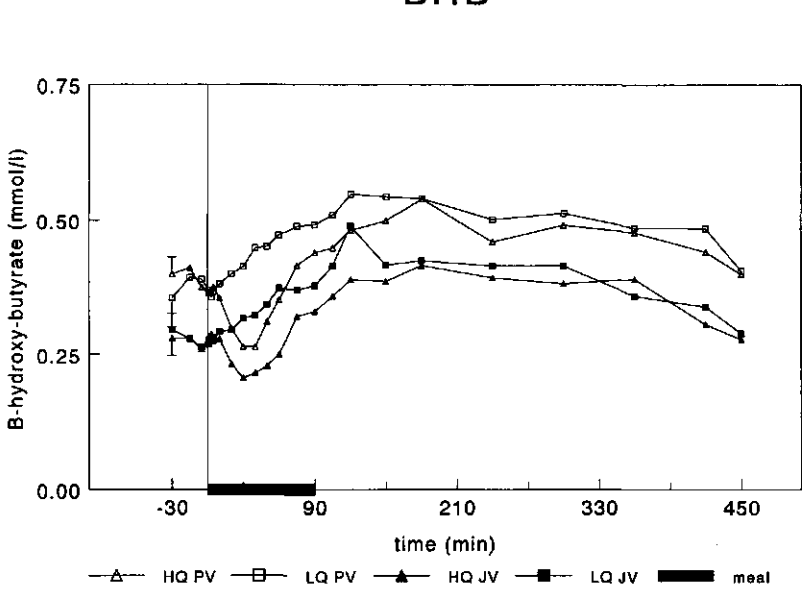

Figure 8: Portal (PV) and jugular (JV) beta-hydroxy-butyrate (BHB) concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

Portal iso-butyrate levels in HQ sheep increased due to higher release in the portal vein since PV-JV difference changed rapidly after meal start (not shown). As with acetate and propionate, the produced iso-butyrate was very accurately removed from the peripheral circulation, probably by the liver (31), leading to unchanged jugular concentrations. The observed long-term decrease in portal and jugular concentration of iso-butyrate in LQ-fed sheep was not due to a enhanced uptake by the liver or peripheral tissues, but probably due to a lower release of iso-butyrate, which may indicate lower ruminal production. dAUC was significantly higher in HQ-fed animals as compared to LQ fed animals. This was likely due to increased fermentation of proteins (27, 36), which were present in a higher percentage in the HQ feed.

As iso-butyrate, iso-valerate is formed by fermentation of proteins (27, 36), leading to the higher portal vein concentrations of iso-valerate in HQ-fed sheep. As a consequence of the higher protein content of the HQ feed, dAUC was significantly higher in the HQ fed sheep.

Decreased BHB levels observed in both the portal and jugular veins of HQ-fed animals are most likely the consequence of the decreased peripheral release of butyrate as described earlier. Hence, BHB release, which is dependent on the availability of substrate (i.e. butyrate)(8), was decreased leading to a lower PV-JV difference.

The increased levels of BHB in the LQ-fed animals, were probably not due to increased metabolization of butyrate following the meal start since PV-JV difference did not change significantly during the test period. Increased levels of BHB after a meal are generally observed (9, 23, 25, 30). dAUC's of BHB, were not different between feeds, despite the enhanced uptake of butyrate in the LQ group by the PDV.

Glucose levels were higher in HQ-fed as compared to LQ-fed animals, which is generally found (5, 13). The observed slight increase in jugular glucose levels of LQ-fed sheep during the first 10 minutes after feed start may be due to increased glucose synthesis. However, since liver uptake of propionate during the first 10 minutes after feed start was presumed to be decreased rather than increased, it is likely that propionate was not used as a precursor. Presumably glucogenic amino acids were used as a substrate for glucose production (7). Reported effects of feeding on glucose levels are variable. Some researchers found a post-prandial increase (3, 4, 6, 26) while others found a decrease in glucose concentration, especially when fed concentrates (9, 10) or no effect (23, 24).

In summary, this paper shows that rapid effects of feeding on VFA's, BHB and glucose can be observed in ruminants. In addition to this, it shows that effect of feeding may largely differ as a result of feed quality. It also shows that differences in peripheral concentration of a blood component are not necessarily a consequence of a higher production of the component. Changes in plasma concentration may not be a result from an altered release only, but can also be a result of changed uptake. In most cases, the observed early changes (until 30 minutes past meal start) were probably due to changes in uptake by the peripheral tissues including the liver rather than changes in release from the PDV. The changes observed after 30 minutes were likely due to changes in release. This is in accordance with the idea of a certain time lag before fermentation of the ingested feed in the rumen is started.

Another important observation is that higher levels of a metabolite do not implicate automatically a higher release. In case of propionate and acetate, dAUC's were not different between feed, while higher jugular levels were observed in LQ-fed animals.

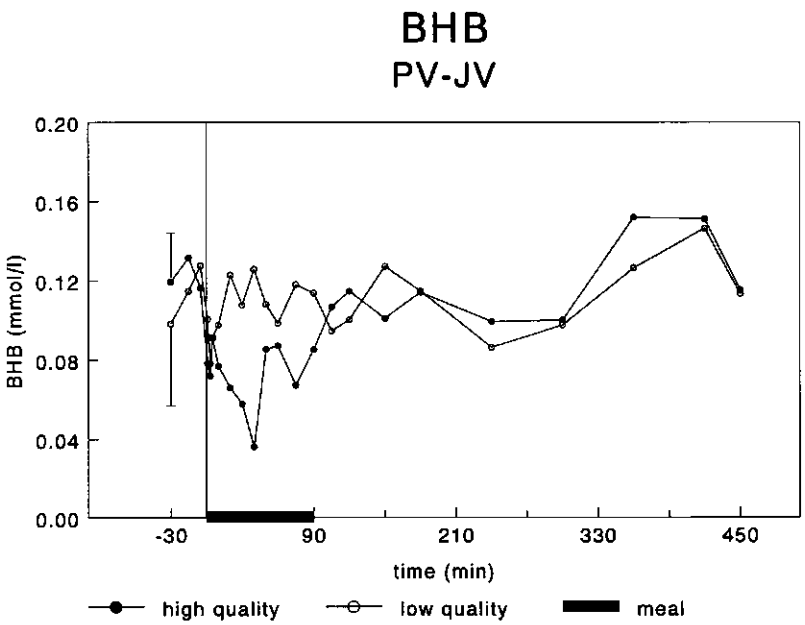

Figure 9: Portal-jugular vein difference of beta-hydroxy-butyrate (BHB) of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

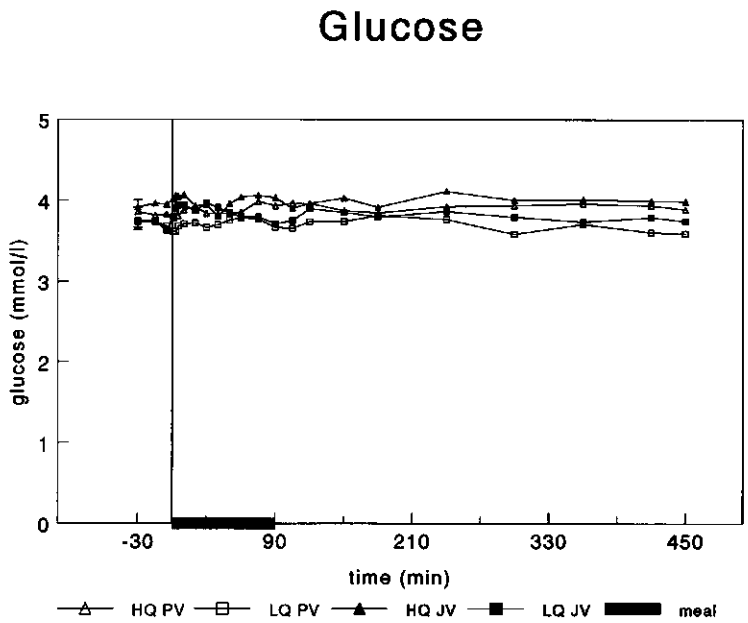

Figure 10: Portal (PV) and jugular (JV) glucose concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

On the other hand, LQ-fed sheep showed higher butyrate levels, which were likely caused by a higher butyrate production in organs not drained by the portal vein.

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CHAPTER 3

Metabolic and gastrointestinal hormones during meal feeding in sheep

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ABSTRACT

Short-term effects of feed intake on portal and jugular levels of metabolic hormones i.e. insulin, glucagon and Growth Hormone (GH), and gastrointestinal hormones i.e. gastrin, pancreatic polypeptide (PP) and cholecystokinin (CCK) were studied in sheep. Two experimental pelleted grass diets, qualified as High Quality (HQ) and Low Quality (LQ) were fed.
It was shown that feeding induced both rapid as well as more sustained changes in hormone concentration. Rapid fluctuations were shown for insulin, glucagon, PP and CCK levels in HQ-fed sheep. Sustained changes were observed for insulin, glucagon and gastrin levels in HQ-fed sheep. LQ-fed sheep showed rapid changes in GH, gastrin, PP and CCK levels. Sustained changes were observed for insulin, GH, gastrin, PP and CCK levels.
The rapid changes in hormone concentration may be due to decreased para-sympathetic activity and/or increased sympathetic activity. More sustained changes are likely nutrient induced. Feed quality mainly affected the magnitude of the meal induced changes in hormone levels, with the HQ-fed sheep showing more pronounced differences.

keywords: insulin, glucagon, growth hormone, cholecystokinin, pancreatic polypeptide, gastrin, feed intake, feed quality, ruminants

INTRODUCTION

For covering their nutritional requirements, ruminants eat discrete meals (13). The entrance of nutrients, such as Volatile Fatty Acids (VFA's), can be considered as a metabolic disturbance (5). Related to this disturbance, blood levels of metabolic hormones like insulin, glucagon and Growth Hormone (GH) are changing. In addition to the changes in metabolic hormones, gastrointestinal hormones like CCK, Gastrin and PP, are also influenced by feeding.
Many hormones related to feeding are released by the Portal Drained Viscera (PDV). Blood from these organs, is collected in the portal vein and transported to the liver. Here, a substantial part of the hormones is cleared from the circulation.
In the search for hunger/satiety signals, attention has been paid to metabolic and gastrointestinal hormones. In ruminants, a relation between feed intake and insulin was postulated. VFA's, especially propionate and butyrate, were found to be insulin releasing nutrients (26). However, experiments with administration of exogenous insulin did not confirm the proposed role of insulin as a satiety signal (9). Much less is known about the relation between feed intake and glucagon or GH in ruminants. Both hormones are influenced by feeding and have been proposed to influence feed intake (2, 11). However, the experimental evidence for this assumption is meagre and mainly arising from experiments performed on monogastrics.
One of the most intensely investigated gastrointestinal hormones is CCK. CCK was characterised as a satiety-inducing hormone in both non-ruminants (3) and in ruminants (12). Although various studies provide evidence for the satiating effect of CCK, others emphasise the potentially malaise inducing properties of CCK (25). Gastrin and PP are less investigated in relation to feeding, but both are affected by feeding (4, 6) and may have satiating characteristics (18).

If a hormone is involved in the regulation of feed intake, it should not only be affecting intake when administered exogenously, but also be influenced by feed intake. Furthermore, the changes in hormone release or level should occur within the time span of a meal. It is surprising that there are very few studies concerning the short-term effects of a meal on metabolic and gastrointestinal hormone changes in the blood of ruminants.
The present study was designed to investigate the effect of feeding on metabolic and gastrointestinal hormone profiles in the jugular and portal veins. We also investigated the effect of feed quality on these parameters.

MATERIALS AND METHODS

Animals
Eight Swifter wether sheep (1.8 ± 0.01 years old, 75 ± 3 kg LW) were housed indoors in ground pens and were kept at room temperature (18 ± 2 °C). Lights were on from 6.00 - 21.00 hour.

Feed
Animals were fed two pelleted grass diets. Diets were based on grass harvested from adjacent pastures, at two different growth stages (early spring and late summer). After harvesting, grass was dried and pelleted. Diets were qualified as High Quality (HQ) and Low Quality (LQ) based on crude protein and fibre contents.
Dry matter of the pellets was determined by drying at 103 °C, ash in an oven at 550 °C, N content according to Kjeldahl and cell wall constituents according to Goering & Van Soest (16). Cellulose was calculated as ADF - ADL, hemicellulose as NDF - ADF, and lignin as ADL. Composition of the feed is shown in Table 1.

Table 1. Chemical composition of the experimental feed (g/kg)

	HQ	LQ
Dry Matter	965	961
In DM		
OM	844	864
CP	241	141
Cellulose	210	253
Hemi-cellulose	284	263
Lignin	23	41

abbreviations: HQ, High Quality; LQ, Low Quality; DM, dry matter; OM, organic matter; CP, Crude Protein.

Feed (45 g/kg$^{0.75}$) was offered three times daily with an eight-hour interval. Feed was offered for 1.5 hour and the refusals were automatically discarded and weighed. Water and salt lick were available ad libitum.

Surgery
Animals were provided with silastic catheters in the portal and jugular veins as described previously (41). Animals were routinely treated post-surgically with analgesics and antibiotics. Catheter patency was maintained by weekly flushing with physiological saline containing heparin (5000 IU/l). If a catheter was blocked, it was treated with streptokinase (22). Experiments were started six weeks after surgery when feed intake and body weight were normal again for at least two weeks, and animals had well adapted to the experimental procedures.

Blood sampling
Blood samples were withdrawn through polyethylene tubes that were connected to the animals' catheters at least 30 minutes before start of the experiment. Blood sampling occurred without handling the animal. In this way, stress related to blood sampling was minimised. Before and after the experiments, sampling lines were cold sterilised with 70% ethanol.
Blood samples were collected in chilled tubes containing EDTA. Tubes were centrifuged (4 °C, 1800 G) and plasma was stored in small aliquots at -20 °C before analysis.

Analyses
Plasma insulin was determined by radioimmunoassay (RIA) using p-Insulin as standard and tracer. The sensitivity of the assay was 1.5 µU/ml. Plasma glucagon was determined using a commercial kit (Linco, nr.GL-32K, St. Louis, USA).
Plasma o-GH was determined by RIA using materials provided by NIDDK-NIH.
Plasma CCK was determined by RIA using T-204 as antibody (20). Pancreatic Polypeptide was analysed as described by Lamers et al (21). Gastrin was also analysed by RIA using G-142-08 as antibody (36).
Portal and jugular CCK, and jugular glucagon were analysed in part of the samples.

Experiments
Animals were fed either a LQ diet or a HQ diet according to a cross-over design. Four sheep were fed a LQ diet and four sheep a HQ diet. Animals were allowed to adapt to the experimental feed for 3 weeks.

Calculations and statistics
For each sheep, portal vein - jugular vein (PV-JV) differences were calculated for each time point as an indicator for portal appearance of hormones produced in the portal drained viscera. As an indicator for total production during the postprandial period, difference between the area under the curve of the PV and JV curves (dAUC), was calculated over the postprandial period for each individual sheep.
All results are expressed as means. If applicable, pooled SE is shown at the first data point. The data were analysed using analysis of variance (GLM procedure, SAS) (34).
For comparison between diets the following model was used:
$y = \mu + sheep + diet + period + error$.
For comparison between veins:
$y = \mu + sheep + diet + error$
For determining differences from basal level (t=-30 and -15):
$y = \mu + diet + error$

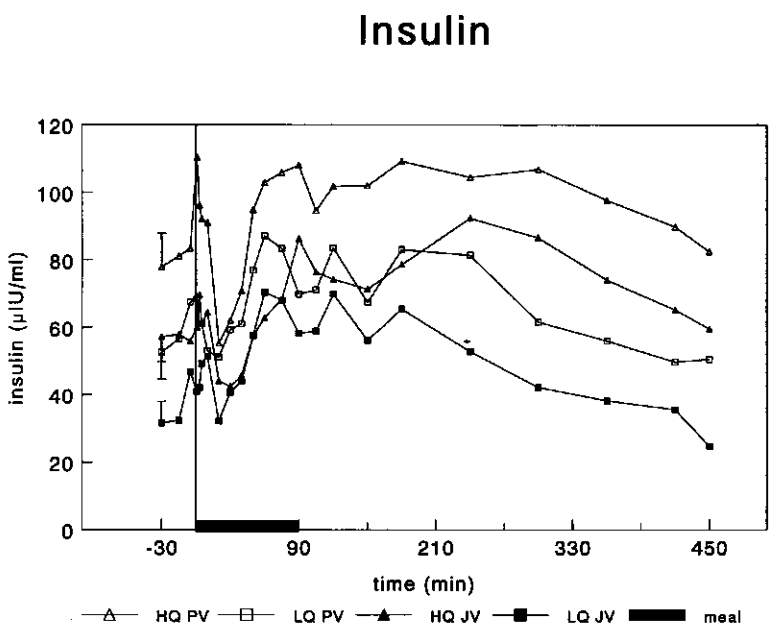

Figure 1: Portal (PV) and jugular (JV) insulin concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

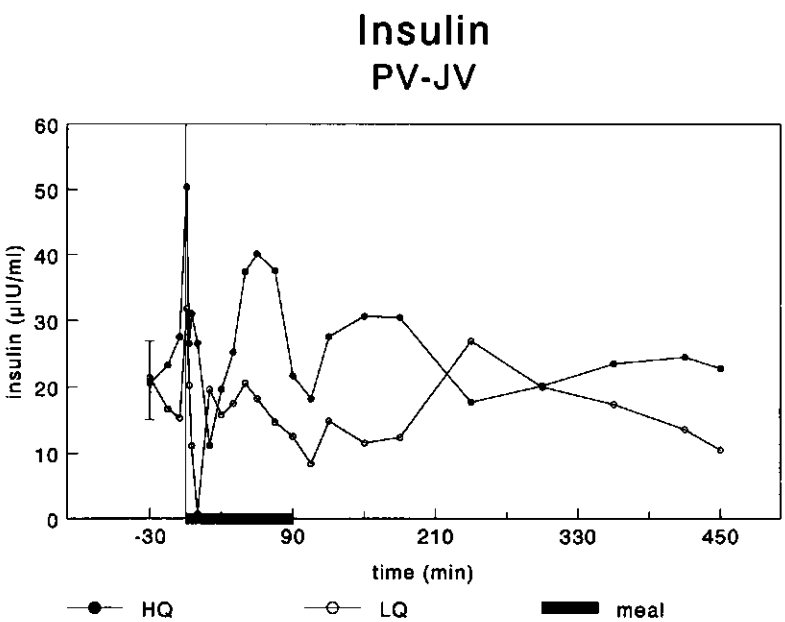

Figure 2: Portal-jugular vein difference of insulin levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

RESULTS

Significant differences were observed between portal and jugular insulin levels of both HQ-fed and LQ-fed sheep (Fig. 1). When comparing pre-feeding levels with levels found during and after a meal, a peak in portal insulin level was found immediately after feed start in HQ fed sheep. Decreased levels were shown at t=20 and t=30, followed by a postprandial increase. Jugular insulin concentrations of HQ-fed sheep showed a similar pattern. Increased insulin levels in LQ-fed sheep were observed postprandially in both portal and jugular veins. A significant effect of feed quality could be demonstrated at t=300 until t=450 where HQ-fed sheep showed higher insulin levels in both veins.
PV-JV insulin differences of HQ-fed sheep (Fig. 2) were increased immediately after meal start and postprandially (t=60 and t=75) but decreased at t=20. LQ fed sheep showed a significantly lower PV-JV difference at t=10.
Portal glucagon levels of both HQ-fed and LQ-fed sheep were significantly higher as compared to jugular levels (Fig. 3). In HQ-fed animals, decreased portal glucagon levels were observed at t=30 and t=40, compared to pre-feeding levels. A small but significant increase in jugular levels could be demonstrated from t=1 until t=5. No difference from pre-feeding glucagon levels could be demonstrated in neither portal nor jugular vein of LQ-fed sheep. Sheep fed a HQ diet showed significantly higher levels during the whole sampling period for both veins compared to LQ-fed sheep.
PV-JV differences (Fig. 4), were significantly lowered at t=30, 40 and 60 min in HQ-fed sheep. No-effect was shown in LQ-fed sheep. HQ-fed sheep showed larger PV-JV differences as compared to LQ-fed sheep during the whole sampling period.

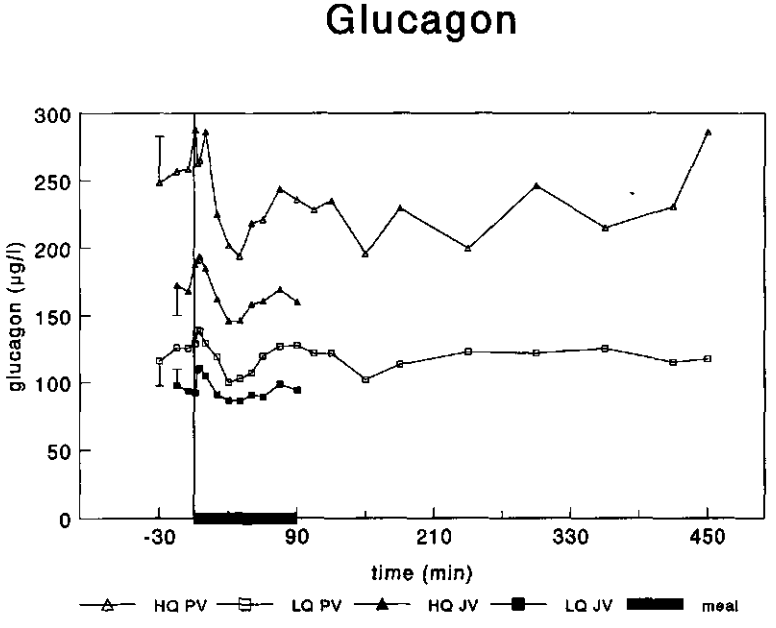

Figure 3: Portal (PV) and jugular (JV) glucagon concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

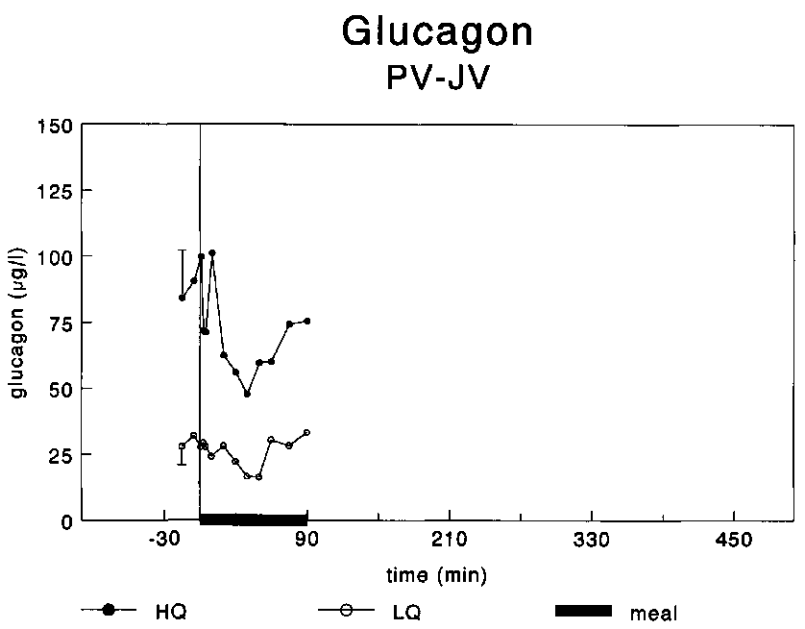

Figure 4: Portal-jugular vein difference of glucagon levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

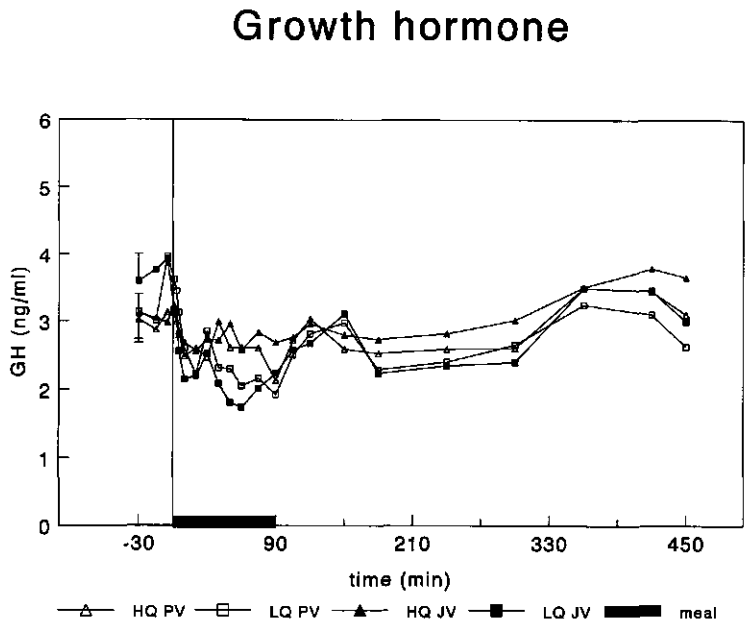

Figure 5: Portal (PV) and jugular (JV) Growth Hormone concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

In Fig. 5, GH levels are given. In HQ-fed sheep, portal and jugular levels were not significantly different. Also, no significant difference from pre-feeding levels could be demonstrated in HQ-fed sheep.
GH levels of LQ-fed sheep were not different between veins. In both veins, levels were significantly decreased from t=5 until t=300, compared to pre-feeding levels. No difference between feed quality could be demonstrated.
Gastrin levels were not significantly different between veins (Fig. 6). In both veins, gastrin levels of HQ-fed sheep were decreased at t=20 and increased from t=75 until t=150. In LQ-fed sheep, decreased levels due to feeding, were found in the portal vein at t=10 and 20, while increased levels were observed at t=50, t=60 and t=150. Jugular concentrations were decreased at t=20 and increased from t=60 until t=120. Animals fed HQ diets showed significantly lowered gastrin concentrations at t=-15, t=-5, t=1 and t=450. No effect of feeding on PV-JV differences was found (data not shown).
Generally, higher portal Pancreatic Polypeptide (PP) levels of HQ-fed sheep were observed as compared to jugular vein concentrations (Fig. 7). Portal PP levels were significantly decreased compared to pre-feeding levels at t=10 and t= 20, while levels were increased at t=150 until t=450. Jugular levels were decreased from t=10 until t=40 and increased from t=150 until t=180. LQ-fed animals showed no difference in PP concentration between veins except for t=1. Feeding a LQ diet resulted in initially increased portal and jugular PP levels followed by a gradual decrease, which led to decreased PP levels from t=105 until t=300. Due to the diet, HQ-fed sheep showed higher portal levels at t=-30 until t=-5 and from t=75 until t=300. Jugular levels were increased due to diet at t=-30 and from t=75 until t=180.
PV-JV difference (Fig. 8) was decreased in HQ feed sheep from t=20 until t=40. LQ-fed sheep showed an increased PV-JV difference at t=1.

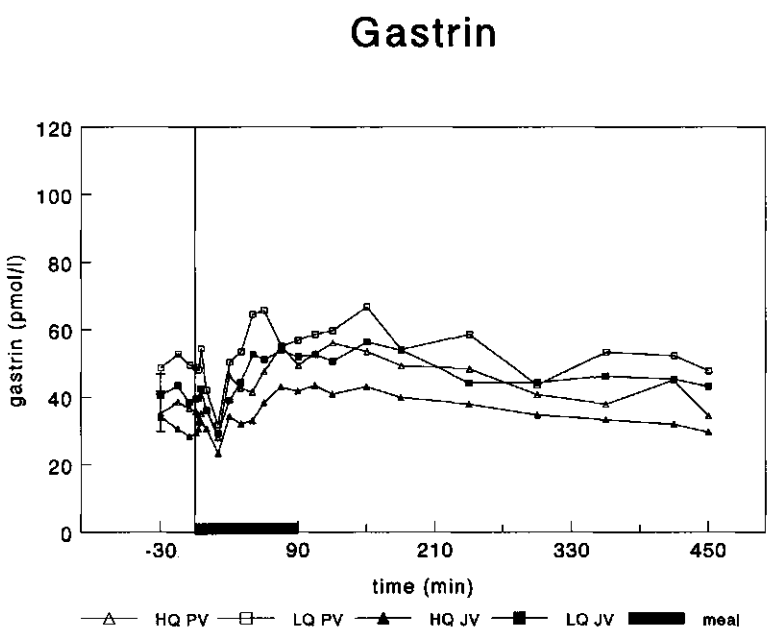

Figure 6: Portal (PV) and jugular (JV) gastrin concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

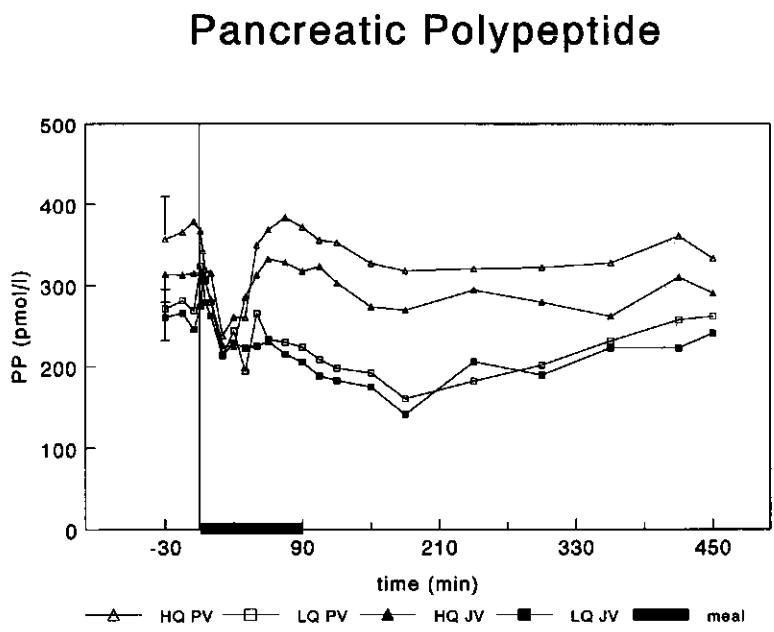

Figure 7: Portal (PV) and jugular (JV) pancreatic polypeptide concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

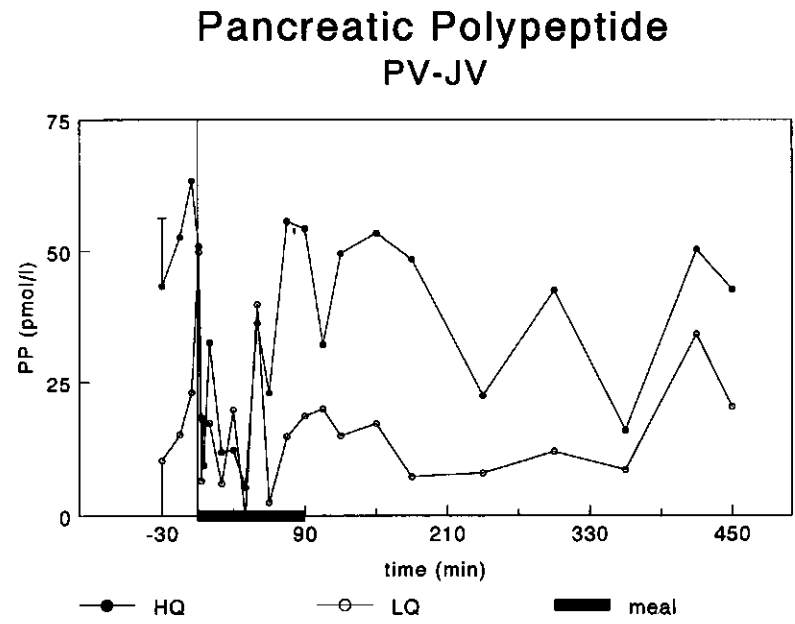

Figure 8: Portal-jugular vein difference of pancreatic polypeptide levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

CCK levels (Fig. 9) were slightly higher in the portal vein of HQ-fed as compared to jugular vein at t=-5, t=5 and t=60. As compared to pre-feeding levels, portal levels were decreased at t=10 and t=20. Feeding did not significantly influence jugular levels. LQ-fed animals showed higher portal levels as compared to jugular levels at t=-5, t=40 and t=60. Immediately after feed start portal CCK levels were decreased due to feeding from t=3 until t=20. At t=60 portal CCK levels were increased as compared to pre-feeding levels. Jugular concentrations were lowered at t=10 and t=20 and increased at t=60 and t=90. Significant feed quality effect could be demonstrated in both veins at t=-5, t=40 and t=60, where HQ-fed sheep showed lower CCK levels as compared to LQ-fed sheep. PV-JV difference was not influenced except at t=3 in LQ fed sheep where PV-JV difference was decreased to 0.45 pmol/l (Fig. 10).
dAUC (Table 2) was significantly higher in HQ-fed sheep for glucagon and PP. dAUC tended to be lower in HQ-fed sheep for CCK.

Table 2. Postprandial AUC (area under curve) of portal vein minus AUC jugular vein of sheep fed either a HQ or a LQ diet.

	HQ		LQ		P
insulin (mIU.min/l)	10.7	± 1.6	7.5	± 1.9	0.13
glucagon (ng.min/l)	5.9	± 1.1	2.3	± 0.4	0.01
gastrin (nmol.min/l)	4.0	± 1.1	3.0	± 1.6	0.15
PP (nmol.min/l)	16.1	± 4.0	4.7	± 0.4	0.01
CCK (nmol.min/l	0.11	± 0.03	0.18	± 0.02	0.06

values are expressed as means ± SE

DISCUSSION

This study provides substantial information about the postprandial responses of metabolic and gastrointestinal hormones. In most cases, changes due to feeding were observed in both jugular and portal veins. Often, hormone profiles were different due to feed quality.
Increases in plasma concentrations of hormones released in the portal vein suggest an enhanced release of the hormone. It should be noticed that decreased removal of hormone from the blood circulation may also lead to increased concentrations.
There is extensive information about the effects of feeding on insulin levels. Changes in insulin levels due to feeding were reported in lambs (7), sheep (1, 30), goats (8) and cattle (39). Only a few studies reported the early insulin peak (2, 8) as observed in the present study. Animals fed a HQ diet showed a peak in portal insulin concentrations probably due to the increased release during the first minutes (PV-JV difference). The proposed increased insulin release was probably due to a central activation as in non-ruminants (35). It is unlikely that the observed insulin peak was induced by enhanced VFA levels, since VFA levels were not changed during the first minutes (23). The significant decrease in insulin levels observed in both the portal and the jugular veins may rather have been due to decreased release of insulin than increased uptake of insulin by the liver. Possible explanation might lie in increased sympathetic activity since the observed decrease was also observed in portal levels of glucagon. Sympathetic stimulation was reported to decrease levels of glucagon and insulin (32).
After the reported decrease, insulin levels increased again reaching a constant level, which gradually decreased to pre-feeding levels just before the end of the experiment. This second increase was probably due to enhanced release of VFA

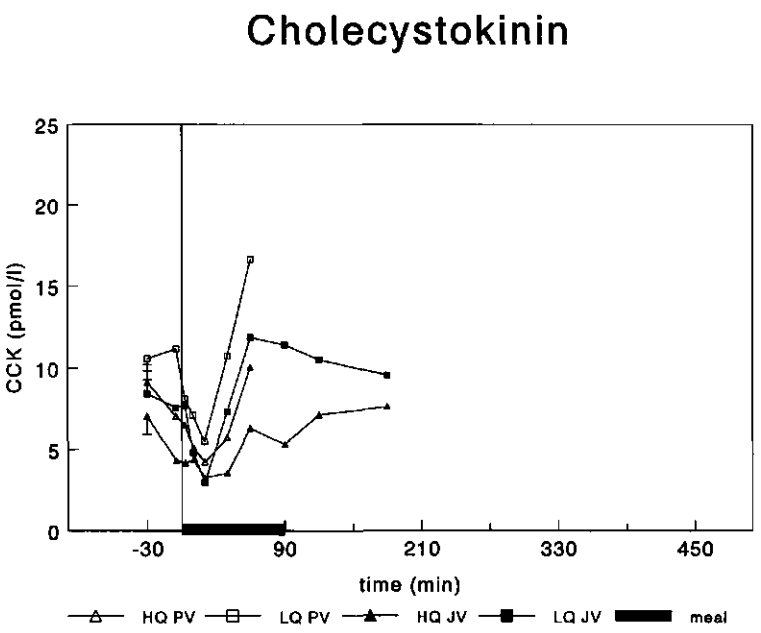

Figure 9: Portal (PV) and jugular (JV) CCK concentration of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

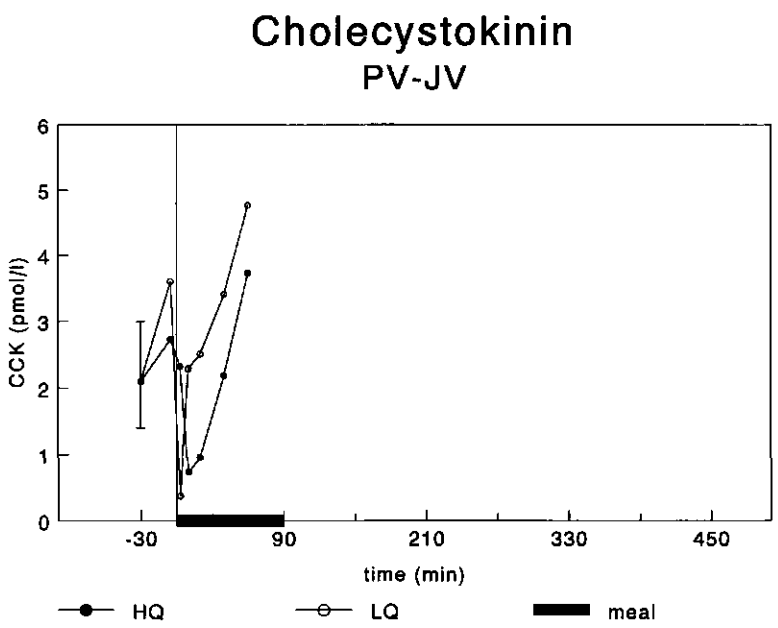

Figure 10: Portal-jugular vein difference of CCK levels of High Quality (HQ) and Low Quality (LQ) fed sheep. Values are means, pooled SE is shown at the first data point.

from the rumen. Several reports show that VFA's are capable of inducing insulin release from the pancreas (30, 33). Insulin levels of LQ-fed animals also showed a bi-phasic pattern, but the magnitude was much smaller probably due to feed characteristics. LQ feed, which contained a higher proportion of slowly degradable constituents, showed a more gradual and possibly smaller release of VFA (23). The effect of feed quality was not clearly observed in dAUC-insulin. Feeding higher amounts of feed or feeding a better feed was reported to be correlated with higher insulin levels (2, 28, 31, 33, 38).

Reports on glucagon levels after feeding are scarce and conflicting (9, 27, 28). In this study, feeding a HQ diet resulted in a small but significant, rapid increase in jugular levels, which is probably para-sympathetically mediated. As with insulin, decreased portal levels were observed after 30 minutes. Surprisingly, levels remained depressed despite the entrance of VFA, which are reported to stimulate glucagon release (30, 32). Both the increase in glucagon levels as well as the decrease resulted from changed release of glucagon in the portal vein. dAUC of HQ-fed sheep was much higher as compared to LQ-fed sheep indicating that glucagon is very sensitive to feed quality.

Growth Hormone levels were not changed when animals were fed the HQ diet. Generally, decreased levels are observed after a meal (1, 2, 9). Although there is no hard evidence, one might assume that activation of the sympathetic nervous system may have abolished the GH decrease. Adrenergic stimulation has been shown to increase GH secretion (40). Alternatively, a decrease in release of somatostatin, may have contributed to the absence of decreased GH concentrations (39). LQ-fed animals showed a postprandial decrease in GH concentration. Explaining the observed decrease is highly speculative since reports concerning the underlying mechanisms are not available. Trenkle postulated that somatostatin release may be increased (39). In this study, no feed quality effect was shown on GH concentration. Other researchers reported a negative relation between GH levels and feed quality (39) and amount of feed supplied (28).

Gastrin levels of HQ-fed and LQ-fed sheep decreased rapidly followed by an increase. Increased levels due to feeding were also reported in calves, lambs, and adult sheep (37) and mono-gastrics (6, 42). The rapid fall in gastrin levels, shortly after meal start was not reported before. Possibly gastrin release was lowered due to a decreased vagal activity since vagal blockade during a meal in dogs led to decreased gastrin levels (6). The postprandial increase most prominently observed in HQ fed sheep may have been caused by entrance of digesta in the duodenum (42). dAUC-gastrin was not significantly influenced by feed quality. The insensitivity of gastrin to feed quality was also shown in cows and sheep (29). In non-ruminants, gastrin release is primarily determined by the direct stimulation of the antral G-cells by amino acids and polypeptides. It is very likely that, due to rumen degradation of protein and microbial protein synthesis, differences in protein amount between diets are partly eliminated (29).

The decreased PP levels of HQ-fed sheep occurred in the same period as the observed decreased levels of insulin, glucagon and gastrin. As with the above mentioned hormones, PP levels were decreased due to a lower release, which might be explained by decreased vagal activity (6, 24). The mild postprandial increase was probably due to entrance of digesta in the small intestine (24). LQ-fed sheep also showed postprandially decreased levels followed by increased levels, but this was preceded by a small but significant increase in PP concentration during the first 5

minutes after feed start. This cephalic response is also reported in monogastrics (24) and less evident in sheep (4).
dAUC-PP was significantly higher in HQ-fed animals as compared to LQ-fed animals. This observation, together with the increased dAUC's of glucagon and less evident insulin, indicate that feeding a HQ feed may have led to larger activation of the pancreas.
Cholecystokinin is thought to induce satiety and is therefore one of the most extensively investigated hormones. Many studies show that CCK or CCK agonist decrease intake in monogastrics (3, 19) and ruminants (1, 6). Whether the reported reductions in feed intake are rather due to induction of malaise than genuine satiety is as yet unclear (17, 25, 37). Surprisingly, very little is known about the effects of a meal on plasma concentration of CCK in ruminants.
Studies in goats (14) and dairy cows (15) revealed no effect of feeding on jugular CCK levels. In the present study, CCK levels were decreased after meal start. Since we measured relatively small number of samples, a clear conclusion on the origin of the observed decreased CCK levels can not be drawn. Considering the decreased PV-JV difference in LQ-fed sheep at t=3 it is tempting to think that decreased release of CCK was responsible for lowered levels. Possibly, decreased vagal stimulation of CCK release may underlie the observed phenomenon (38). In addition to the early decrease in CCK levels, LQ-fed sheep showed slight increased levels after 60 minutes. This post-prandial increase is often shown in monogastrics, due to the entrance of digesta in the duodenum (10).
The present study shows that feeding induces both rapid as well as more sustained changes in hormone concentration. The rapid fluctuations in insulin, glucagon, gastrin, PP and CCK were likely due to decreased para-sympathetic activity and/or increased sympathetic activity. The observed rapid changes as described in the present study were rarely observed in non-ruminants. On the other hand, changes occurring after approximately 30-40 minutes resemble changes in hormone levels reported in non-ruminants.
Generally, magnitude of the meal induced changes was affected by feed quality, with the HQ-fed sheep showing more pronounced differences. Profiles were often more or less similar except for GH.

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CHAPTER 4

Effect of short-term propionate infusion on feed intake and blood parameters in sheep

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ABSTRACT

The hypothesis that propionate is a short-term feed intake regulating agent was studied. Mature wether sheep were infused over 20 min with Na-propionate into the mesenteric vein, while feed intake and feeding pattern were monitored over 1.5 hours. Feed intake was reduced by infusions at 2 mmol/min, which were associated with marked increases in jugular as well as portal concentrations of insulin, glucose and propionate.
In a second experiment, animals were infused with 2 mmol/min Na-propionate into the portal vein. No decrease in feed intake was observed, although there were similar increases in insulin, glucose and propionate as found in mesenteric vein-infused animals. It is concluded that mesenteric propionate in high doses acts as a satiety factor. Possible explanations for the difference between site of infusion may be a different distribution of the infusate over the liver, and/or the presence of propionate sensitive receptors in the mesenteric/portal vein region. It seems unlikely that insulin concentrations are involved in inducing satiety in propionate infused animals.

keywords: volatile fatty acids, insulin, glucose, ruminants

INTRODUCTION

Short-term feed intake (meal) is thought to be controlled by a variety of peripheral signals (7, 8, 12). These signals are transferred to the central nervous system through nervous pathways or blood circulation. The hypothalamus has been ascribed a decisive role in integrating these signals (13, 21, 25). Moreover, it is recognized that there is no overall governor of feed intake (7). This is true for centers in the brain as well as peripheral signals.
In non-ruminants, much work has been done on the glucostatic theory of Mayer. This theory along with other "static theories" like the lipostatic and aminostatic theories proved to be too simple (18, 19). It is more likely that lipostatic, aminostatic and glucostatic mechanisms are working in concert, giving information about the metabolic status of the individual. There is also a great interest in the involvement of the liver. The liver which is strategically well placed in the bloodstream, is a potential target organ as a sensory organ on feed intake (1, 2, 20)
Energy nutrients are candidates for feed intake control. In non-ruminants, it was shown that infusion of glucose decreased feed intake not only because of its osmotic load (26). In ruminants, volatile fatty acids are the major contributors for meeting the energy requirements of the animal (5). Propionate, which is the major precursor for glucose, seems to have some analogies with glucose. It is a potent insulin-releasing agent in ruminants (14, 16, 17, 22), and in some studies it decreased feed intake (9, 10). In addition, it was shown that liver denervation abolished the feed intake-reducing effect of propionate (2). Although the feed intake-reducing effect is supported by a large number of experiments with propionate infusions over wide concentration ranges, there are also contrasting results (23). In an extensive research by De Jong (6) in goats, no effect was found with volatile fatty acids (VFA) infused in a physiological range. Anil and Forbes (2) argued that one of the possible explanations might lie in the site of infusion. In the majority of the trials, infusions were done over longer periods (several hours) while the time spent on eating a meal was much less.

Therefore, the preferred method is to infuse the animals during a much shorter period. The aim of the present study is to determine whether portal propionate acts as a physiological satiety signal in meal-fed animals.

MATERIALS AND METHODS

Animals
Seven adult Swifter wether sheep (98 ± 3 kg LW) were housed indoors in groundpens and were maintained at room temperature (18 ± 2 °C). Lights were on from 06.00 - 21.00.

Surgery
Animals were provided with catheters in the portal, mesenteric, and jugular veins as described previously (28). Animals were routinely treated postsurgically with analgesics and antibiotics. Catheter patency was maintained by weekly flushing with physiological saline containing heparin (5000 IU/l). Experiments were started six weeks after surgery when feed intake and body weight were normal again.

Feed
Animals were fed a pelleted grass diet, containing in dry matter: 14.9% crude protein, 18.9% cellulose, 16.3% hemicellulose, 4.0 % lignin and 16.8 % ash. Feed was analyzed as described by van Bruchem (27).
Feed (45 g/kg$^{0.75}$) was offered three times daily at 8-h intervals. Feed was offered for 1.5 h, and the refusals were automatically discarded and weighed.

Blood sampling
Blood samples were withdrawn through polyethylene tubes that were connected to the animals' catheters at least 30 min before the start of the experiment. Blood sampling occurred without the animal being handled. In this way, stress as a result of blood sampling was minimized. Before and after the experiments, sampling lines were cold sterilized with 70% ethanol.

Analyses
Blood samples were collected in chilled tubes containing EDTA. Tubes were centrifuged (4 °C, 3000 RPM) and plasma was stored in small aliquots at -20 °C before analysis.
Plasma glucose was determined spectrophotometrically by the glucose oxidase-peroxidase anti-peroxidase method using a kit (Boehringer Mannheim No 166391). Plasma insulin was determined by radioimmunoassay using porcine insulin as standard and tracer. The sensitivity of the assay was 1.5 μU/ml. Plasma VFA's were determined in deproteinized plasma by gas chromatography.
Feeding pattern was measured via an infra-red sensor placed in the feeding bin. Whenever the infra-red light-beam was broken, a signal was transferred to a computer that registered the time an animal kept its head in the feeding bin.

Infusions
Na-propionate infusate (Merck, Hohenbrunn, Germany) was adjusted to pH 7.5 with NaOH. Infusates were passed through a 0.2 μm filter and autoclaved before infusion. Osmolality of the solutions was adjusted to .96 osmol/kg H_20 with NaCl. The

control solution was therefore an NaCl solution with the same osmolality as the propionate solutions.
Infusion was performed via polyethylene tubing, which did not adsorb propionate. The solutions were infused with the use of a peristaltic pump (Watson Marlow, Fallmouth, UK) at a speed of 4 ml/min.

Experiment 1
The animals were randomly infused with Na-propionate in the mesenteric vein at a rate of 0, 1 or 2 mmol/min. Each animal received the treatment once. Experiments were performed every second day.
Infusion was started at 8.00 (feed offering), and lasted for 20 minutes. Blood samples were withdrawn from portal and jugular veins 10 minutes before(t_{-10}), 10 minutes during (t_{10}) and 10 minutes after infusion(t_{30}).

Experiment 2
The animals were randomly infused with Na-propionate in the portal vein at a rate of 0 or 2 mmol/min. Each animal received the treatment once. Experiments were performed every second day.
Infusion was started at 8.00 h (feed offering), and lasted for 20 minutes. Blood samples were withdrawn from the jugular vein 10 minutes before(t_{-10}), 10 minutes during (t_{10}) and 10 minutes after infusion(t_{30}). Due to the infusion in the portal vein, no sample was withdrawn from the portal vein during the infusion.

Calculations and statistics
All results are expressed as means (n=7) ± SE. The data were analyzed using analysis of variance (General Linear Modeling procedure; SAS, Cary, NC) (24).The model used was $y = \mu$ + sheep + treatment + vein + (vein x treatment) + error. Data were analyzed by time.
Dose-response relationship was tested with the regression procedure of SAS.

RESULTS

Experiment 1
Infusion of 2 mmol/min Na-propionate into the mesenteric vein resulted in a decrease in feed intake (Fig. 1). However, neither total eating time, nor eating pattern were influenced (Fig. 2).
Plasma levels of propionate, insulin as well as glucose were generally increased in the portal vein (Table 1) as well as the jugular vein (Table 2).
Infusion at the lower rate of 1 mmol/min did not lower feed intake significantly (Fig. 1), but increased plasma levels of insulin in both portal (Table 1) and jugular (Table 2) veins.

Experiment 2
Infusion of 2 mmol/min of propionate into the portal vein did not reduce feed intake (802g ± 114 saline vs. 764g ± 53 Na-propionate). Similar to experiment 1, neither total eating time nor eating pattern was influenced by the infusion (Fig. 3).
Plasma levels of propionate, insulin and glucose were increased in the jugular vein (Table 3). In the portal vein, postinfusion levels were similar to preinfusion levels (data not shown).

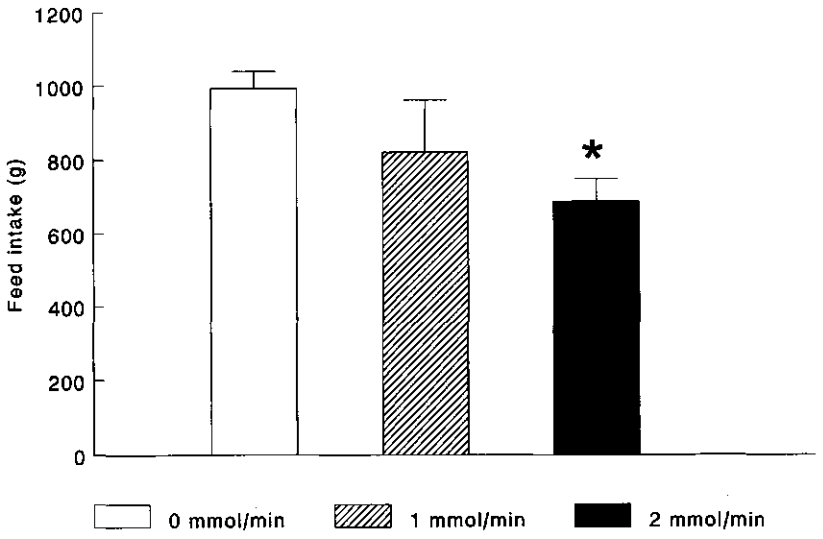

Figure 1: Feed intake (g) over 90 minutes after Na-propionate infusion in the mesenteric vein. Values are means ± SE. * P<0.05

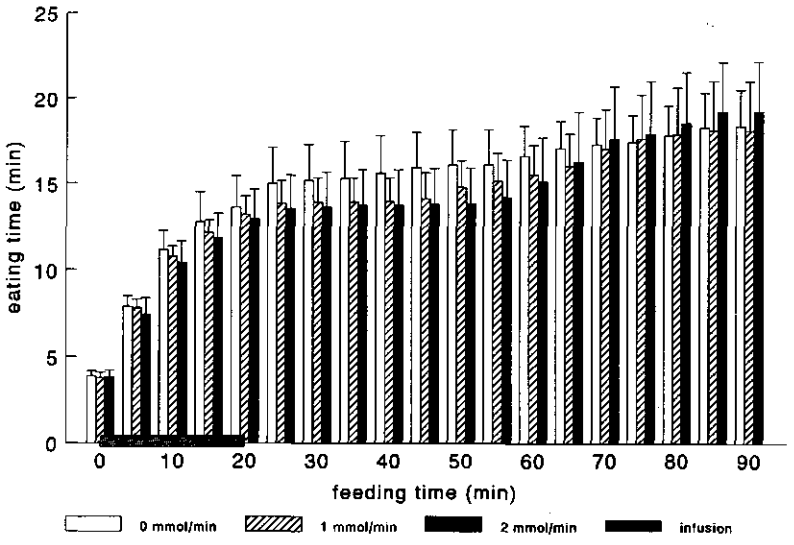

Figure 2: Cumulative eating time, defined as the time spent eating for each successive 5-min period, in mesenteric Na-propionate infused sheep. Values are means ± SE.

Table 1. Portal vein concentrations of propionate (mmol/l), insulin (µIU/ml) and glucose (mmol/l) in relation to a mesenteric Na-propionate infusion

	Propionate, mmol/l	Insulin, µIU/ml	Glucose, mmol/l
		0 mmol/min Na-propionate/min	
Before	0.34 ± .11	78±9	3.1±0.1
During	0.36 ± .07	87±8	3.2±0.1
After	0.41 ± .09	80±11	3.2±0.1
		1 mmol/min Na-propionate/min	
Before	0.31 ± .02	61±3	3.1±0.1
During	1.31 ± .78	149±31a	3.3±0.1
After	0.39 ± .05	72±11	3.2±0.1
		2 mmol/min Na-propionate/min	
Before	0.35 ± .08	68±12	3.1±0.1
During	1.71 ± .91ab	297±40ab	3.6±0.2b
After	0.50 ± .06	76±14	3.1±0.1

Values are means±SE. Statistical significance: a=different from 0 mmol/l, b different from 1 mmol/min ($p<0.05$)

Table 2. Jugular vein concentrations of propionate (mmol/l), insulin (µIU/ml) and glucose (mmol/l) in relation to a mesenteric Na-propionate infusion

	Propionate, mmol/l	Insulin, µIU/ml	Glucose, mmol/l
		0 mmol/min Na-propionate/min	
Before	0.06±.01	56±8	3.1±0.1
During	0.05±.07	72±5	3.3±0.1
After	0.05±.09	61±6	3.2±0.1
		1 mmol/min Na-propionate/min	
Before	0.04±.01	62±3	3.1±0.1
During	0.08±.01	138±22a	3.2±0.1
After	0.04±.01	70±8	3.1±0.1
		2 mmol/min Na-propionate/min	
Before	0.05±.01	68±11	3.0±0.1
During	0.24±.05ab	244±22ab	3.5±0.1b
After	0.07±.02	86±15	3.1±0.1

Values are means±SE. Statistical significance: a=different from 0 mmol/l, b different from 1 mmol/min ($p<0.05$)

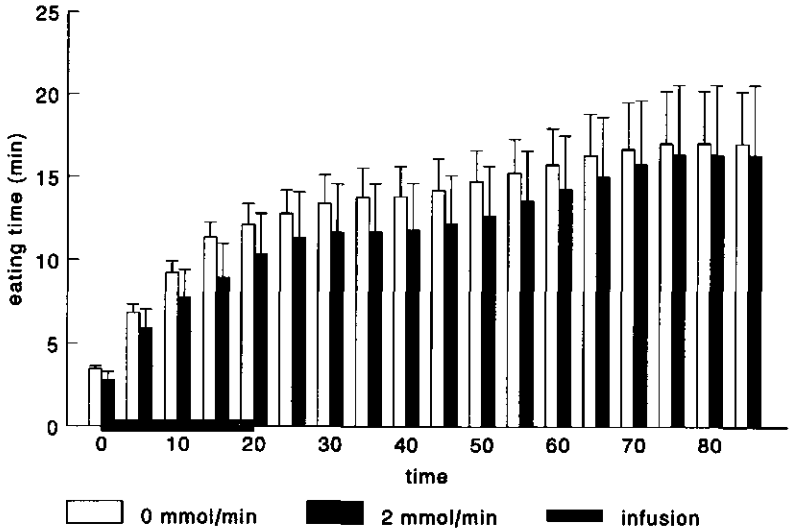

Figure 3: Cumulative eating time, defined as the time spent eating for each successive 5-min period, in portal Na-propionate infused sheep. Values are means ± SE.

Table 3. Jugular vein concentrations of propionate (mmol/l), insulin (µIU/ml) and glucose (mmol/l) in relation to a portal Na-propionate infusion

	Propionate, mmol/l	Insulin, µIU/ml	Glucose, mmol/l
		0 mmol/min Na-propionate/min	
Before	0.04±.01	90±7	3.4±0.1
During	0.06±.01	94±6	3.4±0.1
After	0.06±.01	85±6	3.3±0.1
		2 mmol/min Na-propionate/min	
Before	0.03±.01	88±6	3.3±0.1
During	0.16±.02a	244±44a	3.7±0.1a
After	0.05±.01	125±8a	3.0±0.1

Values are means±SE.
Statistical significance: a=different from 0 mmol/l ($p<0.05$)

DISCUSSION

Infusion of 2 mmol/min propionate into the mesenteric vein resulted in a decreased intake, whereas infusion of 1 mmol/min did not change feed intake. Reduction in intake by infusion of propionate is a well-known phenomenon. In most studies, however, the animals were infused for a prolonged period. In the present study, duration of the infusion was set at 20 minutes, while the animals were allowed to eat for another 70 minutes. The observation that the eating patterns were not different between the groups shows that satiety was not due to sickness. This implies that the infused propionate acted as a signal of satiety that was not counteracted during the meal period. The lowered intake, without a reduction in eating time, may be due to less active eating (smaller bites and/or slower chewing), which also occurs as a meal progresses (11).

Although many studies found a feed reduction in response to propionate infusion, others did not. In Table 4, a summary of Na-propionate infusion experiments in sheep is given.

The overall impression is that infusion rates of 1.2 mmol/min or higher reduced intake, whereas lower infusion rates do not effect feed intake. The only exception is an experiment by Farningham et al. (9) in which 1.2 mmol/min failed to reduce intake. In another paper, Farningham and Whyte (10) showed a dose-dependent relation between feed intake reduction and propionate infusion.

The discrepancy between the two experiments probably lies in the fact that, in the first-mentioned experiment the control animals were not infused with saline. We found in several experiments that animals ate larger amounts of feed during experimental days compared with nontrial days (unpublished data). In the second-mentioned paper, a dose of 1.2 mmol/min reduced intake compared with controls infused with saline.

Infusion of propionate in the portal vein did not result in a decreased intake. The reason possibly lies in the position of the catheter. The tip of the catheter was placed in the hilum of the portal vein, close to the liver. It was postulated by Anil and Forbes (2) that infusion at this site may explain the negative results found by De Jong (6). Our observations are in agreement with this explanation. Portal infusion may result in a poorer distribution over the liver, which proved to be important in the feed intake-reducing action of propionate (2). On the other hand, it could also mean that propionate-sensitive receptors, if present, in the mesenteric/portal vein region would not be reached. Baile (4) postulated the existence of ruminal vein receptors.

Although there is not much data on postprandial levels of propionate in the portal vein, it may be assumed that increases of 0.1-0.6 mmol/l occur after a meal. In sheep, net portal fluxes are calculated in the range of 0.3 mmol/min (3) to 1.6 mmol/min (10), with portal blood flows between 1 and 3 l/min. Jugular propionate concentrations following a meal can reach levels of 0.05-0.06 mmol/l when fed a hay diet, which is about twice basal level (unpublished data).

In the present study, infusion of 1 mmol/min tended to increase portal as well as jugular vein propionate concentrations only moderately, whereas infusion of 2 mmol/min resulted in a more than fourfold increase in both veins ($P<0.05$). Linear regression analyses showed a dose-response relationship in both the jugular as the portal veins (Table 5). It is questionable whether the increases found with the 2 mmol/min infusions are within a physiological range, since both jugular as well as portal increases are larger than those observed after a meal.

Table 4. Results of low-dose propionate infusion studies in the hepatic region of sheep

Diet	regime	BW	sex	inf route	dose/min	dos/min.kg bw	duration	intake red	ref
50% alfalfa + 50% corn	ad lib	78	female	Portal	1	0,0131	240 min	none	22
Hay	63 % maint	79	female	Portal	1	0,0131	240 min	none	22
hay + concent	ad lib	40-50	female	Portal	1,2	0,027	120 min	none	9
hay + concent	ad lib	40-50	female	Portal	2,4	0,053	120 min	40 %	9
hay + concent	ad lib	60-90	female	Portal	0,6	0,008	180 min	none	10
hay + concent	ad lib	60-90	female	Portal	0,9	0,012	180 min	none	10
hay + concent	ad lib	60-90	female	Portal	1,2	0,016	180 min	42 %	10
hay + concent	ad lib	60-90	female	Portal	1,8	0,024	180 min	57 %	10
hay + concent	ad lib	60-90	female	Portal	2,5	0,033	180 min	58 %	10
50%grass pellet 50% barley	ad lib	42-57	wether	Portal	0,6	0,012	180 min	none	1
50%grass pellet 50% barley	ad lib	42-57	wether	Portal	1,2	0,024	180 min	81 %	1
Unknown	ad lib	35-55	male	Portal	1,2	0,026	180 min	71 %	2

Propionate infuison into the portal vein. Ad lib, ad libitum

Insulin levels in both veins increased as a result of Na-propionate infusion in both experiments. The insulin releasing activity of propionate has been shown before (14, 16, 17). Linear regression analyses showed a linear dose-response relationship for the mesenteric vein infused animals (Table 5). The increases due to 1 mmol/min are within a range normally observed after a meal (15). However, infusion of 2 mmol/min led to supra-physiological insulin levels of over 200 μIU/ml in both experiments.
Propionate, the major precursor for glucose in ruminants, enhanced glucose levels significantly during infusion of 2 mmol/min propionate. A weak linear regression was only found between portal glucose and Na-propionate infusion in the mesenteric vein infused animals (Table 5). The extraordinary insulin increase may be a consequence of both the increase in propionate as well as glucose.

Table 5. Dose-response relation of propionate, insulin and glucose in mesenteric vein infused animals.

	intercept	slope	r^2	P
		Jugular vein		
Propionate	0.03±0.03	0.09±0.02	0.47	0.001
Insulin	65±19	86±15	0.08	0.0001
Glucose	58±2	2±1	0.47	0.23
		Portal vein		
Propionate	0.43±0.31	0.67±0.23	0.39	0.01
Insulin	75±22	104±25	0.59	0.001
Glucose	57±2	1.5±1	0.33	0.03

Values are means±SE. Regression parameters calculated at t=10 min.

Because levels of propionate, insulin and glucose were increased due to infusion of 2 mmol/min Na-propionate in both experiments, it can therefore be concluded that neither insulin nor glucose contributed to the feed intake reduction due to propionate infusion in this particular experiment.
Although not significant, the intake of animals infused with saline, was lower in the second experiment compared to the first experiment. This may be due to the somewhat higher insulin levels, which may be a result of a slightly higher glucose concentration of the animals used in experiment 2.
This study provides evidence that propionate can induce satiety without disturbing the normal eating pattern. Because levels of propionate inducing satiety were supraphysiological, it is not likely that satiety is normally evoked by propionate solely. However, it is to be expected that propionate is one of a whole set of satiety signals.

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CHAPTER 5

Mesenteric infusion of propionate induces changes in VFA, insulin and gastrointestinal hormone concentrations

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ABSTRACT

During a 90 minutes feeding period, sheep provided with jugular, portal and mesenteric catheters were infused via the mesenteric catheter with 0, 1.5 or 6 mmol/min Na-propionate for 20 minutes. Blood was frequently sampled from jugular and portal veins.
Infusion of 6 mmol/min Na-propionate decreased feed intake but also induced discomfort. Portal levels of propionate, glucose and insulin were increased while decreased levels of butyrate, beta-hydroxy-butyrate, gastrin, pancreatic polypeptide (PP) and CCK were observed. Jugular levels generally showed similar patterns as portal levels except for butyrate. Jugular butyrate was immediately increased after start of the meal, presumably due to a smaller liver uptake of butyrate.
Infusion of 1.5 mmol/min Na-propionate resulted in elevated levels of propionate and insulin while gastrin and PP concentrations were decreased.
It was concluded that propionate is not a major factor influencing meal size. However, it is possible that effects found during and after a meal on insulin, gastrin, and PP can be attributed to propionate.

keywords: insulin, cholecystokinin, pancreatic polypeptide, gastrin, feed intake, propionate, volatile fatty acids, ruminants.

INTRODUCTION

In the search for blood constituents contributing to satiety in ruminants, considerable attention was paid to propionate (8, 10). It was shown that after a meal, propionate levels are increasing and represent feeding level or feed quality (7, 12). Infusions of propionate in the rumen and blood were effective in decreasing intake, but levels infused are often high (10, 16). Physiological doses infused in the portal system have minor or no effect, weakening the hypothesis that propionate is a major factor contributing to satiety (5, 10).
Additionally, propionate is reported to stimulate release of insulin and glucagon, often accompanied by enhanced glucose production (4, 17, 18). Feeding can induce remarkable changes in blood levels of insulin and glucagon (11). Other hormones, which may also be involved in satiety, like the gastrointestinal hormones CCK, gastrin and pancreatic polypeptide, are also influenced by feed intake (11). The effects of propionate infusion on gastrointestinal hormones in ruminants are not known.
It is possible that the intake reduction found after propionate infusion may be an indirect effect via other metabolites or hormones (9). The present study was designed to investigate the effect of propionate infusion on feed intake and blood concentrations of metabolites and hormones related to feeding.

MATERIALS AND METHODS

The study was performed with 7 wether sheep (98 ± 3 kg LW) provided with permanent catheters in the jugular, portal and mesenteric veins (19). Animals were fed a grass pellet diet containing (in dry matter): 831 g/kg organic matter, 150 g/kg crude protein, 189 g/kg cellulose, 162 g/kg hemi-cellulose and 40 g/kg lignin.

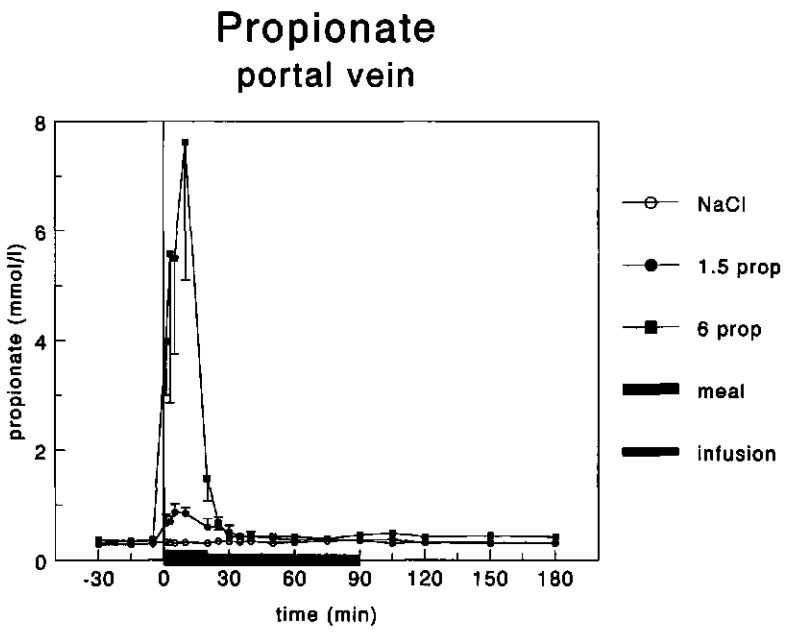

Figure 1: Portal propionate concentration in mesenteric Na-propionate infused sheep. Values are means ± SE.

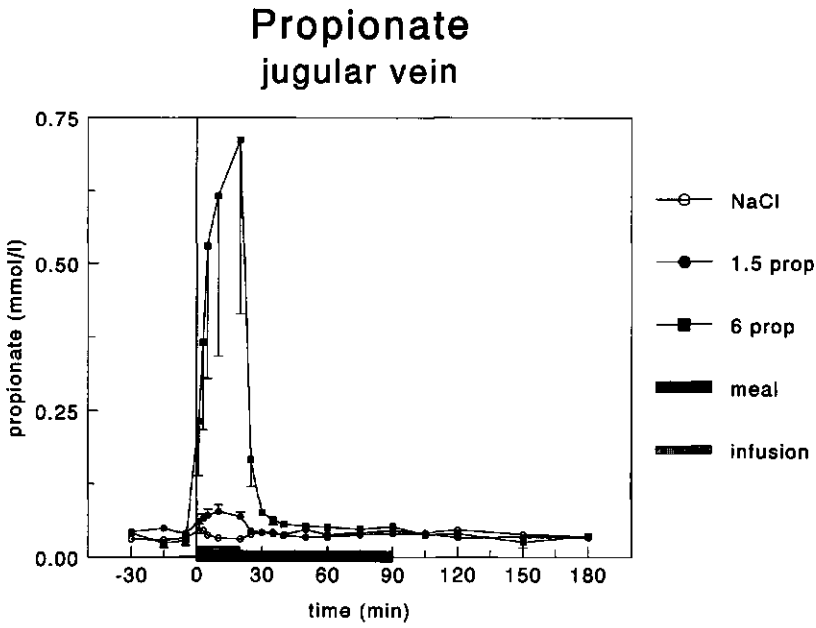

Figure 2: Jugular propionate concentration in mesenteric Na-propionate infused sheep. Values are means ± SE.

Feeding regime, chemical analyses, sampling procedures, experimental set up and statistical analyses were as described previously (11, 12).
Concisely, feed was provided three times daily with 8-hour intervals. Each meal period, feed was available for 90 minutes. Residues were discarded automatically and weighed. On trial days, animals were attached to sampling and infusion devices. Blood was withdrawn during 3.5 hours, before, during and after a meal period of 90 minutes. Animals were infused with the experimental solutions during the first 20 minutes of the meal period.

Infusate
Na-propionate infusate (Merck, Germany) was adjusted to pH 7.5 with NaOH. Infusates were passed through a 0.2 µm filter and autoclaved before infusion. Na-propionate concentrations of the infusate were 0, 0.375 and 1.5 mol/l. Osmolality of the control solution was adjusted to 0.72 osmol (same osmolality as the 0.375 mol/l propionate solution).
Infusion was performed via polyethylene tubing, which did not adsorb propionate. Solutions were infused using a peristaltic pump (Watson Marlow, UK) at a speed of 4 ml/min.
Animals were randomly infused with Na-propionate in the mesenteric vein at a rate of 0, 1.5 or 6 mmol/min. Each animal received the control and the 1.5 mmol/min treatment once. Only 3 animals were infused with the 6 mmol/min infusion due to abnormal behaviour of the animals.
Experiments were performed once a week.

Calculations
Portal vein - jugular vein differences were calculated for each time point as an indicator for portal appearance of nutrients produced in the portal drained viscera. Difference in area under the curve (dAUC) of the portal and jugular curves were calculated over the infusion period (dAUC-inf) and post-infusion (dAUC-post) period, as indicator for production during and after the infusion period.

RESULTS

Feed intake of sheep infused with 6 mmol/min (454 ± 80 g) was significantly lower as compared to NaCl (951 ± 30 g) and 1.5 mmol/min (898 ± 56 g) infused animals.
Infusion of propionate at a rate of 1.5 mmol/min as well as 6 mmol/min increased portal propionate levels as compared to basal level during the infusion period (Fig. 1). Jugular levels of 6 mmol/min infused sheep were increased during infusion as compared to basal levels, while no difference was demonstrated for the control and 1.5 mmol/min treated animals (Fig. 2).
Figure 3 shows that acetate levels remained at pre-feeding levels in the portal vein. No differences between treatments could be demonstrated. Jugular acetate levels also showed no changes, but were significantly lower as compared to portal vein levels (data not shown).
Portal butyrate levels increased in both the NaCl and 1.5 mmol/min Na-propionate infused groups after meal start (Fig. 4). Sheep infused with 6 mmol/min Na-propionate showed lower portal levels from t=20 until t=35 minutes. After t=90 min levels were increased above basal levels.

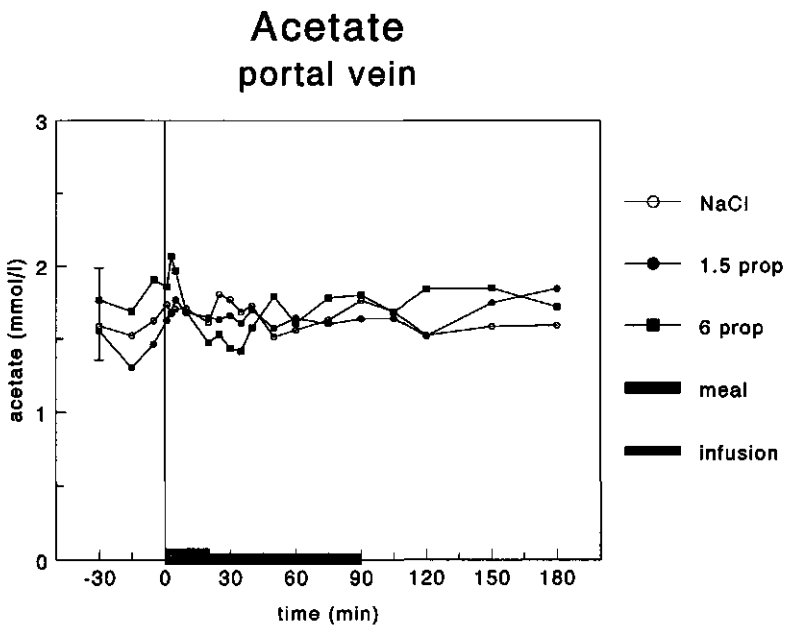

Figure 3: Portal acetate concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

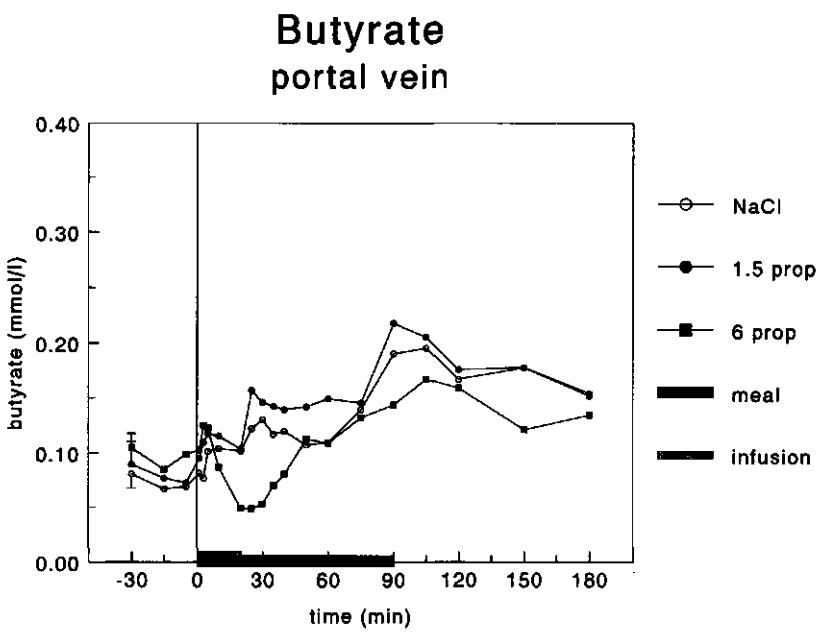

Figure 4: Portal butyrate concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

Portal beta-hydroxy-butyrate (BHB) concentrations are shown in figure 5. In NaCl and 1.5 mmol/min Na-propionate infused animals, levels were elevated postprandially. Infusion of 6 mmol/min Na-propionate resulted in lowered BHB levels immediately after end of the infusion period, after which levels returned to control levels. Jugular BHB levels showed similar patterns as portal levels, but concentrations were significantly lower (data not shown).
Portal glucose levels (Fig. 6) remained unchanged in the NaCl treated group. Infusion of 1.5 mmol/min Na-propionate resulted in a small but significant increase from t=5 until t=20 min. Increased glucose levels due to 6 mmol/min Na-propionate infusion were shown from t=5 until t=25. At t=60 and t=75 glucose levels were significantly below basal levels. At t=40 and 50 min, only a tendency could be demonstrated ($p<0.1$).
Jugular glucose levels showed similar patterns as portal glucose levels and were generally slightly lower as compared to portal levels (data not shown).
Insulin levels in the portal vein (Fig. 7) were increased in the 1.5 mmol/min Na-propionate infused group from t=3 until t=20 min. 6 mmol/min infusion of Na-propionate resulted in major increased insulin concentrations from t=1 until t=35. Jugular insulin levels showed similar pattern but were generally lower than portal levels (data not shown).
Portal CCK levels are shown in figure 8. No differences from basal level could be demonstrated except for the 6 mmol/min infused group which showed lower levels from t=20 until t=40. Jugular levels showed similar and were not significantly different from portal levels (data not shown).

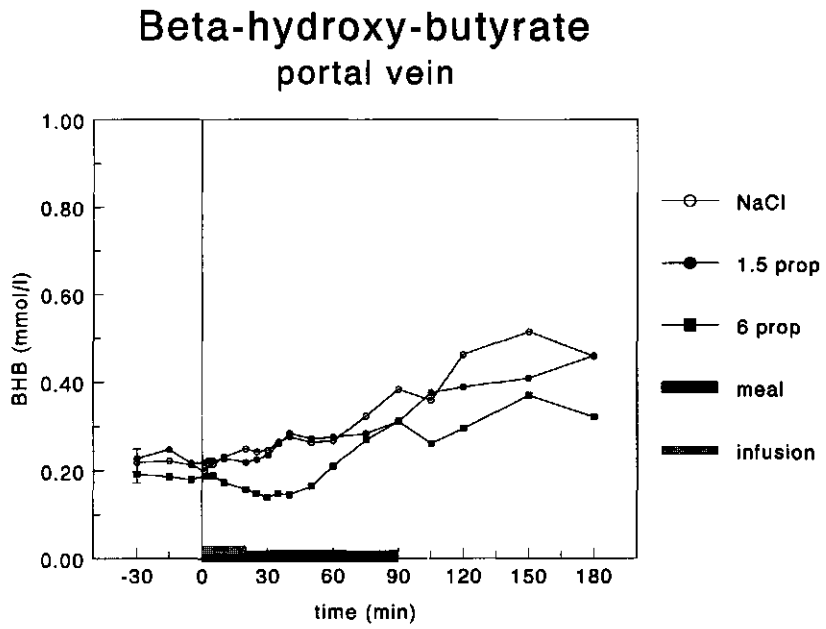

Figure 5: Portal beta-hydroxy-butyrate concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

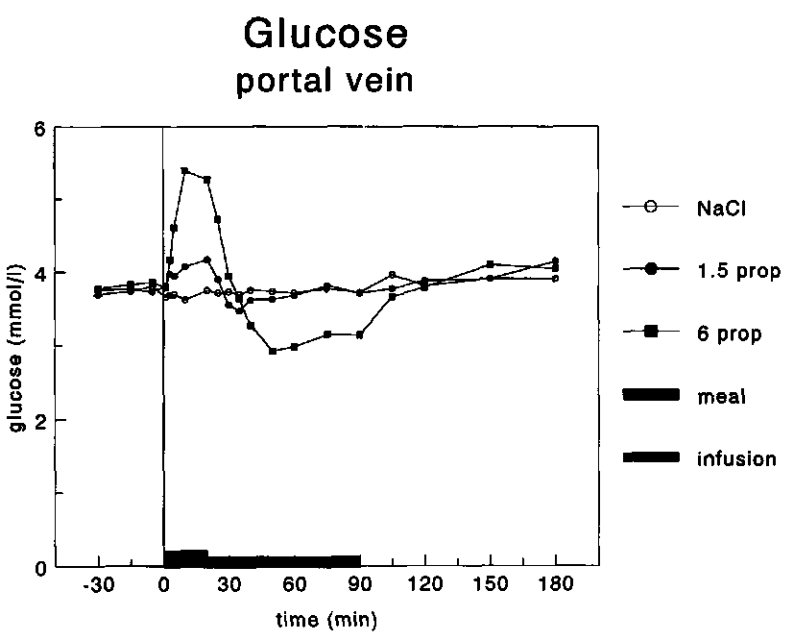

Figure 6: Portal glucose concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

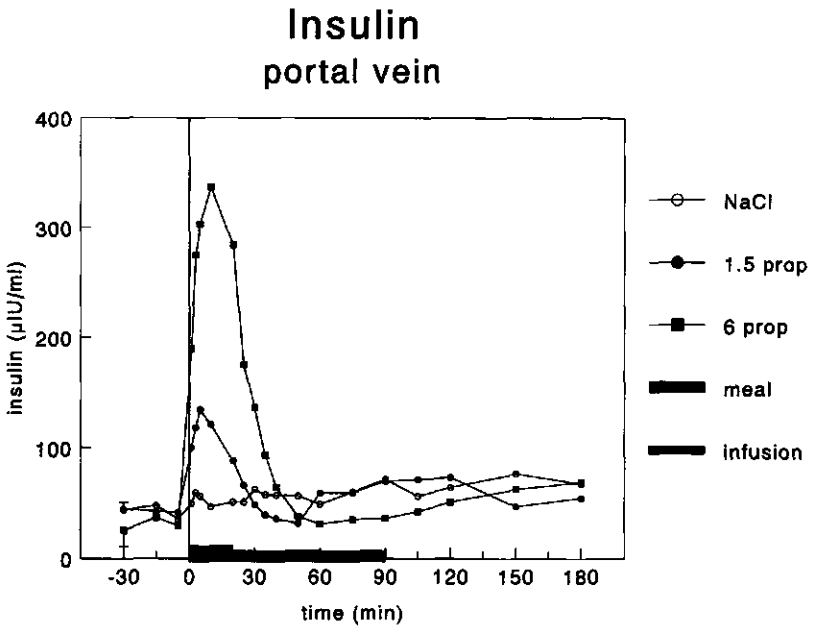

Figure 7: Portal insulin concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

Portal gastrin levels (Fig. 9) were decreasing below basal level in the 1.5 mmol/min Na-propionate infused group from t=10 until t=30 minutes. Animals infused at a rate of 6 mmol/min Na-propionate showed decreased levels from t=10 until t=50.
Portal Pancreatic Polypeptide (PP) levels are shown in figure 10. Animals infused with NaCl showed lowered PP levels from t=90 until t=180 compared to basal level. Infusion of 1.5 mmol/min Na-propionate led to decreased concentrations from t=10 until t=30 min and from t=105 until t=180 min. 6 mmol/min Na-propionate infused animals showed lower PP levels from t=10 until t=60 min and from t=120 until t=180 min as compared to basal levels.Estimated production during infusion and during the whole sampling period are given in Table 1. dAUC-butyrate was significantly decreased during infusion of 6 mmol/min Na-propionate. dAUC-glucose was increased during infusion of 6 mmol/min Na-propionate. dAUC-insulin was increased during infusion of Na-propionate at both 1.5 mmol/min as well as 6 mmol/min. Post infusion dAUC-insulin was enhanced in the 1.5 mmol/min Na-propionate infused group. dAUC-CCK was enhanced during infusion of 6 mmol/min Na-propionate.

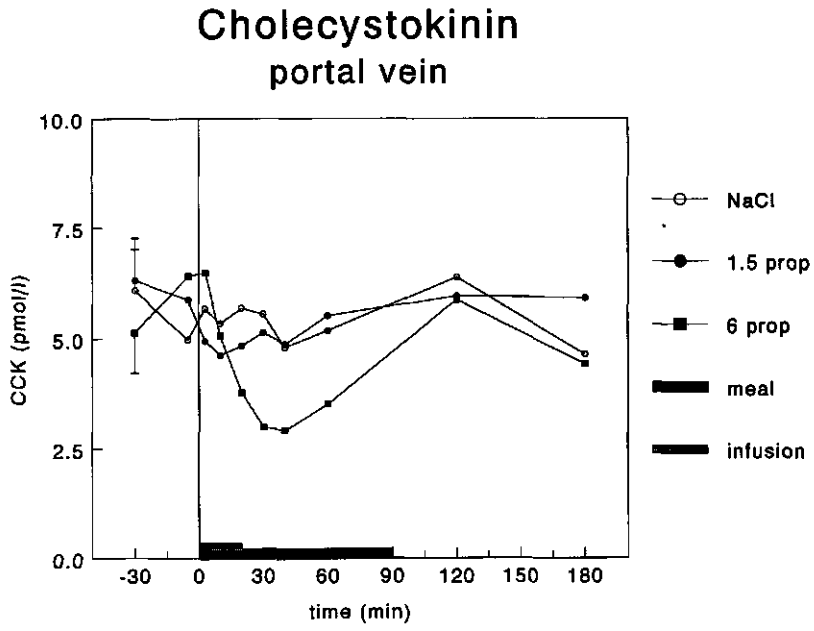

Figure 8: Portal CCK concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

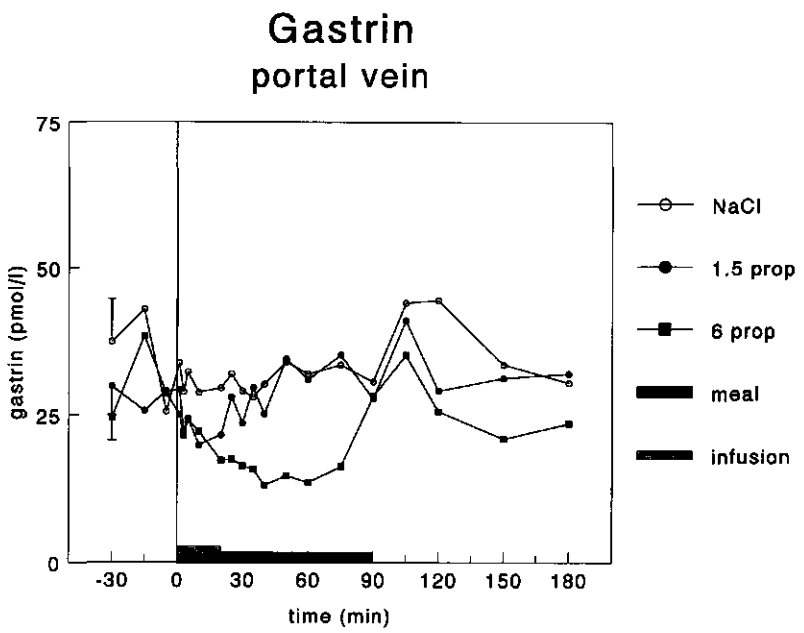

Figure 9: Portal gastrin concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

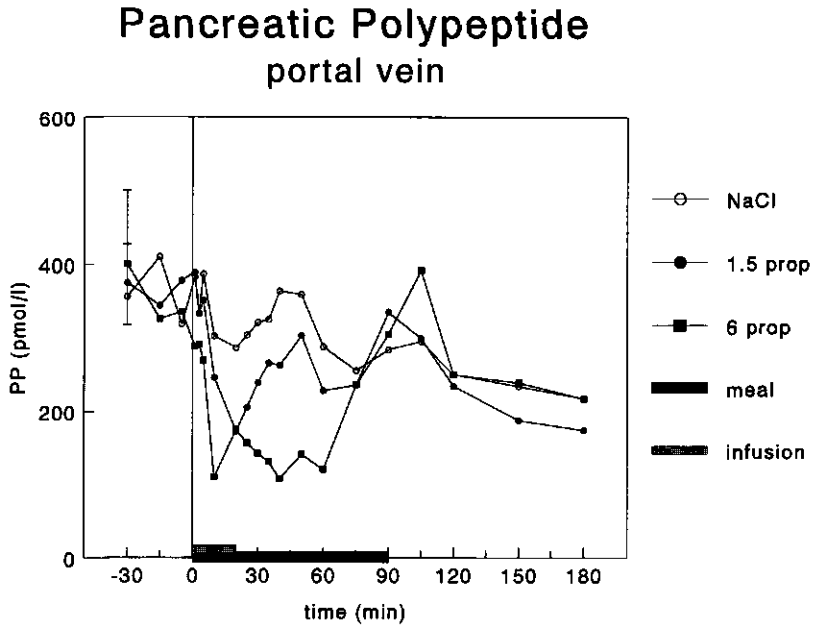

Figure 10: Portal PP concentration in mesenteric Na-propionate infused sheep. Values are means, pooled SE is shown at the first data point.

DISCUSSION

Many studies report the intake reducing effect of propionate (6, 8, 10). Although one could argue that levels of infusion necessary to influence intake are non-physiological, the effect is thought to be specific and not due to the osmotic pressure changes (6, 9). In the present study, infusion of a high dose of Na-propionate at a rate of 6 mmol/min resulted in a significant reduction in intake. However, intake reduction was probably due to malaise. Sheep infused with a 6 mmol/min Na-propionate were shivering and seemed uncomfortable. The abnormal behaviour of the sheep made us decide to limit the number of animals infused with this apparently very high dosage. Infusion of 1.5 mmol/min Na-propionate, which can be considered as a physiological dosage, did not influence intake. This may strengthen the hypothesis that regulation of spontaneous meals is not mediated through propionate (4, 10).

In contrast to the effects of propionate infusion on intake, less is known about the effects on other metabolites and hormones. In the present study, we investigated the effects of Na-propionate infusion on other metabolites and hormones, which are related to feeding. It is obvious that infusion of Na-propionate led to increased portal propionate levels. Propionate concentrations in the jugular vein were much lower than portal levels due to a very high extraction of propionate by the liver (1, 2). Jugular levels were increased due to infusion of Na-propionate at a rate of 6 mmol/min but not by infusion at a rate of 1.5 mmol/min indicating that the liver extracted the major part of the infused Na-propionate.

Portal butyrate levels of NaCl and 1.5 mmol/min Na-propionate infused animals increased gradually. This was likely due to the intake of feed as reported earlier (12). Portal butyrate levels were significantly decreased in the 6 mmol/min Na-propionate infused animals. dAUC-butyrate was decreased during infusion of 6 mmol/min Na-propionate. This could imply a lower uptake from the rumen. On the other hand it could also mean that more butyrate was metabolised by the portal-drained viscera (3, 13). In this case it is more likely that less butyrate was absorbed from the rumen since also BHB levels decreased from t=20 until t=70 min in the 6 mmol/min infused group.

As with butyrate levels, portal BHB levels were increased after meal start in NaCl infused animals. BHB levels in 1.5 mmol/min Na-propionate infused animals were also increasing albeit at a later time point (from t=50 until t=180 min). Animals infused with Na-propionate at a rate of 6 mmol/min showed initially decreased levels followed by increased levels. Lowered BHB levels were most likely due to reduced availability of butyrate which is a precursor of BHB (3).

Glucose levels were increased during the infusion of Na-propionate since propionate is the major precursor for glucose (2, 3). During infusion, dAUC-glucose was increased in both the 1.5 as well as the 6 mmol/min Na-propionate infused group. Decreased levels were most likely due to a massive release of insulin. Infusion of Na-propionate resulted in enhanced insulin release. The insulin releasing properties of propionate are well described (14, 17, 18). In the present study the increase in insulin concentrations may have been intensified by the increase in glucose levels.

Infusion of Na-propionate at a rate of 6 mmol/min resulted in lowered CCK levels. In contradiction, dAUC-CCK was increased in 6 mmol/min Na-propionate infused animals indicating that uptake was enhanced to an even larger extent. The enhanced CCK release may have promoted the enhanced insulin levels since CCK is known to stimulate insulin release in sheep (15). However, it seems unlikely that

Table 1. Difference between AUC-portal and AUC-jugular of propionate/saline infused sheep during (INF) and after infusion (POST)

		Control	1.5 mmol/min	6 mmol/min
acetate	INF	34.1±4.9	37.8±4.8	33.5±7.5
(mmol.min/l)	POST	197.5±29.7	264.3±36.6	251.9±29.9
butyrate	INF	2.9±0.4	3.4±0.5	2.1±0.2 *#
(mmol.min/l)	POST	31.1±3.1	36.1±3.4	24.6±2.4
BHB	INF	5.0±0.5	4.6±0.5	4.3±0.9
(mmol.min/l)	POST	42.0±1.5	45.7±3.9	36.2±7.4
glucose	INF	-0.9±4.3	7.4±2.6	17.7±1.9*#
(mmol.min/l)	POST	31.4±9.5	35.9±6.9	41.7±24.7
insulin	INF	0.46±0.11	0.78±0.14*	3.09±0.98*#
(mIU.min/l)	POST	4.0±0.5	5.1±0.7*	4.1±0.5
CCK	INF	28.8±12.5	25.4±6.9	45.2±2.2*
(pmol.min/l)	POST	74.7±134.6	261.2±43.7	148.3±29.8

* different from NaCl infusion, # significantly different from 1.5 mmol/min Na-propionate infusion. $p<0.05$

circulating CCK played a major role in the insulin release in the present study since CCK levels were depressed during infusion of Na-propionate.

Gastrin levels were decreased as a result of infusion of Na-propionate, with the 6 mmol/min Na-propionate infused animals showing the largest decrease. Decreased levels of gastrin, following a meal were reported in sheep (11). It is highly speculative to point out which factor was responsible for the decrease in gastrin levels found in the present study, since we did not measure jugular gastrin levels.

Pancreatic Polypeptide (PP) levels were decreased in 1.5 mmol/min and even more in 6 mmol/min Na-propionate infused sheep. A reduction in plasma PP concentration was also observed in NaCl infused animals which is in line with earlier reports on the effect of feeding on PP levels (11).

In conclusion, it can be stated that propionate is probably not a major factor influencing meal size. On the other hand, it is possible that some of the effects found during and after a meal on insulin, gastrin and PP concentrations can be attributed to propionate. It is not likely that the observed decrease in CCK levels after a meal is due to the same mechanism as the propionate induced CCK decrease.

It is also clear, that infusion of a high dose of Na-propionate has little physiological value and should not be used as a tool for explanation of physiological mechanisms. As shown in this study, high doses of propionate may affect other nutrients and hormones largely, and may induce discomfort to the animals. One should consider this when explaining the effects of propionate infusion on intake.

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CHAPTER 6

Effect of mesenteric insulin infusions on intake, intake behaviour and hormones in sheep

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ABSTRACT

During a 90 minutes feeding period, sheep provided with jugular, portal and mesenteric catheters were infused via the mesenteric catheter with 6.7 mU/min insulin or saline for 20 minutes. Blood was frequently sampled from jugular and portal veins. The study was performed on two diets differing in quality.
Infusion of insulin did not decrease feed intake but decreased feeding time. Portal insulin levels of sheep receiving an insulin infusion were increased in animals fed a low quality diet but not in animals fed a high quality diet. Insulin levels in the jugular vein were not influenced by infusion of insulin compared to saline infusion. No differences due to infusion of insulin were shown on glucose, glucagon, gastrin, and pancreatic polypeptide levels. Effects of diet composition were reflected by glucagon levels but not by other hormones.
It was concluded that insulin might be a factor involved in satiety, but not by regulation of meal size. It was also postulated that regulation of insulin release might be more sensible in animals fed a higher feed quality.

keywords: insulin, pancreatic polypeptide, gastrin, feed intake, feeding pattern, feed quality, ruminants.

INTRODUCTION

In the search for blood constituents contributing to satiety in ruminants, considerable attention has been paid to nutrients such as propionate (6, 8). Infusion of VFA in the rumen and blood were often effective in decreasing intake, but levels infused were often high (8, 11). Additionally, VFA's are reported to stimulate release of insulin (2, 12, 13). Possibly, the effects of VFA's on intake may be addressed to insulin (7). Other important observations are the remarkable changes in blood levels of insulin due to feeding (1, 3, 9, 10, 15). Papers concerning the effects of insulin infusion indicate that insulin may attribute to satiety (4, 5, 7).
Other hormones that may also be involved in satiety, like the gastrointestinal hormones Cholecystokinin (CCK), gastrin and pancreatic polypeptide (PP), are also influenced by feed intake (9, 14). However, the effects of insulin infusion in ruminants on gastrointestinal hormones are not known.
The present study was designed to investigate the effect of insulin infusion on feed intake, feeding pattern and blood concentrations of metabolites and hormones related to feeding and feed quality.

MATERIALS AND METHODS

The study was performed with 8 wether sheep (93 ± 5 kg LW) provided with permanent catheters in jugular, portal and mesenteric veins (16). Animals were fed grass pellet diets addressed as High Quality (HQ) and Low Quality (LQ) (Table 1). Feeding regime, chemical analyses, sampling procedures, experimental setup and statistical analyses were as described previously (9).

Concisely, feed was provided three times daily with 8-hour intervals. Each meal period, feed was available for 90 minutes. Residues were discarded automatically and weighed. On trial days, animals were attached to sampling and infusion devices.

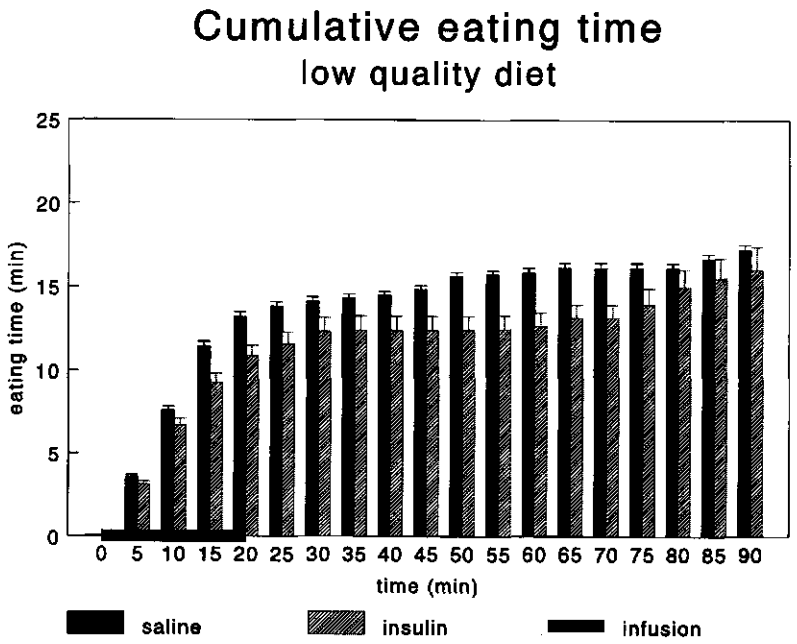

Figure 1: Cumulative eating time of mesenteric insulin/saline infused sheep fed a low quality diet. Values are means (SE).

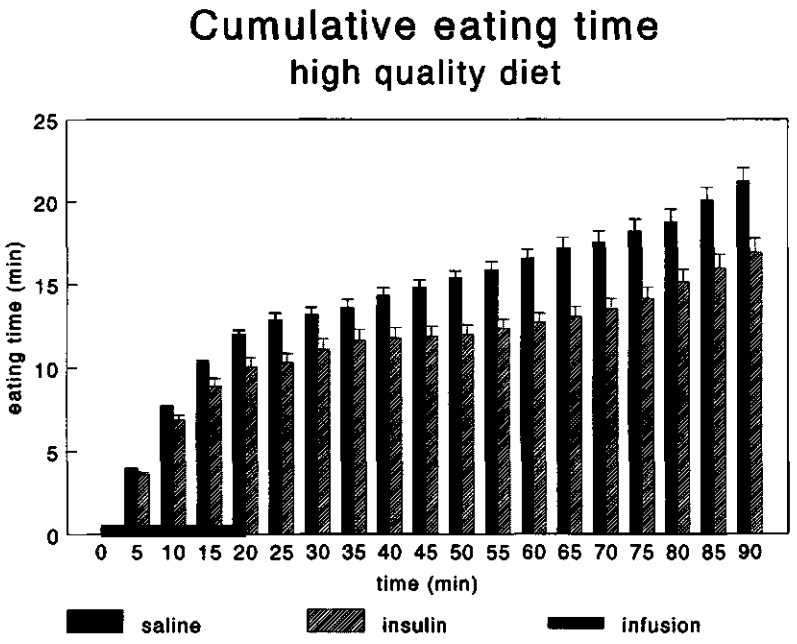

Figure 2: Cumulative eating time of mesenteric insulin/saline infused sheep fed a high quality diet. Values are means (SE).

Blood was withdrawn during 3.5 hours, before, during and after a meal period of 90 minutes via the portal and jugular catheters. Animals were infused with the experimental solutions during the first 20 minutes of the meal period.

Table 1. Chemical composition of the experimental feeds (g/kg)

	LQ	HQ
Dry Matter	967	962
In DM		
OM	866	827
CP	127	170
Cellulose	268	238
Hemi-cellulose	218	181
Lignin	47	21

abbreviations: HQ, High Quality; LQ, Low Quality; DM, dry matter; OM, organic matter; CP, Crude Protein.

Infusions

Ovine insulin (Sigma, I9254) solution was freshly prepared immediately before start of the experiment.

Infusion was performed via polyethylene tubing, which did not adsorb insulin. Solutions were infused using a peristaltic pump (Watson Marlow, UK), with mersilene tubing (Gilson, UK) at a speed of 4 ml/min.

Animals were randomly infused with insulin (6.7 mU/min) or saline. Each animal received each treatment once. Experiments were performed once a week.

Calculations

Area under the curves (AUC) was calculated for portal insulin levels of each individual sheep. Increments from basal were calculated using the mean of the first 3 samples (t=-30, t=-15 and t-5 min) as basal value. AUC-inf was calculated during the infusion period (t=1 until t=20 min) while AUC-post was calculated starting at the end of the infusion until end of the experiment (t=20 until t=180).

RESULTS

Feed intake of insulin infused sheep did not differ from saline infused sheep neither in LQ-fed sheep (789 ± 126 g vs. 848 ± 40 g) nor in HQ-fed sheep (812 ± 48 g vs. 792 ± 134 g).

Infusion of insulin decreased cumulative feeding time in the LQ-fed group from t=15 until t=70 min (Fig. 1). Cumulative feeding time was decreased from t=20 until t=90 in the HQ-fed group due to insulin infusion (Fig. 2).

Insulin infusion resulted in slightly increased portal insulin levels during infusion in the LQ- fed group (Fig. 3). Portal levels of HQ -fed sheep were not significantly changed due to infusion (Fig 4). Jugular levels were not significantly changed neither in LQ nor in HQ-fed animals (data not shown).

Portal glucagon levels were not influenced by infusion of insulin in LQ-fed sheep (Fig. 5) nor in HQ-fed sheep (Fig. 6). However, generally glucagon levels of LQ-fed

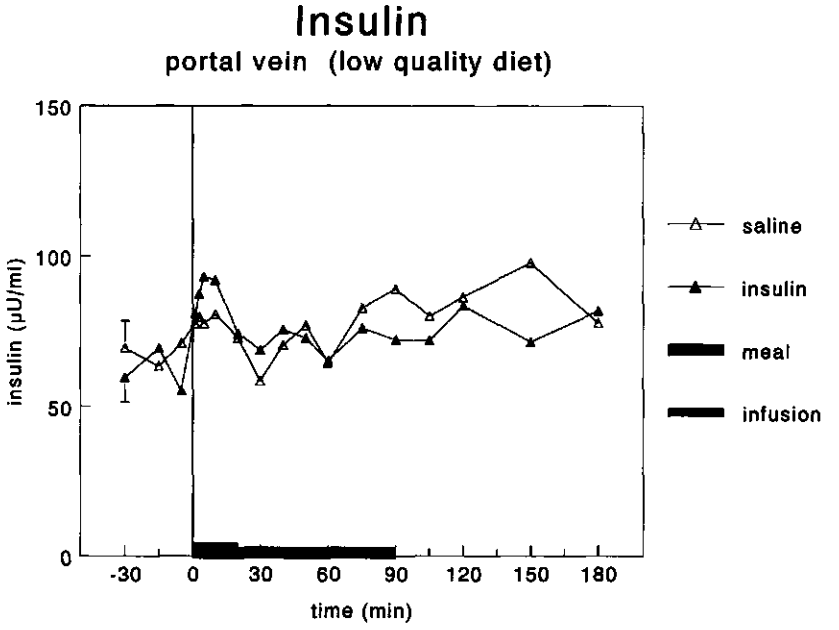

Figure 3: Portal insulin concentration in mesenteric insulin/saline sheep fed a low quality diet. Values are means, pooled SE is shown at the first data point.

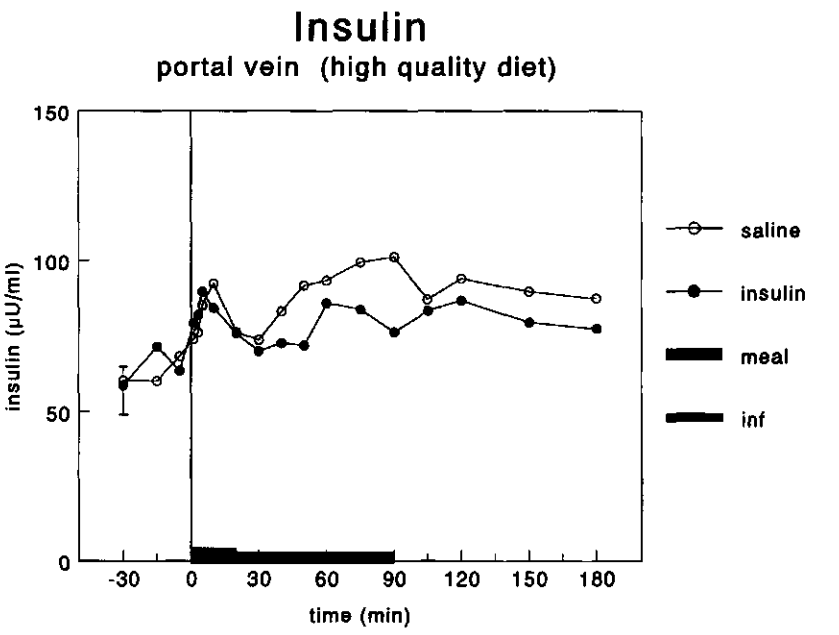

Figure 4: Portal insulin concentration in mesenteric insulin/saline sheep fed a high quality diet. Values are means, pooled SE is shown at the first data point.

sheep were lower as compared to HQ-fed sheep. Jugular levels showed the same characteristics as portal levels (data not shown)
Pancreatic Polypeptide levels were not influenced by infusion of insulin in both the LQ-fed group (Fig. 7) as well as the HQ-fed group (Fig. 8).
Gastrin levels were not changed due to infusion of insulin (data not shown).
Portal glucose levels were not changed as a result of insulin infusion in LQ-fed sheep (Fig. 9) and HQ -fed sheep (data not shown). Jugular levels were similar to portal levels (data not shown).
In table 2, portal AUC during (AUC-inf) and after infusion (AUC-post) is shown. Portal AUC during insulin infusion was increased in LQ-fed sheep as compared to controls. Post infusion AUC was decreased in HQ-fed sheep, which received an insulin infusion, as compared to saline infused sheep.

Table 2. Portal AUC-insulin during and after infusion of insulin or saline in HQ and LQ-fed sheep.

	AUC-inf	AUC-post
SALINE LQ	186 ± 131	2360 ± 653
INSULIN LQ	477 ± 65 *	2564 ± 777
SALINE HQ	402 ± 75	4797 ± 698
INSULIN HQ	341 ± 66	2760 ± 521 *

* different from control treatment ($p<0.05$).

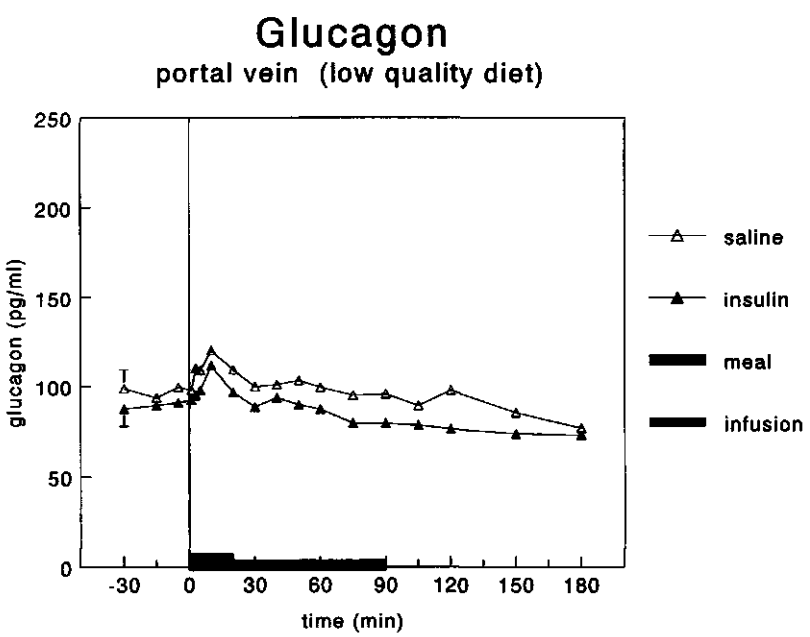

Figure 5: Portal glucagon concentration in mesenteric insulin/saline sheep fed a low quality diet. Values are means, pooled SE is shown at the first data point.

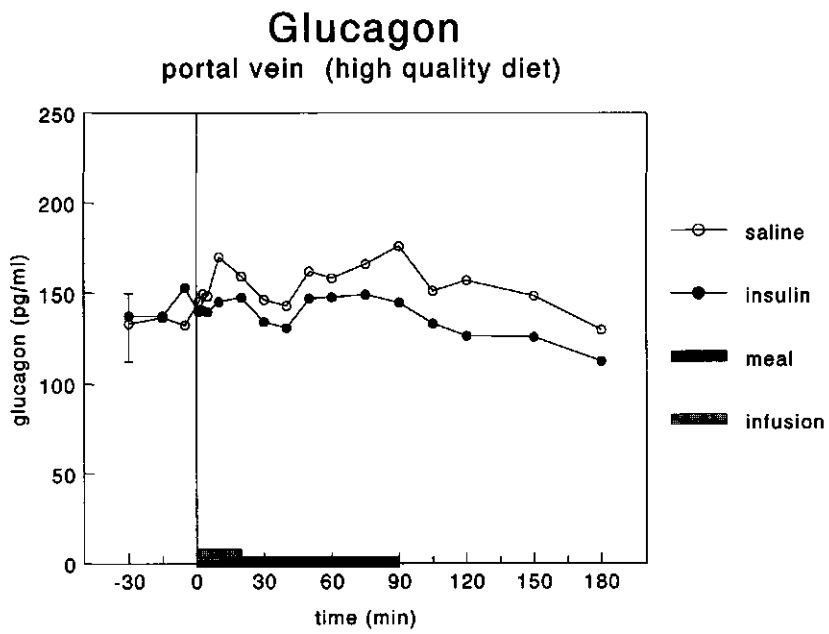

Figure 6: Portal glucagon concentration in mesenteric insulin/saline sheep fed a high quality diet. Values are means, pooled SE is shown at the first data point.

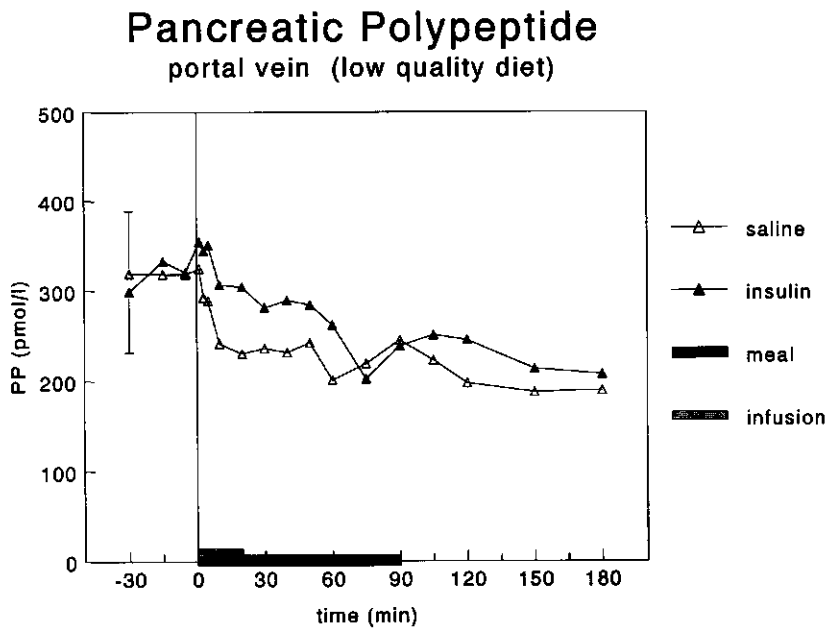

Figure 7: Portal pancreatic polypeptide concentration in mesenteric insulin/saline sheep fed a low quality diet. Values are means, pooled SE is shown at the first data point.

DISCUSSION

The intake response to insulin is complex. Grovum postulated that the effects of insulin on intake resemble one cycle of a sine wave (7). Insulin administration at a low rate would induce satiety, intermediate administration, resulting in insulin levels increased 10-15 times, might be ineffective. However, higher insulin levels would induce hyperphagia due to hypoglycemia while even higher (pharmacological) levels would induce CNS disturbances. In the present study, we intended to increase insulin levels mildly by infusion of a low dose of insulin into the portal system. In contrast to earlier findings by Deetz and Wangness (4, 5), we did not observe a change in amount of feed ingested. Although we did not find an effect on feed intake, feeding behavior was clearly influenced by infusion of insulin in both the LQ as well as the HQ-fed group. Insulin infusion induced a more rapid uptake of feed as shown by the cumulative eating time.

The effects shown are not likely to be attributed to enhanced insulin concentration since only portal levels of LQ-fed sheep infused with insulin were increased compared with saline infused sheep. Also portal AUC-inf was increased in LQ-fed sheep as compared to HQ-fed sheep. This observation implicates that endogenous release of insulin was not influenced by the exogenous insulin supply in LQ-fed animals. However, exogenous insulin supply was completely compensated by a decrease in endogenous insulin release in HQ-fed animals since neither portal insulin levels nor AUC-inf, were different in insulin infused animals as compared to saline infused sheep. Portal AUC-post was significantly lower in the insulin infused group as compared to controls indicating an enhanced insulin clearance and/or a decreased insulin release. Since portal and jugular insulin concentrations were not significantly different, we could not use the portal-jugular difference as an indicator of endogenous production.

Both the LQ as well as the HQ-fed sheep showed no effect of insulin infusion on jugular levels. This indicated that peripheral insulin levels were regulated tightly in both diet groups but mechanisms may be different. In LQ-fed animals clearance of insulin was increased (possibly by the liver), while in HQ-fed animals decreased insulin release contributed to the unaltered jugular concentrations.

Despite the differences in insulin level characteristics, feeding behavior was influenced similarly in LQ and HQ-fed sheep. This diminishes the importance of circulating insulin as the factor responsible for the observed effect on feeding behavior. Also endogenous insulin release is not likely to be involved.

No effects were found on glucose levels. It therefore can be excluded that the eagerness of the animals to eat was increased due to hypoglycemia. This is also reflected in the glucagon levels that were not changed. However, glucagon levels of LQ fed sheep were generally lower than levels of HQ -fed sheep as shown before (9). It seems that glucagon levels are very sensitive to feed quality since none of the other hormones was influenced by feed quality.

Effects of insulin on gastrointestinal hormones were not observed in the present study. Pancreatic Polypeptide (PP) was measured since PP and insulin levels were reported to be influenced by feeding in a similar way (9) and insulin induced hypoglycemia increased PP (14). As PP, gastrin levels are also diminished in insulin induced hypoglycemia (14). No effects of insulin infusion were found on gastrin levels. Although no firm conclusion can be drawn, it appears that PP and gastrin release is neither influenced by insulin release nor by portal insulin concentrations,

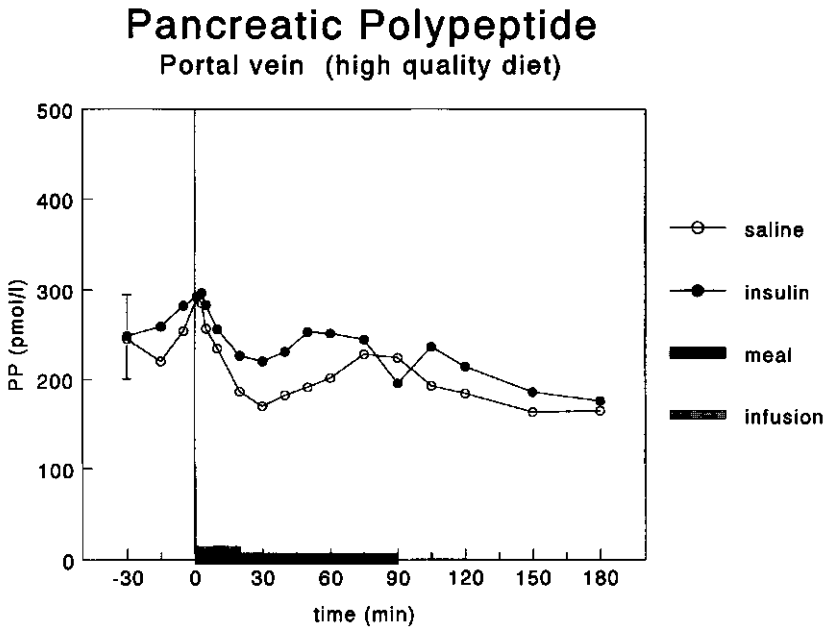

Figure 8: Portal pancreatic polypeptide concentration in mesenteric insulin/saline sheep fed a high quality diet. Values are means, pooled SE is shown at the first data point.

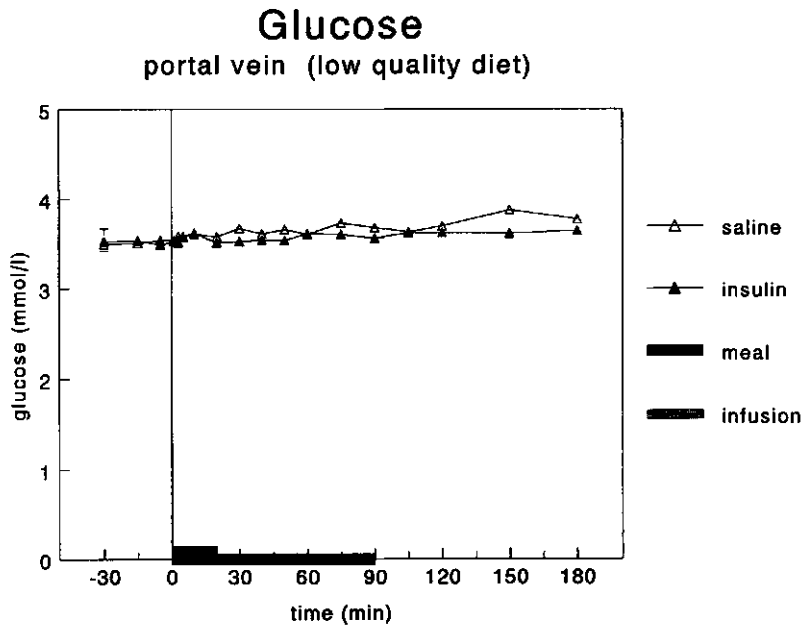

Figure 9: Portal glucose concentration in mesenteric insulin/saline sheep fed a low quality diet. Values are means, pooled SE is shown at the first data point.

since both PP and gastrin levels were not altered neither in HQ-fed sheep (lower endogenous release) nor in LQ-fed sheep (higher portal insulin concentrations).
In summary, it can be concluded that feeding behavior is influenced by insulin infusion. However, the mechanism remains unsolved but may be different in HQ-fed sheep and LQ-fed sheep. Another observation is that peripheral insulin levels are tightly regulated in both dietary groups although mechanisms may differ.

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CHAPTER 7

Changes in circulating gastrointestinal hormones and cortisol resulting from mesenteric CCK-8 infusion in sheep

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ABSTRACT

During a 90 minutes feeding period, sheep provided with jugular, portal and mesenteric catheters were infused via the mesenteric catheter with 0, 1.2 or 2.4 nmol/min CCK-8 for 20 minutes. Blood was frequently sampled from jugular and portal veins.
Infusion of CCK-8 increased levels of CCK-8 in the portal vein but not in the jugular vein. A very accurate clearance of CCK-8 by the liver may have attributed to this observation. Infusion of both 1.2 and 2.4 nmol/min CCK-8 decreased portal and jugular CCK-33 levels, indicating a decreased endogenous release of CCK. Portal Pancreatic Polypeptide levels were decreased as a result of 2.4 nmol/min CCK-8 infusion. This may be due to a decrease in release or a enhanced portal blood flow.
Cortisol concentrations, as an indicator of stress, were decreased during infusion of saline but increased as a result of CCK-8 infusion. It was concluded that CCK-8 may have induced some discomfort. Despite the increased portal CCK-8 levels and the increased cortisol levels no effect was found on feed intake.

keywords: cholecystokinin, CCK-8, CCK-33, pancreatic polypeptide, feed intake, ruminants.

INTRODUCTION

Evidence is increasing that CCK may act as a satiety factor in ruminants (8, 9, 13). CCK is heterogeneous consisting of several different molecular forms containing the bioactive C-terminus. Different forms are found in all animals, but length of the CCK forms differ between species. In humans, CCK molecules containing 4, 8, 22, 33, 39 and 58 amino acids are reported (4). In ruminants, CCK-8, CCK-33, CCK-39 and CCK-58 can be present (7, 12).
Many studies show that infusion of several forms of CCK result in meal termination in humans (22, 25), laboratory animals (1, 3) and less evident in ruminants (8, 26, 28). Most studies used the sulfated octapeptide form of CCK (CCK-8) which is thought to be the biologically active form. The physiological significance of the effect on feed intake is uncertain because often some malaise is induced probably by altered gastric contractions (2, 4, 10, 17). Infusion of CCK is reported to increase cortisol and prolactin concentration in sheep which can be considered as a stress response (6).
In ruminants, CCK-8 and CCK-33 inhibited feeding in sheep when infused peripherally by various routes(8, 13). It was shown by Farningham that effects of CCK-8 are probably mediated through CCK-B receptors (8). In the same study, evidence for both anterograde and retrograde axonal transport of CCK-8 through vagal fibers is provided.
Although many infusion experiments in relation to feeding were performed in ruminants, very little is known about the blood levels of CCK following a meal. In the present paper, we describe the effects of mesenteric infusion of CCK-8 on peripheral and portal levels of CCK-8 and CCK-33. We also investigated the effect on plasma Pancreatic Polypeptide (PP) and gastrin levels because CCK stimulates PP release (23) but may inhibit gastrin release (16).

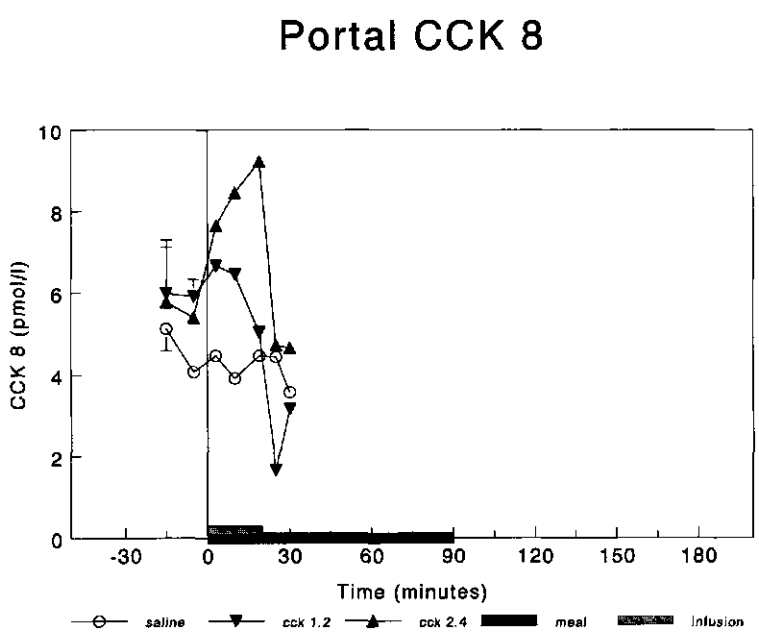

Figure 1: Portal CCK-8 concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

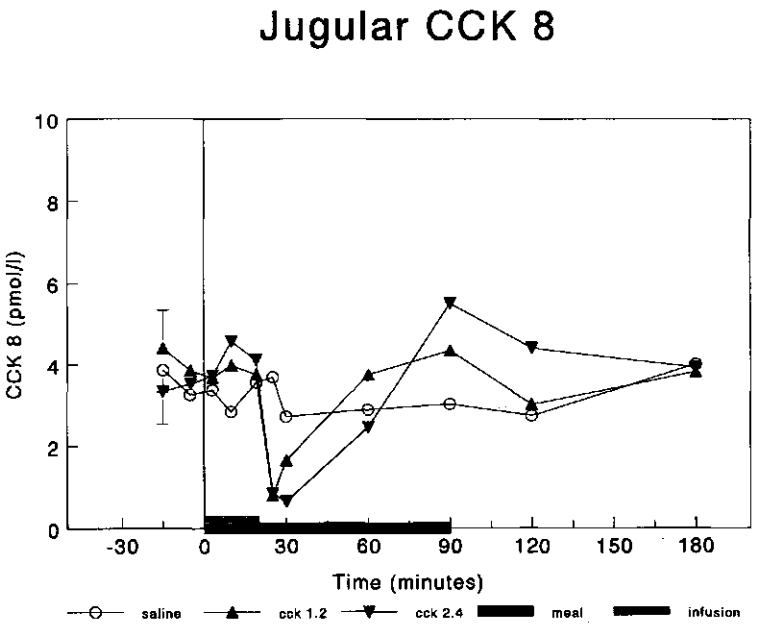

Figure 2: Jugular CCK-8 concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

MATERIALS AND METHODS

The study was performed with 8 wether sheep (77±3 kg LW) provided with permanent catheters in the jugular, portal and mesenteric veins (29). Animals were fed a grass pellet diet containing (in dry matter): 846 g/kg organic matter, 143 g/kg crude protein, 209 g/kg cellulose, 199 g/kg hemi-cellulose and 27 g/kg lignin. Feeding regime, chemical analyses, sampling procedures, experimental setup and statistical analyses were as described previously (20, 21).
Concisely, feed was provided three times daily with 8 hour intervals. Each meal period, feed was available for 90 minutes. Residues were discarded automatically and weighed. On trial days, animals were attached to sampling and infusion devices. Blood was withdrawn during 3.5 hours, before, during and after a meal period of 90 minutes via jugular and portal catheters. Animals were infused via the mesenteric catheter with the experimental solutions during the first 20 minutes of the meal period.

Infusate
Sulphated CCK-8 (Sigma, C2175) solution was freshly prepared immediately before start of the experiment.
Infusion was performed via polyethylene tubing, which did not adsorb insulin. Solutions were infused using a peristaltic pump (Watson Marlow, UK) , with mersilene tubing (Gilson, UK) at a speed of 4 ml/min.
Animals were randomly infused with CCK-8 in the mesenteric vein at a rate of 0, 1.2 or 2.4 nmol/min. Each animal received each treatment once.
Experiments were performed once a week.
A small aliquot of the infusion solution was stored and analyzed on CCK-8 concentration.

Radioimmunoassays
Measurement of immunoreactive CCK was performed by radioimmunoassay (RIA). For estimation of total CCK, the antibody t204 which binds to all sulphated CCK-fragments longer than 7amino acids was used. Another antibody (t1703) with affinity to CCK-fragments containing more than 14 amino acids was used to estimate CCK-33 concentration. Substraction of the levels found with T1703 from levels found with T204 in each individual sheep led to CCK-8 concentrations.
Pancreatic Polypeptide and gastrin were also measured by RIA as described earlier (18, 27). Cortisol was estimated as described by Janssens et al (14).

RESULTS

Feed intake of saline infused sheep was not different from CCK infused sheep.
Portal CCK-8 levels (Fig. 1) were significantly increased as a result of 2.4 nmol/min CCK-8 infusion but not by infusion of 1.2 nmol/min. Immediately after the end of the infusion period, CCK-8 levels of 2.4 nmol/min infused animals decreased to normal levels.
Jugular levels were not changed during the infusion period (Fig. 2). Ending the infusion, led to decreased levels which returned gradually to control levels.

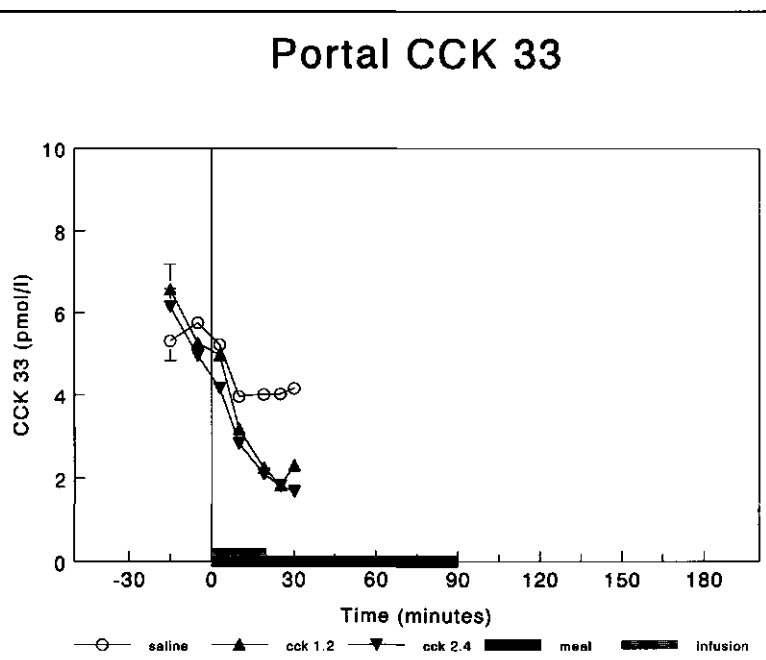

Figure 3: Portal CCK-33 concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

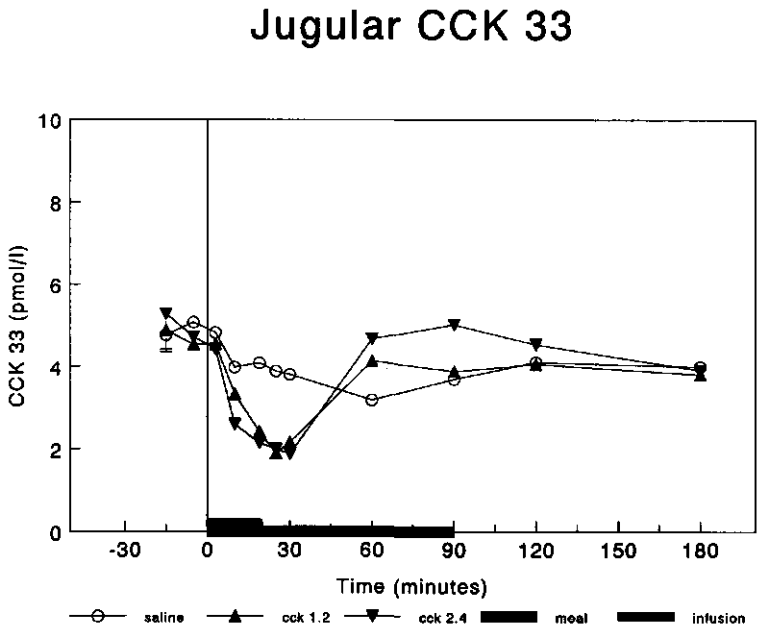

Figure 4: Jugular CCK-33 concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

Infusion of CCK-8 led to significantly decreased CCK-33 levels in both the 1.2 and 2.4 nmol/min infused sheep (Fig.3). A slight but significant decrease in CCK-33 was observed in saline infused sheep shortly after meal start.
Figure 4 shows that in analogy to portal levels, jugular CCK-33 levels decreased during CCK-8 infusion.
Portal PP levels decreased slowly during infusion of CCK-8 (Fig. 5). A slow decrease in all experimental groups is observed compared to pre-meal levels.
Portal gastrin levels (Fig. 6), were increased largely due to infusion of CCK.2.4 nmol/min infusion led to higher levels than1.2 nmol/min.
Jugular cortisol increased to reach maximal levels at the end of the infusion period in both 1.2 and 2.4 nmol/min infused sheep (Fig. 7). After the end of the infusion, levels decreased again.

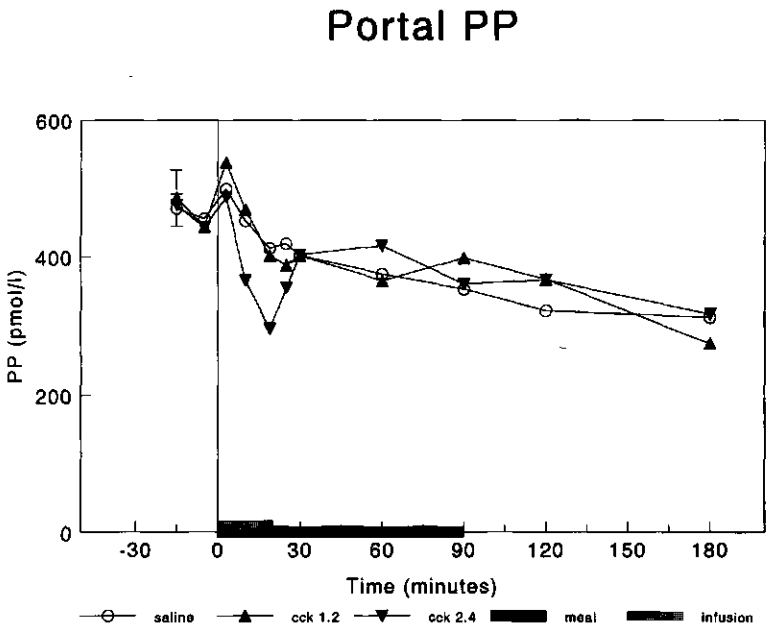

Figure 5: Portal Pancreatic Polypeptide (PP) concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

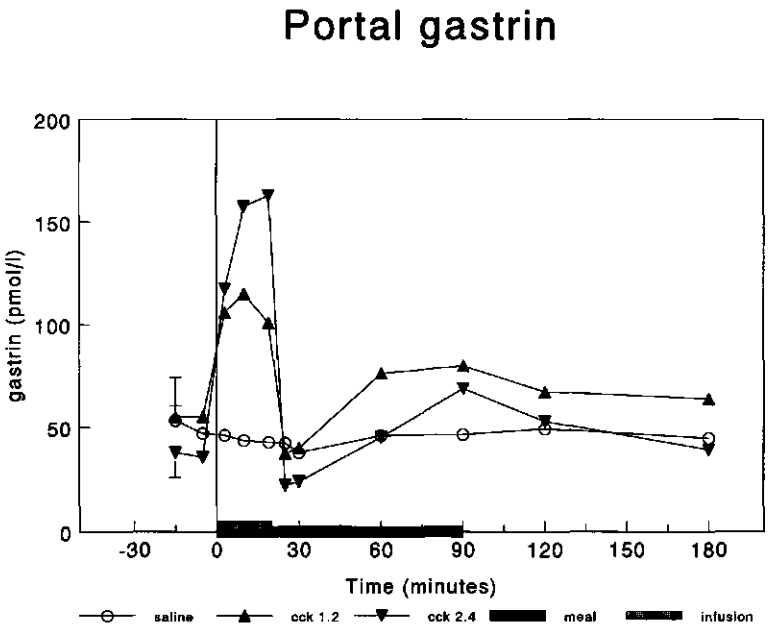

Figure 6: Portal gastrin concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

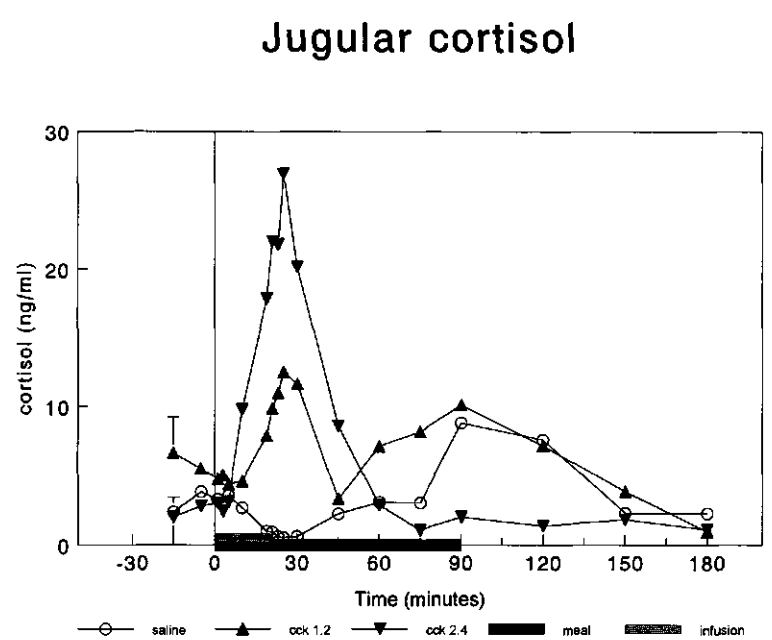

Figure 7: Jugular cortisol concentration in mesenteric CCK-8 infused sheep. Values are means, pooled SE is shown at the first data point.

DISCUSSION

Mesenteric CCK-8 infusion in sheep in the present study did not affect feed intake. Infusion of about 0.8 nmol/min in a study from Farmingham showed a similar result. Grovum reported that peripheral infusion of CCK-8 (±2 nmol/min) did not affect feed intake in sheep, but CCK-33 lowered intake when infused at a rate of about 1 nmol/min (13). Both authors suggest that the amount infused may be physiological, but were not able to measure CCK levels. As could be expected, portal CCK-8 levels increased as a consequence of CCK-8 infusion. Basal levels measured in the present study of about 5 pmol/l are in accordance with those found in goats and dairy cows (11, 12). Theoretically, infusion of 1.2 nmol/min or 2.4 nmol/min in the mesenteric vein should have led to large increments in portal levels. The present study showed only minor increments. This was due to the observation that only 1.5% of the infused solution was immunoreactive CCK-8. No immunoreactive CCK-33 was found in the infusion solution. Possibly, the solution contained smaller fragments or non-sulfated forms, which could not be recognized by the antibodies used. This suggestion is strengthened by the observation that gastrin levels were increased to a large extent. The antibody used for the estimation of gastrin was reported to show less then 5% cross reactivity with the sulphated form of CCK-8 and CCK-33. Infusion of 1.2 or 2.4 nmol/min CCK-8 solution may have led to binding to the anti-body. It is therefore unlikely that the observed gastrin increases are of biological value. Furthermore one has to consider the possibility that non-sulphated CCK-8 may have concerted its action through binding to CCK-B receptors, which are abundant in ruminants (8, 19, 24).

Increased CCK-8 levels in the portal vein were not found in the jugular vein. Probably clearance by the liver is responsible for this observation (4). Effects found in this study must find their origin in the portal vein/hepatic region, most likely through binding to vagal binding sites (8).

Plasma CCK-33 levels were decreased immediately after start of the infusion of both 1.2 and 2.4 nmol/min CCK-8. In humans, Jebbink showed that infusion of CCK-8 did not affect the meal induced CCK-33 increase (15). This indicates that the decrease in CCK-33 concentration may be restricted to ruminants and/or the decrease is mediated through binding to CCK-B receptors. Decreased CCK-33 levels may be due to enhanced clearance from the circulation and/or decreased release. In all experimental groups, CCK-33 levels decreased as a consequence of feeding which we reported earlier (20).

Pancreatic Polypeptide release is reported to be stimulated by CCK-8 in man (23). In contrast to the observation in man, PP levels were decreased as a result of CCK-8 infusion at a rate of 2.4 nmol/min. After the end of the infusion period, levels returned to control values.

Increased levels of cortisol due to infusion of CCK were reported in mono gastrics (24, 25), and ruminants (6). In the present study, cortisol levels increased slowly reaching maximum levels at the end of the infusion period indicating some discomfort. The mechanism by which cortisol is increased is unclear. In pigs blockade of CCK-A or CCK-B receptors did not abolish the effect of CCK-8 infusion on cortisol levels (24). Possibly, CCK-8 was transported retrograde within the vagus, reaching the brain (8). Central administration of pentagastrin, which binds to the CCK-B receptor, evoked cortisol release in sheep (5).

In summary, mesenteric infusion of CCK-8 lead to decreased CCK-33 levels possibly through an autocrine feed back mechanism. Increased levels of cortisol

indicating some discomfort are likely mediated by binding to CCK receptors in the portal vein/ hepatic region and/or retrograde vagal transport.

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CHAPTER 8

Effects of mesenteric CCK-8 and propionate infusion on volatile fatty acids, glucose and insulin in meal-fed sheep

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ABSTRACT

During a 90 minutes feeding period, sheep provided with jugular, portal and mesenteric catheters were infused via the mesenteric catheter with 0, or 2.4 nmol/min CCK-8 or 0.5 mmol/min propionate or a combination of CCK and propionate, for 20 minutes. Blood was frequently sampled from jugular and portal veins.
Feed intake was reduced by combined infusion of CCK-8 and propionate. Increased levels of propionate and insulin were observed following the propionate infusion. Infusion of CCK induced decreased propionate, acetate, butyrate, and glucose levels while insulin levels were initially increased followed by a decrease. Combined infusion of CCK and propionate induced similar blood concentration as infusion of CCK solely on acetate, butyrate, glucose and insulin, while propionate levels were decreased compared to propionate infused animals but increased compared to CCK infused sheep.
It is postulated that decreased levels of volatile fatty acids and insulin may be due to increased portal flow and that mechanisms of insulin secretion of propionate and CCK may be different.

keywords: cholecystokinin, propionate, CCK-8, volatile fatty acids, glucose, insulin, feed intake, ruminants.

INTRODUCTION

Evidence is increasing that there is no single factor essential for normal feed intake. The various theories of intake control should be looked upon as complementary and contributing to a multifactorial system. In ruminants, propionate and CCK are two factors that received considerable interest in relation to intake regulation. Both CCK and propionate may act as a satiety factor in ruminants (4-6). Many studies show that infusion of several forms of CCK result in meal termination in humans (13, 18), laboratory animals (1, 3) and less evident in ruminants (4, 22, 23). Propionate infusions were also effective in reducing meal size although levels may be supra-physiological (7, 9). Besides the effects on intake, infusion of CCK or propionate may induce changes in blood concentrations of other hormones and metabolites. Both propionate and CCK increase release of insulin and glucagon (8, 9, 16, 17, 21). Since propionate is one of the main precursors for glucose, glucose levels are usually increasing as a result of propionate administration (9).
A potential synergistic effect of propionate and CCK on intake was shown by Farningham (4). However, in this study possible changes in hormone or metabolite levels were not included.
In the present study we investigated the effect of a combined, short-term infusion of CCK and propionate on feed intake and plasma levels of Volatile Fatty Acids (VFA), glucose and insulin.

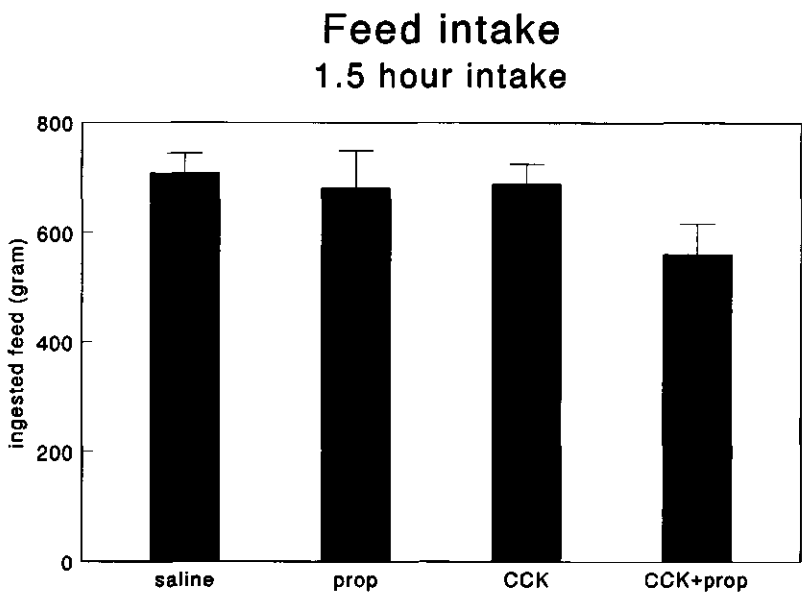

Figure 1: 1.5-hour feed intake of 20 minutes infused sheep. Values are means ± SE.

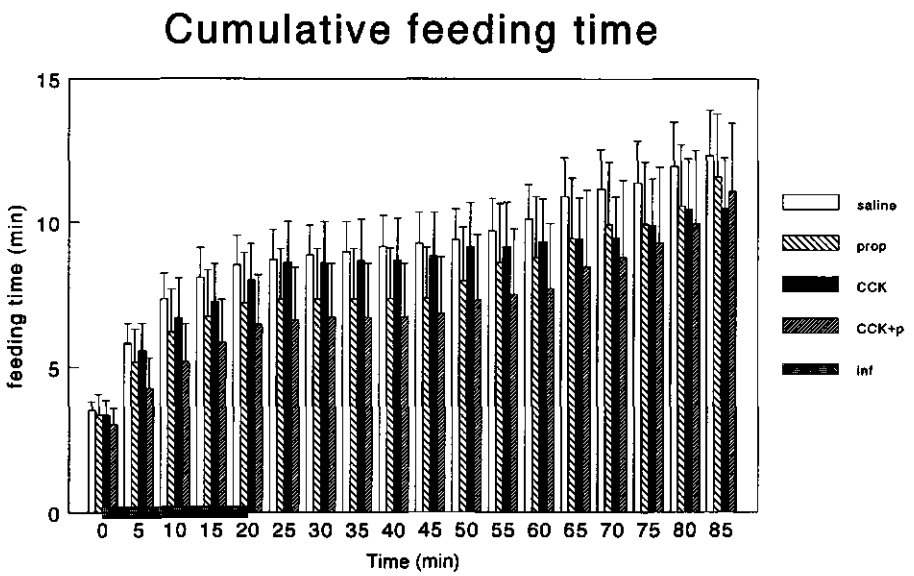

Figure 2: Cumulative feeding time of 20 minutes infused sheep. Values are means ± SE.

MATERIALS AND METHODS

The study was performed with 8 wether sheep (LW 77.0±2.7 kg) provided with permanent catheters in the jugular, portal and mesenteric veins (24). Animals were fed a grass pellet diet containing (in dry matter): 846 g/kg organic matter, 143 g/kg crude protein, 209 g/kg cellulose, 199 g/kg hemi-cellulose and 27 g/kg lignin. Feeding regime, chemical analyses, sampling procedures, experimental setup and statistical analyses were as described previously (9, 10, 12).
Concisely, feed was provided three times daily with 8 hour intervals. Each meal period, feed was available for 90 minutes. Residues were discarded automatically and weighed. On trial days, animals were attached to sampling and infusion devices. Blood was withdrawn during 3.5 hours, before, during and after a meal period of 90 minutes. Animals were infused with the experimental solutions during the first 20 minutes of the meal period.

Infusates
Sulphated CCK-8 (Sigma, C2175) solution was freshly prepared immediately before start of the experiment. Na-propionate infusate (0.125 mol/l)(Merck, Germany) was adjusted to pH 7.5 with NaOH. Infusate were passed through a 0.2 µm filter and autoclaved before infusion.
Animals were randomly infused with 0 or 2.4 nmol/min CCK-8 or 0.5 mmol/min propionate or a combination of CCK and propionate. As a control animals were infused with saline.
Infusions were performed via polyethylene tubing that did not adsorb CCK or propionate. Solutions were infused using a peristaltic pump (Watson Marlow, UK), with mersilene tubing (Gilson, UK) at a speed of 4 ml/min.
Each animal received each treatment once. Experiments were performed once a week.

Calculations
Difference in area under the curve (dAUC) of the portal and jugular curves for each individual sheep was calculated over the infusion period (dAUC-inf) and post-infusion (dAUC-post) period, as indicator for production during and after the infusion period.

RESULTS

No difference in feed intake was observed between saline (707 ± 37 g), propionate (680 ± 50 g) or CCK (670 ± 50 g) infused sheep (Fig. 1). Combined infusion of CCK and propionate led to decreased intake (560 ± 56 g, $p<0.05$). Cumulative feeding time was not different between treatments (Fig. 2).
Portal propionate (Fig. 3) showed a gradual increase in saline infused sheep, while infusion of propionate induced enhanced levels which returned to control levels after the infusion period. CCK infusion induced a small decrease in propionate levels. Propionate levels during combined infusion of propionate and CCK led to propionate levels that were significantly increased compared to CCK infused sheep. Combined infusion led to lower propionate levels compared to propionate infusion.
Acetate levels in the portal vein (Fig. 4) were slightly lowered after the end of the infusion period (t=25 until t=40 min) in CCK and CCK+propionate infused sheep.

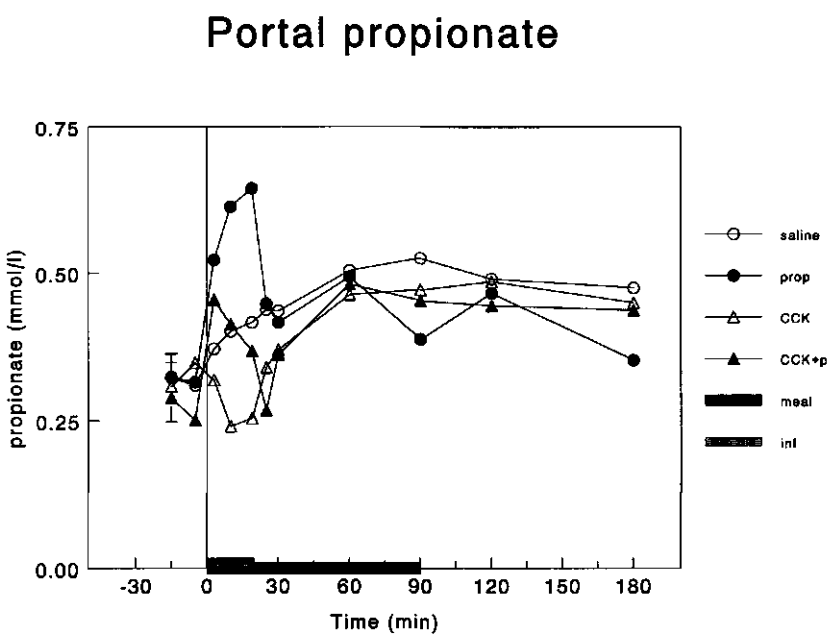

Figure 3: Portal propionate concentration in mesenteric CCK-8 and/or propionate infused sheep. Values are means, pooled SE is shown at the first data point.

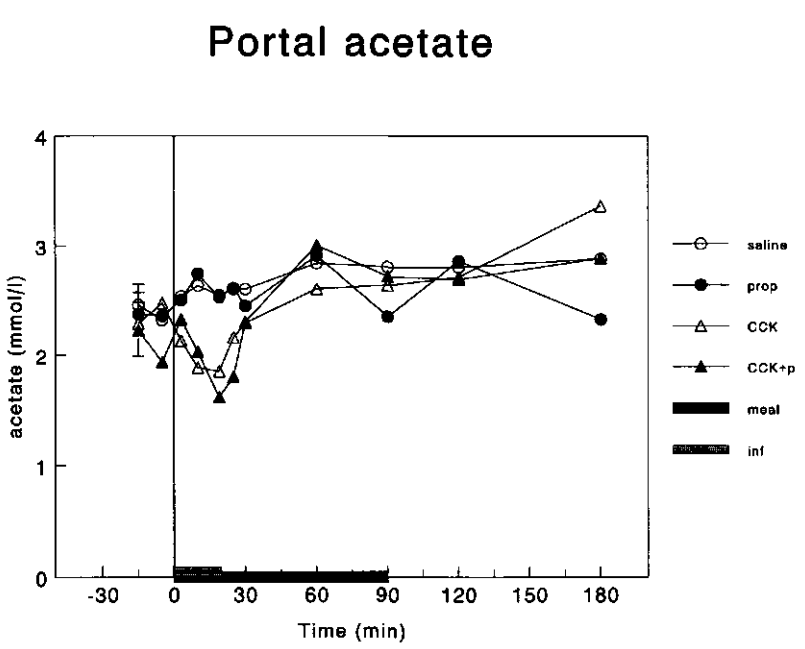

Figure 4: Portal acetate concentration in mesenteric CCK-8 and/or propionate infused sheep. Values are means, pooled SE is shown at the first data point.

Butyrate levels (Fig. 5) increased after start of the meal period in saline and propionate infused sheep. Infusion of CCK and CCK+propionate decreased butyrate levels, but levels reached control levels after termination of the infusion.
Portal glucose (Fig. 6) was not changed in control and propionate infused sheep. Infusion of CCK and CCK+propionate reduced glucose levels from t= 19 until t=30 minutes.
Infusion of propionate induced an increase in portal insulin levels which decreased to control levels after termination of the infusion (Fig. 7). Both CCK and a combination of CCK and propionate induced a rapid peak in insulin levels followed by a decrease in insulin levels. After the infusion period, plasma insulin concentrations slowly reached control levels.
Table 1 shows that dAUC-propionate is increased in the propionate infused sheep. CCK infusion resulted in a decreased dAUC of propionate during infusion. Combined infusion of CCK and propionate led to decreased dAUC compared to propionate infusion but increased compared to CCK infusion.
dAUC-butyrate was decreased during infusion of both CCK and CCK+propionate.
Infusion of propionate resulted in a significantly higher dAUC-glucose and dAUC-insulin compared to control infusion.

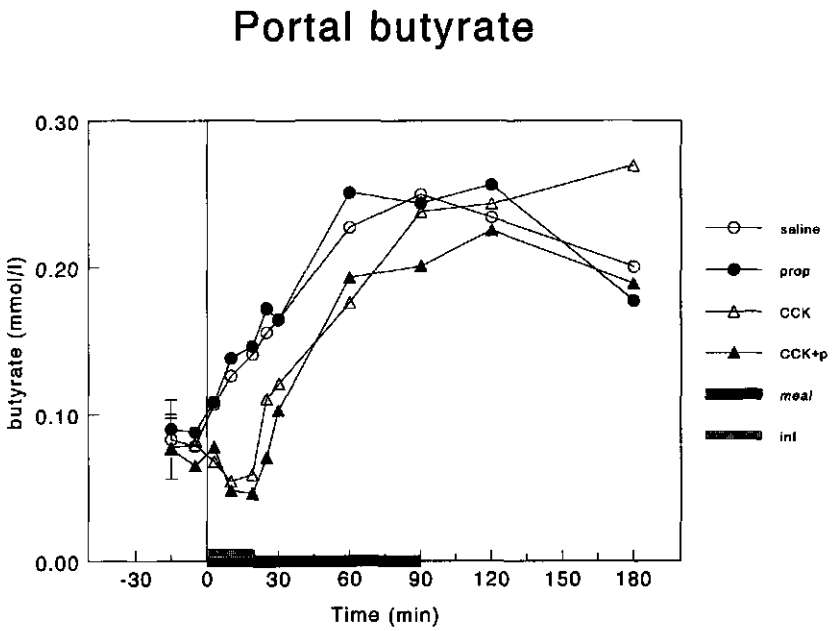

Figure 5: Portal butyrate concentration in mesenteric CCK-8 and/or propionate infused sheep. Values are means, pooled SE is shown at the first data point.

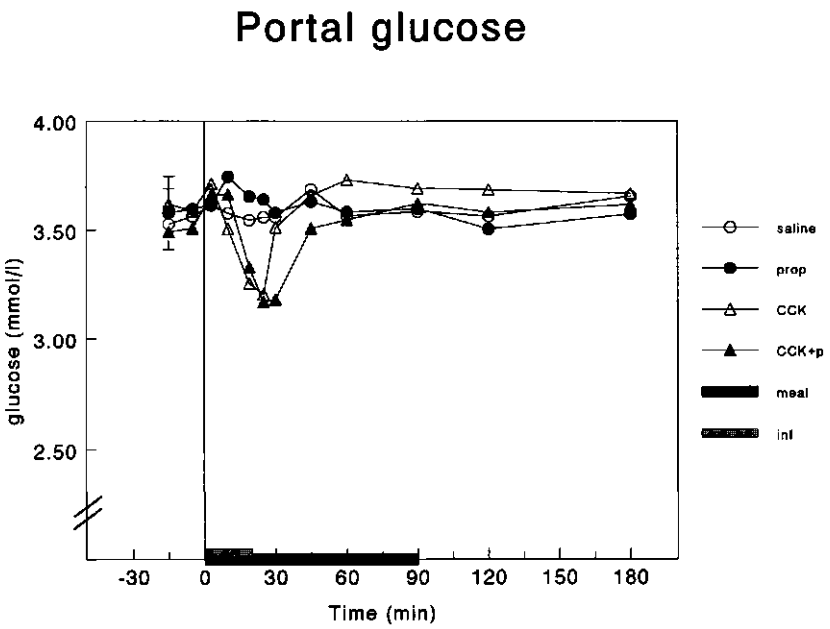

Figure 6: Portal glucose concentration in mesenteric CCK-8 and/or propionate infused sheep. Values are means, pooled SE is shown at the first data point.

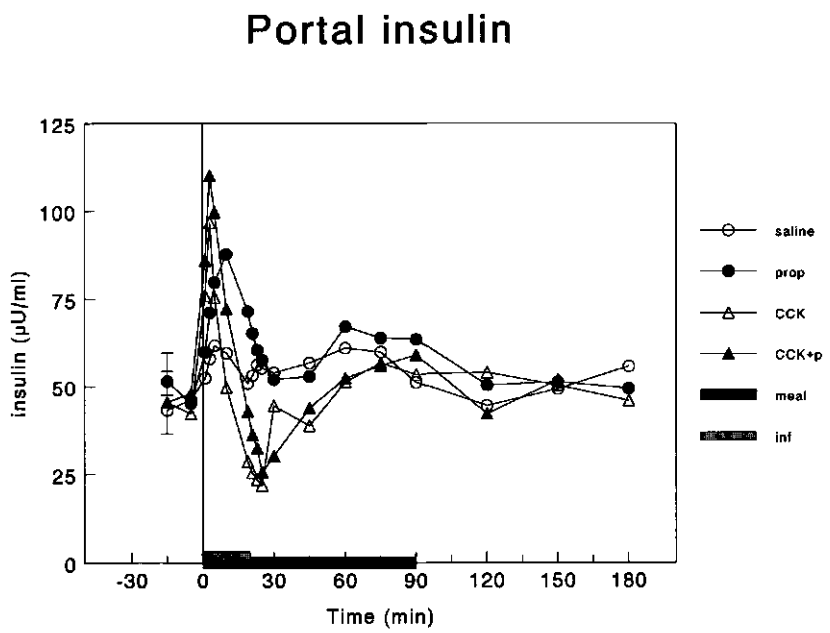

Figure 7: Portal insulin concentration in mesenteric CCK-8 and/or propionate infused sheep. Values are means, pooled SE is shown at the first data point.

DISCUSSION

In the present study, combined short-term CCK and propionate infusion reduced meal size in meal fed sheep. This was also shown in a study of Farningham where sheep were infused during 2 hours (4). Despite the decreased intake no significant effect was shown on cumulative feeding time. Similar results were obtained in an earlier study where infusion of 2 mmol/min propionate, which reduced intake, also showed no effect on feeding time (9).
As expected, propionate levels were increased due to propionate infusion. Infusion of CCK lowered propionate levels, while combined infusion of propionate and CCK resulted in levels lower as compared to propionate infusion but higher as compared to CCK infusion. The propionate concentration increasing effect of propionate infusion and the propionate concentration decreasing effect of CCK infusion were counteractive, leading to intermediate propionate levels. Lowered concentrations following the CCK or CCK+propionate infusion were also observed on acetate and butyrate levels. This may indicate that CCK enhanced portal flow in the present study as was shown in dogs (19). Difference in AUC (dAUC) between portal vein and jugular vein was used as an indication of the release of substances formed in the portal drained viscera (PDV). Decreased dAUC's were observed for propionate and butyrate, following a CCK infusion, which also implicate that portal flow was enhanced.

Table 1. Difference between portal and jugular AUC of sheep during (INF) and after infusion (POST) of saline, propionate (0.5 mmol/min), CCK (2.4 nmol/min) or CCK+propionate.

		control	propionate	CCK	CCK + prop
propionate	INF	7.3±0.5	10.0±0.4*	4.8±0.5*#	7.4±0.6#
(mmol.min/l)	POST	65.4±4.5	57.6±1.5	63.9±3.1	61.1±70
acetate	INF	30.4±3.3	28.0±1.3	22.9±3.7	23.5±3.6
(mmol.min/l)	POST	250.5±20.0	211.1±13.8	243.3±12.3	223.4±23.2
butyrate	INF	2.3±0.8	2.7±0.1	1.0±0.1*	0.9±0.1*
(mmol.min/l)	POST	27.1±2.4	23.0±1.6	29.7±1.8	24.1±2.7
glucose	INF	-1.1±0.8	1.0±0.6*	-0.12±1.1	0.6±0.6
(mmol.min/l)	POST	1.5±4.9	6.8±11.2	3.8±7.3	2.7±7.9
insulin	INF	0.20±0.03	0.26±0.03*	0.21±0.03	0.34±0.09
(mIU.min/l)	POST	1.07±0.16	1.84±0.38	1.44±0.32	1.34±0.27

* different from control, # different from propionate infusion. $p<0.05$

Glucose levels were also decreased by infusion of CCK. This probably resulted from the increased insulin levels. In propionate infused sheep, glucose appearance (dAUC-inf) is positive which implicates absorption of glucose from the gastrointestinal tract. We observed this effect also in sheep infused with higher dosages of propionate (11). In forage fed ruminants, net PDV appearance is negative, while concentrate fed ruminants often show a positive net appearance

(14). Increased propionate availability probably increased glucose production from propionate by the liver and spared other glucogenic precursors such as lactate or amino acids (2). Increased availability of nutrients for the PDV may lead to less utilization of glucose.
Insulin levels were increased by infusion of propionate which is reported several times (9, 15, 20, 21). Increased insulin levels following CCK infusion, were also shown by Mineo et al in sheep (16, 17). In his studies, comparable dosages resulted in increased insulin levels but not to the same extent as in the present study. This may be due to the enhancing effect of nutrients as proposed by Mineo (16, 17). Although CCK infusion lasted 20 minutes, insulin levels were decreasing after 10 minutes, which is in agreement with previous reports (16, 17). The proposed increased portal flow may have induced the lowered insulin levels at the end of the infusion period. This effect may be exclusive for combined infusion of CCK and feeding, since levels of insulin and glucagon during infusion of CCK without feeding were never below pre-infusion levels even though dosages of CCK were much higher (16, 17).
In conclusion, combined infusion of low dosages of CCK and propionate decreased intake in meal fed sheep. The possibly synergistic effect is not shown on any of the measured blood borne substances. Furthermore, both CCK and propionate increased insulin levels, but mechanisms may be different. CCK infusion also appeared to increase blood flow since levels of PDV produced nutrients were decreased following a CCK infusion.

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CHAPTER 9

Modelling of plasma concentration of metabolites and hormones

In the preceding chapters elaborate information about the responses of nutrients and hormones is given. In most cases, fluctuations in concentration of metabolites and hormones in response to feeding were influenced by feed quality or infusions. In general, also differences between sampling sites, i.e. portal and jugular veins, could be observed. To facilitate interpretation of the nutrient/hormone dynamics at both sampling sites, a conceptual model has been defined in which the portal vein and jugular vein are considered as representatives of mutually interacting compartments. The whole body can be considered as a compilation of a large number of compartments. For describing the whole body as the integrated equivalent of all compartments, one would have to know for each compartment: release, uptake and plasma flow. Several studies on fluxes of nutrients, hormones and electrolytes have been performed on well-defined compartments, for example the liver (6, 16), rumen epithelium (17) and portal vein drained viscera (9, 10, 20-22). In those studies only one compartment was studied, which can be very suitable for estimating fluxes over a certain organ.
The here presented model was developed as a tool for explaining the observed changes in concentration in the two compartments sampled (i.e. portal and jugular veins). Schematically, the model is presented in figure 1. Mathematical formulas describing the model are given in appendix A.

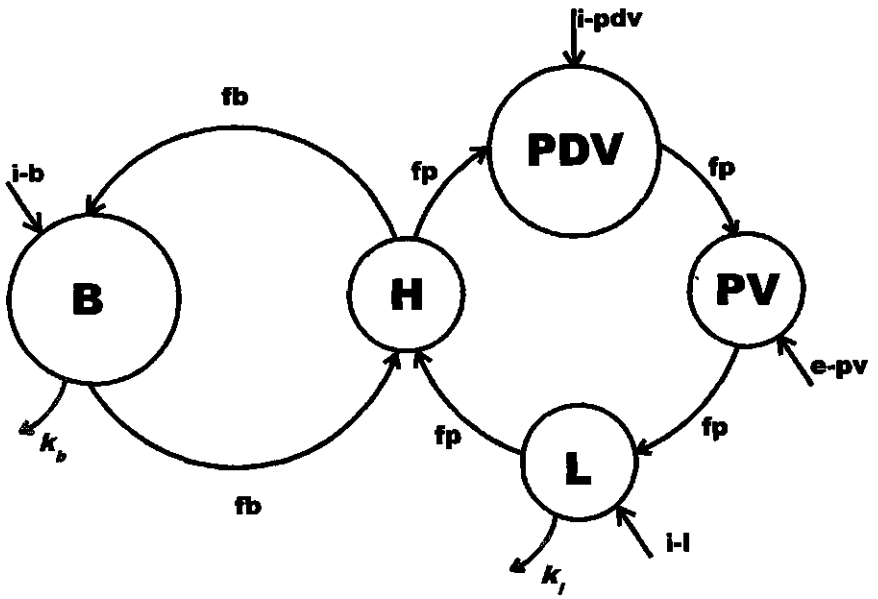

Figure 1: Diagrammatic representation of the mathematical model. Circles enclosed by solid lines indicate compartments. Curved arrows indicate plasma flows. Straight arrows indicate fluxes. Abbreviations used: B, Body; H, Heart; PDV, Portal Drained Viscera; PV, Portal Vein; L, Liver; i-b, release in B compartment; i-pdv, release in PDV compartment; i-l release in L compartment; e-pv, (exogenous) release in the PV compartment; k_b, fractional clearance from B compartment; k_l, fractional clearance from L compartment; fp= portal plasma flow; fb= plasma flow in body.

MODEL CONSTRUCTION

The five compartments defined are PDV, PV, L, H and B representing respectively the blood present in the Portal Drained Viscera, Portal Vein, Liver, Heart and the rest of the Body. The sampling sites used were the portal vein (PV), and the jugular vein, representing the Heart (H).
Input in the PDV compartment represents ruminal, gastrointestinal or pancreatic release of metabolites or hormones, and is depicted as the i-pdv arrow in Fig. 1. i-pdv can be calculated as the PV-H difference multiplied by fp (portal flow). Input in L (i-l) represents release by the liver and input in B (i-b) represents release by the rest of the body. Uptake (i.e. negative release) from the system can occur in the B, L and PDV compartments. Input in the PV compartment (e-pv) represents exogenous infusion. When no infusion was performed e-pv is zero. Volume of the infused solution was neglected.
Clearance constants k_b and k_l were estimated using numerical integration with time steps of 0.02 minutes from half-life time and fractional liver uptake obtained from literature. Constants used are given in table 1 in appendix A.
Volumes of the compartments were estimated as follows. The blood volume of a sheep of 75 kg is approximately 6 litres. This implies that total plasma volume is about 4 litres. In sheep, 40% of the blood volume can be assigned to the portal-drained viscera including the liver (VPDV + VPV + VL) (18). Estimated volume of the portal vein (VPV) was 0.08 l and the plasma volume of the liver (VL) 0.12 litres. Plasma volume of the heart (VH) was set at 0.2 litres, leaving 2.2 litres for the rest of the peripheral circulation (VB).
Plasma flows (fp and fb) were variable in time dependent on feed quality and time after feed supply. Due to absence of a reliable technique for determining blood flow for longer periods on a minute to minute basis, blood flow was estimated using literature data. Flows used are given in table 2 in Appendix A.
By fitting i-b and i-l by numerical integration with time steps of 0.02 min, the physiological release into, or most commonly uptake from, the compartments B and L, portal or jugular levels can be estimated.
Net uptake from the compartments B and L is built up by two factors:
1. Fractional clearance (kl and kb).
2. Physiological clearance (i-b and i-l).
Fractional clearance from a compartment is quantitatively important with high concentrations. Biologically, fractional clearance may be important in removing undesired surpluses for example after a bolus injection or infusion. Physiological clearance (i-b and i-l) may be equivalent with the physiological need or requirement. One might argue that part of hormones or nutrients cleared by fractional clearance also meets part of the physiological need, which is valid. Therefore in the following part of this chapter, uptake will be considered to be the sum of fractional and physiological uptake.

MODELLING POSTPRANDIAL CHANGES

As discussed earlier, concentrations of nutrients and hormones may vary following feeding. Often, these changes may be observed in both portal and jugular veins. Changes in concentration in a given compartment (vein) may be due to one or a combination of the next phenomena

1. Changed release of a substance into this compartment.
2. Changed uptake of a substance from this compartment.
3. Changed flow through this compartment with blood with a different concentration.

It is obvious that due to feeding enhanced release of nutrients or hormones occurs. From a biological point of view it is also very likely that requirements for nutrients may vary leading to changed uptake.
Several studies provide evidence that feeding influences blood flow. Since blood flow was not measured in the experiments presented in this thesis, the model was fed with flows obtained from literature data. In monogastric animals and man, the cardiovascular responses to feeding are well documented (5, 11). In general, two phases can be distinguished. The first phase is the ingestion phase, characterised as a generalised systemic response with a time span of 5-30 minutes. During this phase, cardiac output is increased (20-50%)(5). The vascular resistance of the digestive organs is generally increased while the vascular resistance of the rest of the body is decreased (5), leading to an enhanced peripheral flow rate but unchanged portal flow rate. When the ingestion phase is waning, the absorptive phase is starting. The blood flow is directed towards the digestive organs (8, 14), and is partly compensated by a decreased flow to skeletal muscle (5).
In ruminants however, only a limited amount of work has been done on the relation between feeding and blood flow. Distribution of blood to non-digestive organs is increased shortly after the meal start, without an increased blood supply of the digestive organs (2). This may indicate that the reported ingestion phase in monogastric animals is also occurring in ruminants, but possibly to a lesser extent (2). More is known about the digestive phase. Blood flow towards the digestive organs is increased reaching its peak 2-4 hours after feeding (7, 12). Portal blood flow is increased 25-100% following a meal (12, 13).
Long-term enhanced availability of nutrients, either by increased intake or increased feed quality, results in an increased portal blood flow in ruminants (15, 19, 20) and non-ruminants (8, 14). To our knowledge, no reports on long-term effects of a higher input of nutrients on cardiac output have been published.
Plasma flows through the compartments were approximated as presented in Table 2 in Appendix A. During the first 20 minutes, plasma flow in the peripheral circulation was supposed to be enhanced as a consequence of the increased cardiac output. Peripheral plasma flow was increased maximally 25%. Portal plasma flow was increasing after 20 minutes reaching its maximum (25% above basal) after 90 minutes and subsequently returning to basal levels after 105 minutes. It was assumed that plasma flow in LQ-fed sheep was 10% lower than in HQ-fed sheep (5, 18).

Modelling postprandial propionate concentrations
As an example of the modelling procedure, modelling of propionate levels of sheep fed a low quality diet will be discussed.
Plasma flows and compartment volumes were chosen as described above. From the infusion study performed with a high dosage of propionate (chapter 5), half-life time was estimated to be approximately 4 min, which is accordance with literature data (3).
In table 3a and 3b in appendix A, raw fitting data are presented.

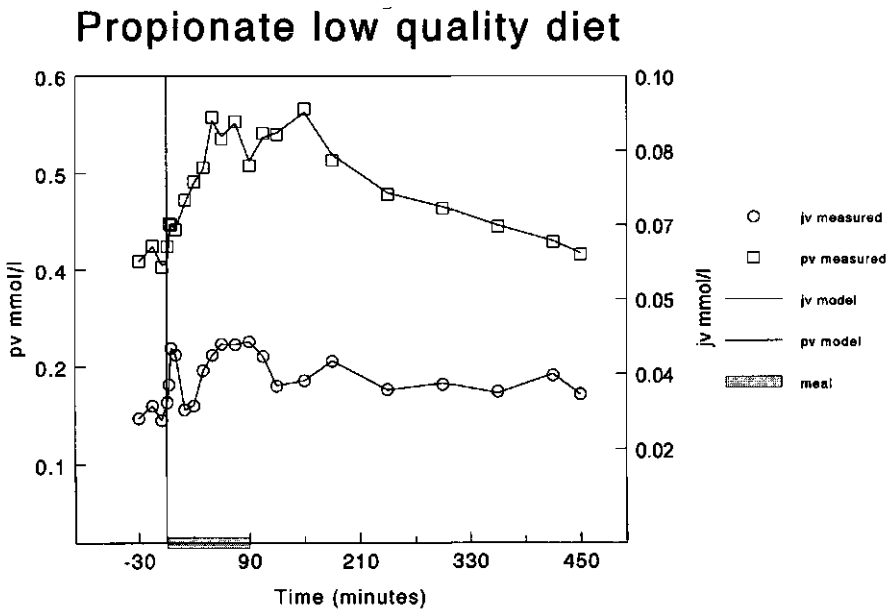

Figure 2: Comparison of observed and modelled propionate concentrations in the jugular and the portal veins of low quality fed sheep.

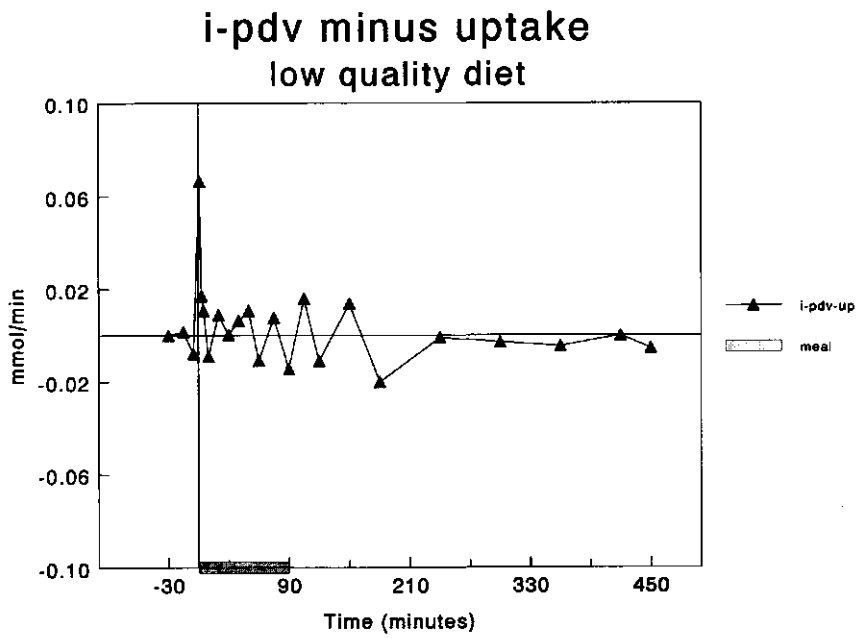

Figure 3: Modelled i-pdv reduced with uptake of low quality fed sheep.

In figure 2, both measured as well as modelled propionate levels are shown. It is obvious that the model fitted measured levels very accurately. It is also clear that propionate levels in the portal vein were usually about 10 fold higher as compared to jugular vein levels. This can be attributed to the very high extraction by the liver of approximately 95% (1, 4). Comparing the two lines, it is remarkable that the pattern in the portal vein does not resemble the pattern in the jugular vein. While levels in the portal vein gradually increased, a bi-phasic pattern was observed in the jugular vein. The observed increase in the jugular vein during the first minutes following feed start, probably arose from a slight mismatch between production (i-pdv) and total uptake (i-l + i-b and the fractional clearance from liver and body compartment) as shown in figure 3. It is very likely that uptake by the liver was slightly lowered resulting in a small increase in propionate escaping liver clearance. Although this may seem logical, it should be borne in mind that the proposed mismatch between i-pdv and uptake was very small relative to the amount of propionate released as shown in figure 4. Comparing figures 4 and 2 shows that the second increase observed in both peripheral and portal levels (Fig. 2) was probably due to enhanced portal appearance as shown in figure 4. This example illustrates that increased levels in peripheral blood are not automatically induced by higher release but may result from lowered uptake.

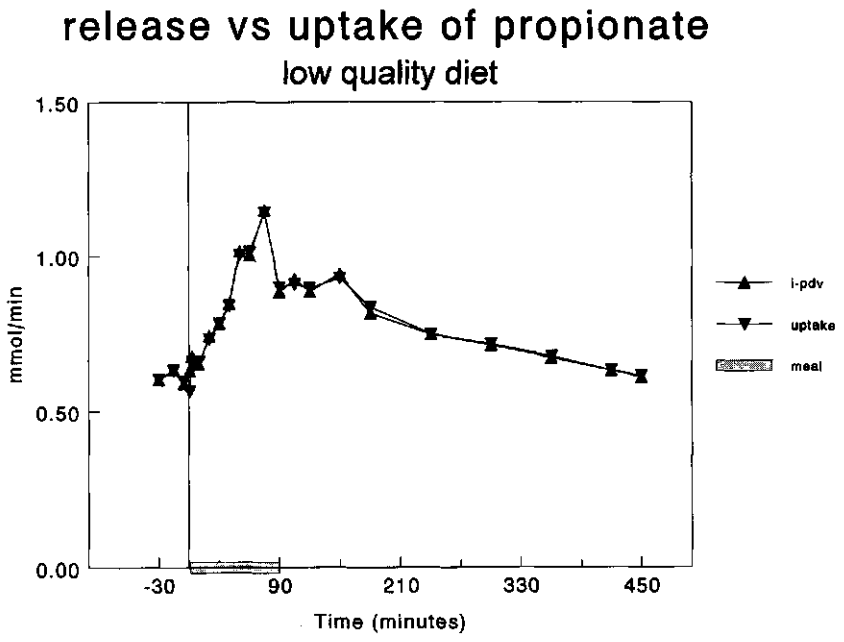

Figure 4: Modelled i-pdv versus uptake of low quality fed sheep.

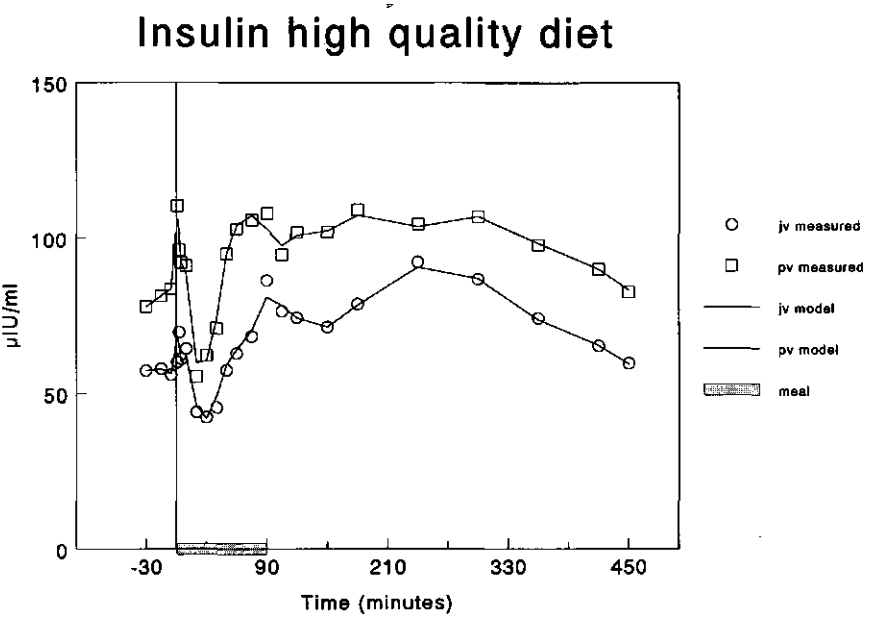

Figure 5: Comparison of observed and modelled insulin concentrations in the jugular and the portal vein of high quality fed sheep.

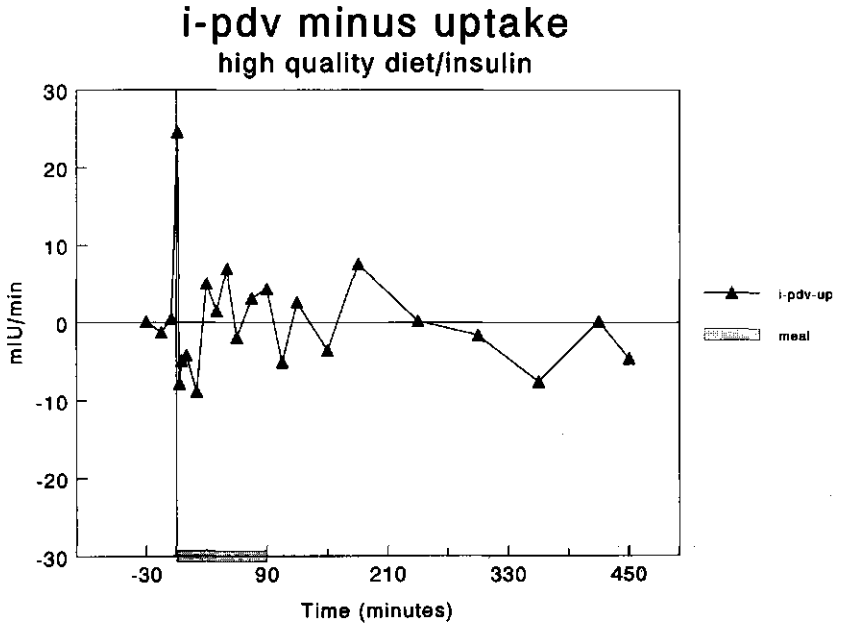

Figure 6: Modelled i-pdv reduced with uptake of high quality fed sheep.

Modelling postprandial insulin concentrations

Rapidly increased concentrations following meal start were also observed in insulin levels of HQ-fed sheep. Modelling these observations led to apparently similar observations as for propionate levels. As for propionate, insulin levels were fitted satisfactory (Fig. 5) and a mismatch between uptake and portal release was observed following meal start (Fig. 6). In case of insulin, this mismatch was not due to diminished uptake but rather due to enhanced release of insulin as shown in figure 7. Although relatively large fluctuations were observed, uptake and release were usually closely matched. In conclusion, fluctuations in hormone and metabolite concentrations arise from relatively small mismatches between release and uptake. Furthermore, it should be noticed that changed (peripheral) blood levels may not result from changed release of a substance.

Biologically, the amount of nutrients absorbed from the gastrointestinal tract or hormone released (estimated by i-pdv), is more important than circulating concentrations. In table 1, estimated 8-hour production/release (i-pdv) is given for some nutrients and hormones. From this table it is clear that feeding HQ feed resulted in increased portal release of nutrients and induced higher (pancreatic) hormone release. It is usually found that feed quality and/or rate of feeding induces enhanced production of VFA's (3, 23, 26) and pancreatic hormones (13, 24, 25).

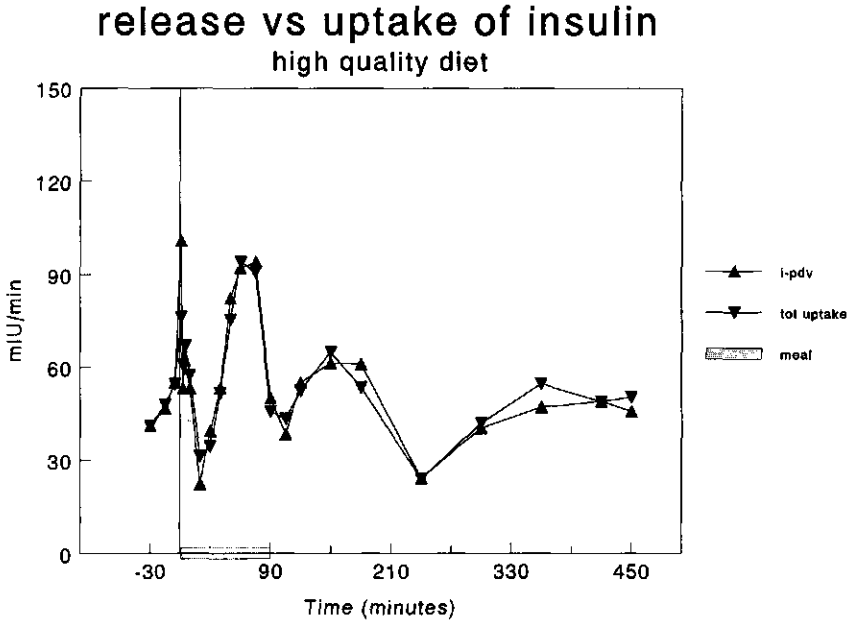

Figure 7: Modelled i-pdv versus uptake of high quality fed sheep.

Table 1. Calculated 8 h portal release (i-pdv) of sheep fed either a HQ or a LQ diet.

	HQ	LQ	p
Acetate (mmol)	1519± 142	1363± 194	0.135
Propionate (mmol)	445± 26	370± 124	0.060
Butyrate (mmol)	-30± 7	-77± 17	0.020
Iso-butyrate (mmol)	21± 1	15± 2	0.002
Iso-valerate (mmol)	15± 1	11± 1	0.002
BHB (mmol)	88± 23	104± 32	0.263
Insulin (IU)	25.1± 3.1	15.5± 4.1	0.05
Glucagon (µg)	73.0± 12.5	25.7± 5.1	0.005
Gastrin (nmol)	6.9± 3.2	7.3± 3.9	0.32
CCK (nmol)	2.3± 0.6	3.0± 0.5	0.24
PP (nmol)	37.8± 10.1	14.9± 7.4	0.05

values are expressed as means ± SE (mmol).

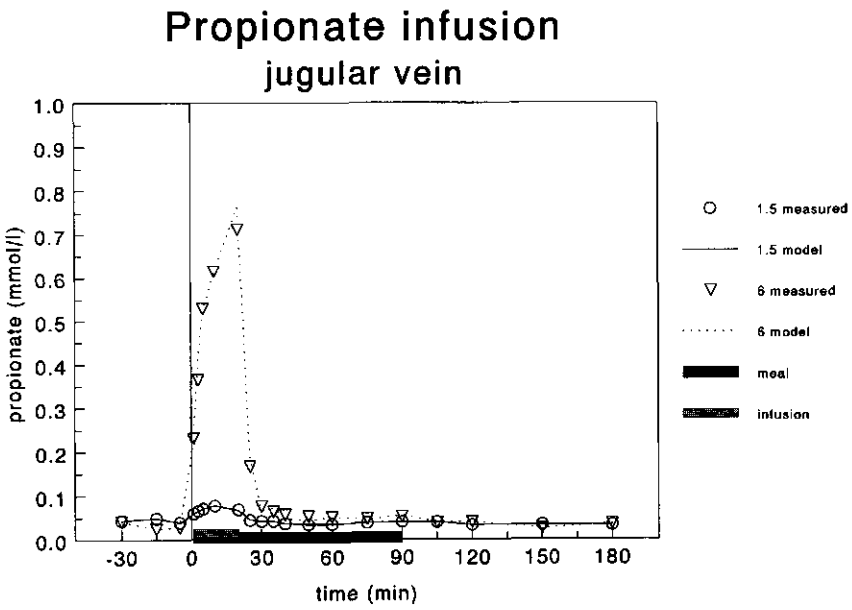

Figure 8: Modelled jugular propionate levels versus measured levels in 1.5 or 6 mmol/min propionate infused sheep. Fitted for jugular levels.

MODELLING OF INFUSIONS

The model can also be used to estimate nutrient or hormone levels that can be obtained theoretically during infusion. In this thesis, experiments are described in which animals received an infusion via the mesenteric vein. Since the tip of the infusion catheter was placed at the entrance of the portal vein it is assumed in the model that infusions were performed in the portal vein compartment. This means that portal vein concentrations are influenced by endogenous release (i-pdv) and exogenous supply (e-pv). It also means that the reasoning that i-pdv can be estimated from the portal vein - jugular vein (PV-JV) difference is not valid during the infusion period. i-pdv during infusion of propionate was therefore chosen to resemble i-pdv during infusion of a control solution. Two procedures can be followed namely:

1. Fitting jugular levels by estimating i-b and i-l.
2. Fitting measured levels of control infused animals (e-pv is zero), followed by running the model with the appropriate exogenous infusion rate (e-pv).

Infusion of propionate

As an example of the first mentioned procedure, the infusion study described in chapter 6 is used. In this study, sheep were infused with 0, 1.5 or 6 mmol/min propionate.

Fitting the experimentally obtained propionate levels during infusion of propionate led to very accurately fitted jugular vein levels as shown in figure 8. In figure 9 accompanying portal levels are shown. Measured portal vein propionate concentrations of the 1.5 mmol/min infused sheep were slightly lower as calculated by the model but not dramatically. In contrast to the 1.5 mmol/min infusion, calculated levels of the 6 mmol/min infusion were much lower as measured levels. Possible explanation for this may lie in incomplete mixing of the infused propionate with the portal blood. This is strengthened by the large variation found in portal propionate levels during infusion in Chapter 6.

Comparing total amount of portal appearance of propionate (i.e. i-pdv + e-pv) with the calculated uptake (i.e. i-b + i-l + fractional clearance) it is striking that uptake and production are closely matched (Fig. 10). As with the postprandial increases as described in the preceding section, only small discrepancies between uptake and release were responsible for the observed increased levels. This is demonstrated in figure 11, where portal release minus uptake is shown. After the infusion period the largest deviations were observed which is due to overestimation of portal release (i-pdv) which is estimated using PV-JV differences.

Infusion of insulin

A second approach in modelling an infusion study is using the control infusion to estimate the fitting parameters (i-b and i-l), followed by running the model with the exogenous infusion rate (e-pv). In other words, control animals receive an imaginary infusion. To show this procedure, data of the insulin infusion study as described in chapter 4 is used. In this study, animals fed two different diets received insulin at a rate of 6.7 mIU/min.

In figure 12, calculated insulin levels are compared to measured insulin levels in sheep fed a low quality diet. Insulin concentrations of control sheep receiving the imaginary infusion (calculated levels), highly resemble that of actually infused

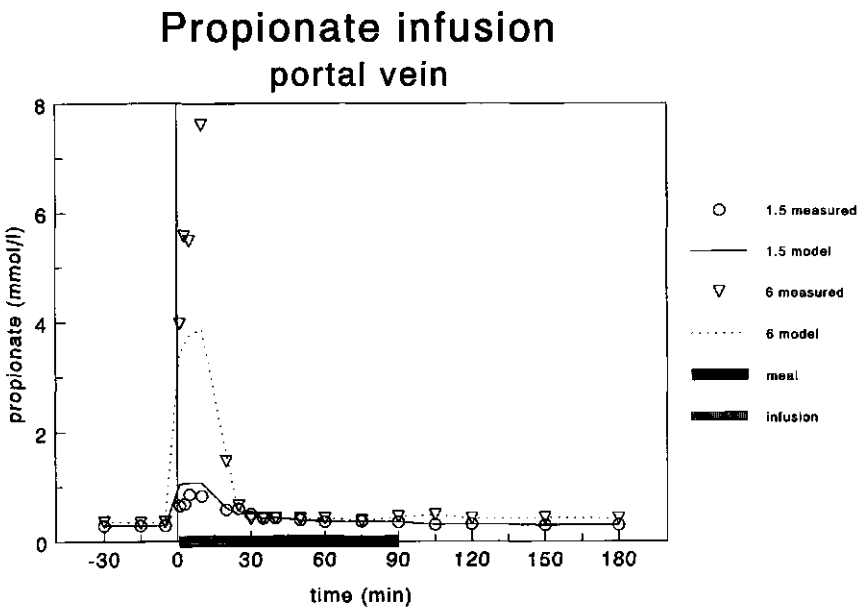

Figure 9: Modelled portal propionate levels versus measured levels in 1.5 or 6 mmol/min propionate infused sheep. Fitted for jugular levels.

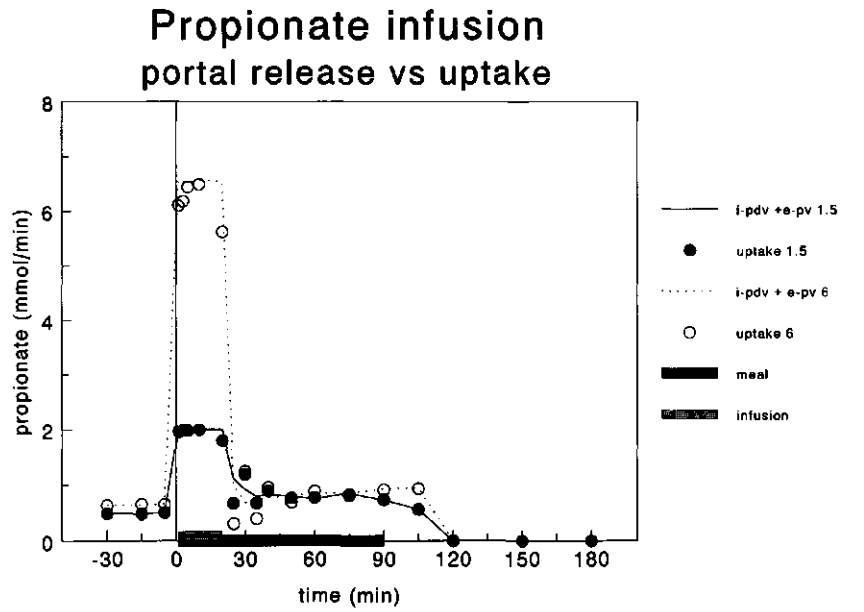

Figure 10: Modelled i-pdv versus uptake of 1.5 or 6 mmol/min propionate infused sheep.

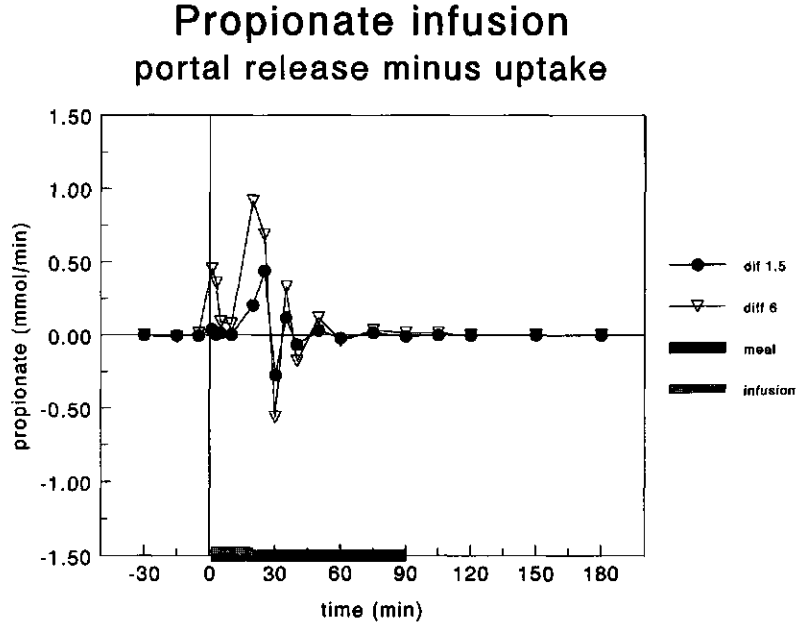

Figure 11: Modelled i-pdv reduced with uptake of 1.5 or 6 mmol/min propionate infused sheep.

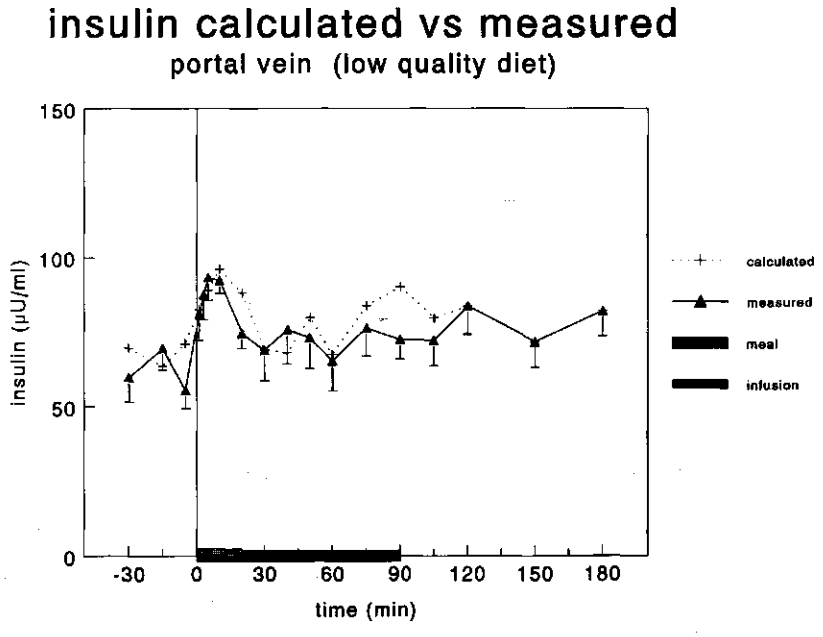

Figure 12: Comparison of observed and modelled portal insulin concentrations in 6.7 mIU/min insulin infused sheep fed a low quality diet

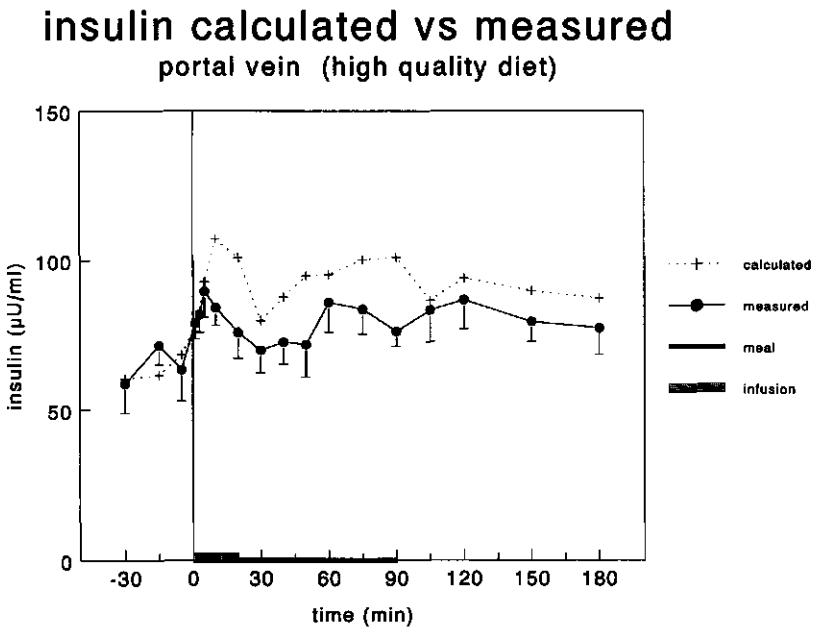

Figure 13: Comparison of observed and modelled portal insulin concentrations in 6.7 mIU/min insulin infused sheep fed a high quality diet

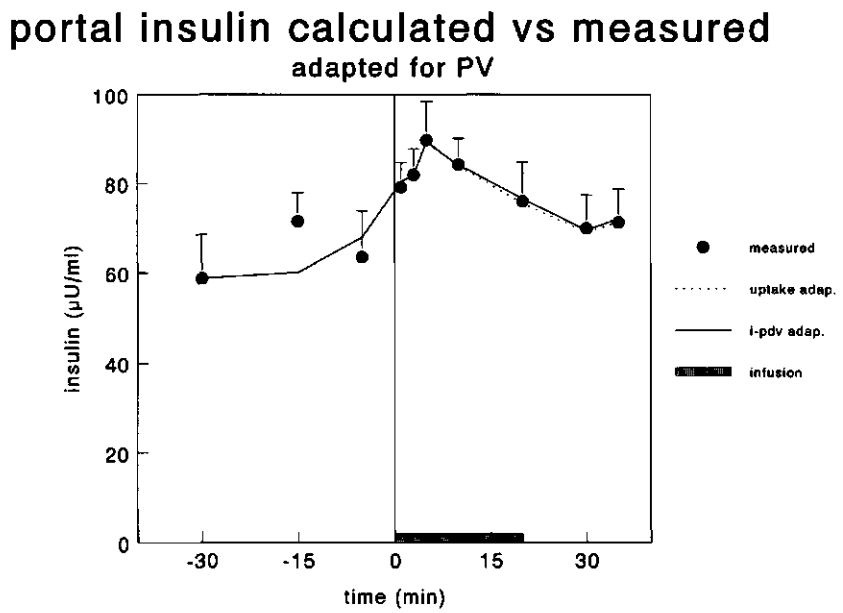

Figure 14: Measured versus modelled portal insulin concentration. Fitted for portal levels. Dotted line: uptake adapted, solid line, i-pdv adapted.

animals. This indicates that insulin infusion did not change endogenous insulin release (i-pdv) or uptake.
On the other hand, measured insulin levels of actually infused sheep fed a high quality diet are lower compared to calculated level (Fig. 13). This means that either i-pdv is lowered to compensate the exogenous supply (e-pv) or peripheral uptake is increased leading to decreased arterial (or jugular) levels. Both situations i.e. decreased i-pdv or increased uptake can be investigated by fitting i-pdv or i-b and i-l to obtain calculated portal levels that resemble measured levels. The result of this procedure is shown in figure 14. During infusion it is possible to fit PV levels very accurately by adjusting i-b and i-l (dotted line) or adjusting i-pdv (solid line). Accompanying jugular levels are shown in figure 15. Fitting i-pdv led to well matched measured and modelled levels. Modelling uptake resulted in jugular levels that are lower as compared to measured levels. In other words, exogenous supply of insulin probably led to reduced endogenous insulin release.

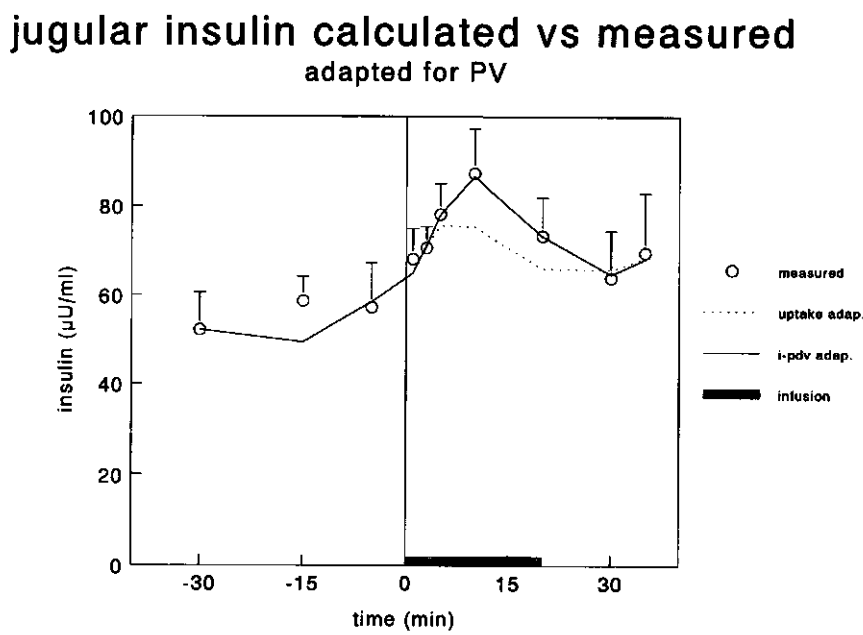

Figure 15: Measured versus modelled jugular insulin concentration. Fitted for portal levels. Dotted line: uptake adapted, solid line, i-pdv adapted.

CONCLUSIONS

Despite the simplicity of the model, it proved useful in explaining experimentally obtained data. One of the major observations was that release and uptake are usually very tightly matched even during infusion of large amounts of propionate. It also showed that changed plasma concentration may arise from changed uptake (in case of the early peak in jugular propionate following feeding) or changed release (in case of insulin increases following feeding).
However, it should be borne in mind that the quantitative data should be interpreted cautiously due to the absence of experimental data on blood flow.

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Mathematically, the model is based on the following equations:

$$dpdv/dt=((-fp \times cpdv) + (fp \times ch) + i\text{-}pdv)/vpdv$$
$$dpv/dt=((-fp \times cpv) + (fp \times cpdv))/vpv$$
$$dl/dt=((-fp \times cl) + (fp \times cpv) - (k_l \times vl \times cl) + i\text{-}l)/vl$$
$$db/dt=((-fb \times cb) + (fb \times ch) - (k_b \times vb \times cb) + i\text{-}b)/vb$$
$$dh/dt=((-fb \times ch) - (fp \times ch) + (fp \times cl) + (fb \times cb))/vh$$

with cpdv, cpv, cl, cb, ch = concentration in the compartments PDV, PV, L, B and H (mmol/l).
fp=flow through the PV compartment (l/min)
fb=flow through the B compartment (l/min)
k_b= clearance constant from the B compartment calculated from half-life time and the relative liver uptake (/min)
k_l= clearance constant from the L compartment calculated from half-life time and the relative liver uptake (/min)
i-pdv= net release in the PDV compartment (mmol/min)
calculated as the measured portal vein -jugular vein difference * fp
i-l= net release in the L compartment (mmol/min)
i-b= net release in the BV compartment (mmol/min)
e-pv= exogenous release in the PV compartment (mmol/min)
vpdv, vpv, vl, vb, vh= volume of the compartments PDV, PV, L, B and H.

Table 1. k_h, k_l, fractional liver uptake, and half-life time for several metabolites.

	k_b (min^{-1})	k_l (min^{-1})	liver uptake	t ½ (min)
Acetate	0.7	0	0%	2
Propionate	0.1	0.1	95%	8
Butyrate	0.1	3	50%	5
Iso-butyrate	0.1	3	50%	5
Iso-valerate	0.1	3	50%	5
BHB	0.1	1	50%	6
Glucose	0.1	0	0%	12

Table 2. Blood flow in the portal circulation (fp) and the body circulation (fb) for both HQ and LQ diets used in the mathematical model.

	HQ		LQ	
Time	fp	fb	fp	fb
-30-0	2.0	5.0	1.8	4.5
0-5	2.0	5.5	1.8	4.9
5-10	2.0	6.0	1.8	5.4
10-20	2.0	6.0	1.8	5.4
20-30	2.0	5.5	1.8	4.9
30-40	2.1	5.2	1.9	4.7
40-50	2.2	5.0	2.0	4.5
50-60	2.3	5.0	2.1	4.5
60-75	2.5	5.0	2.3	4.5
75-90	2.3	5.0	2.0	4.5
90-105	2.1	5.0	1.9	4.5
105-450	2.0	5.0	1.8	4.5

dimension: l/min

Table 3a. Measured versus modelled jugular vein (jv) and portal vein (pv) levels and the relative deviation of calculated concentrations from measured concentrations.

	Measured			Calculated			% deviation	
Time	jv	pv	pv-jv	jv	pv	pv-jv	jv	pv
-30	0.026	0.361	0.334	0.026	0.361	0.334	0.000	0.000
-15	0.029	0.381	0.352	0.029	0.380	0.351	0.000	-0.243
-5	0.026	0.353	0.327	0.026	0.356	0.330	0.000	0.826
1	0.030	0.380	0.350	0.030	0.359	0.329	-0.005	-5.484
3	0.034	0.410	0.376	0.034	0.399	0.365	0.012	-2.603
5	0.041	0.409	0.367	0.041	0.408	0.367	-0.062	-0.124
10	0.040	0.402	0.362	0.040	0.404	0.364	0.092	0.612
20	0.028	0.441	0.412	0.028	0.436	0.408	-0.071	-0.997
30	0.029	0.464	0.435	0.029	0.463	0.434	0.052	-0.269
40	0.037	0.482	0.446	0.037	0.480	0.444	-0.039	-0.389
50	0.040	0.547	0.507	0.040	0.542	0.502	0.025	-0.878
60	0.042	0.520	0.477	0.042	0.523	0.480	-0.019	0.606
75	0.042	0.541	0.499	0.042	0.539	0.497	0.011	-0.350
90	0.043	0.485	0.442	0.043	0.489	0.446	-0.009	0.840

Table 3b. Summary of modelled parameters of postprandial propionate changes as described in table 3a and chapter 9.

Time	i-pdv	i-b	i-l	c-b	c-l	Total uptake
-30	0.6021	-0.0266	-0.5590	-0.0009	-0.0156	-0.6021
-15	0.6330	-0.0279	-0.5873	-0.0008	-0.0154	-0.6314
-5	0.5891	-0.0264	-0.5563	-0.0007	-0.0136	-0.5970
1	0.6302	-0.0246	-0.5189	-0.0006	-0.0198	-0.5639
3	0.6768	-0.0291	-0.6127	-0.0010	-0.0169	-0.6596
5	0.6607	-0.0284	-0.5986	-0.0012	-0.0216	-0.6499
10	0.6508	-0.0290	-0.6109	-0.0014	-0.0186	-0.6600
20	0.7422	-0.0325	-0.6838	-0.0008	-0.0162	-0.7332
30	0.7827	-0.0347	-0.7307	-0.0008	-0.0163	-0.7825
40	0.8467	-0.0372	-0.7822	-0.0009	-0.0199	-0.8402
50	1.0145	-0.0445	-0.9369	-0.0009	-0.0215	-1.0039
60	1.0022	-0.0449	-0.9458	-0.0011	-0.0213	-1.0131
75	1.1480	-0.0507	-1.0664	-0.0007	-0.0225	-1.1403
90	0.8846	-0.0396	-0.8334	-0.0011	-0.0212	-0.8954

Abbreviations: i-pdv, i-l, i-b as described in model construction, c-l and c-b: fractional clearance from the compartments L and B. Total uptake = i-b + i-l + c-l +c-b

CHAPTER 10

DISCUSSION

The mechanisms by which feed intake is regulated may include very different pathways. In the general introduction, some of these pathways have been introduced. In this thesis, the role of several hormones and metabolites in the regulation of feed intake was emphasised. It is clear that no single factor is essential for normal feed intake. In the search for factors involved in intake regulation one should not focus on one factor but bear in mind that intake regulation is multifactorial. The various factors shown to be effective in altering feed intake should therefore be looked upon as complementary in stead of alternatively. In case of blood-borne factors the general thought that a) feeding should induce a change in this particular factor and b) infusion of this particular factor should induce a change in intake may be worthwhile to keep in mind.
In chapters 3 and 4, it was shown that feeding induces changes in concentrations of many hormones and metabolites. It was also shown that changes in hormone or metabolite level depend on the type of feed supplied to the animal. This may raise the question whether intake regulation may involve similar mechanisms irrespective of the type of feed ingested. Considering this question, it may be worthwhile to focus in future research on the role of glucagon and/or pancreatic polypeptide, which showed to be very sensitive to feed quality and showed rapid fluctuations following a meal.

The role of metabolites in the regulation of intake

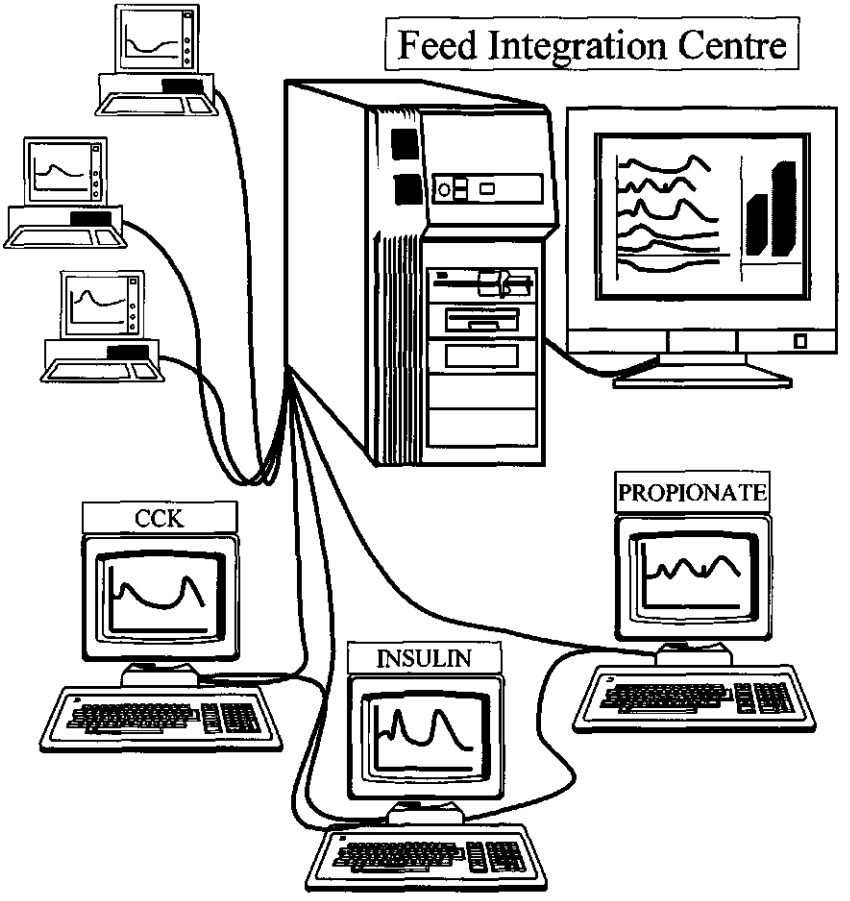
Since depletion of body reserves induces feeding, it is very tempting to think that the organism is monitoring the nutrient availability. In the light of this thought, many researchers focused on the effect of nutrients or metabolites on feed intake. While in monogastrics glucose is one of the main energy suppliers, this is not true in ruminants. Ruminants depend for their energy supply largely on the formation of Volatile Fatty Acids (VFA's) by the microflora in the rumen. Propionate is formed in considerable amounts and has repeatedly been shown to reduce intake. Furthermore, propionate is one of the main precursors of glucose and is insulinotrophic. Since propionate levels following infusion, are usually much higher as observed after a meal some experiments were performed in which animals received small dosages of propionate. In chapters 4 and 5 it was concluded that it is unlikely that propionate is the sole factor regulating intake. It is more likely that it is only one of the (many) factors involved. In future research more attention should be paid to butyrate which is usually produced less than propionate and acetate but portal and jugular levels may vary considerable following feeding. Taking into account the high sensitivity of the endocrine pancreas to butyrate one might assume that butyrate may be involved in feed intake regulation

The role of hormones

Insulin is reported to be involved in the regulation of feed intake. In chapter 6, an attempt was made to elucidate the question whether the observed postprandial insulin increase may be involved in satiety. A mild infusion of insulin did not result in reduction of intake but reduced eating time. Although the experimental set up for estimation of feeding behaviour may be questioned, this observation is very interesting. In this study it was also postulated that exogenous supply of insulin may be counter regulated by the endogenous release and that this may be dependent on feed quality. It may therefore be possible that the variability found in reaction to

exogenous insulin supply in literature is (partly) caused by differences in susceptibility of the animal, due to differences in feed quality.
Cholecystokinin (CCK) is also thought to be involved in feed intake regulation. Many studies show a reduction of intake following an infusion of CCK. CCK-8 is most commonly used and was therefore also used in the studies described in Chapters 7 and 8. It was shown that low dosages of CCK, resulting in very mildly increased portal blood levels, were incapable to reduce intake. Surprisingly, exogenous supply of CCK reduced endogenous CCK release probably through a local pathway since peripheral CCK levels were not influenced. This phenomenon was not reported earlier and deserves more attention. It is possible that this mechanism is exclusive for ruminants since this has not been reported thus far in humans or laboratory animals.

The concerted roles of hormones and metabolites
In Chapter 8, it was shown that combined infusion of a metabolite (propionate) and a hormone (CCK) led to decreased intake. This is in line with the hypothesis that intake is not regulated by a sole factor but by the integration of many factors including blood-borne components. The components investigated in this thesis may directly or indirectly contribute to satiety. Schematically the integration of signals is shown in the subjoined figure.



In this scheme, the body is represented as a computer network. The hypothesised decisive centre may be represented by the 'server'. This server receives and sends information from and to the 'workstations'. In case of the regulation of feed intake, the "feed integration computer" receives information from various workstations throughout the body. These workstations can also share information and interact. By this way signals from a certain workstation may vary in importance.
In this thesis only three of them were investigated but many other are also important. Further research at multifactorial level will elucidate the magnitude of the many signals investigated over the years.

CHAPTER 11

Summary

Samenvatting

SUMMARY

In the search for factors involved in the regulation of feed intake, many experiments have been performed in various species. In ruminants, very little is known about the physiological background of the mechanisms involved in feed intake regulation. In earlier experiments, much attention was paid to physical regulation suggesting that the capacity of the digestive tract is the most important limiting factor in feeding.
Since ruminants are capable of meeting their energy requirements under a wide range of circumstances and feedstuffs, the concept of physiological regulation was introduced. Physiological regulation (or metabolic regulation) can be defined as feed-back signals arising from sensors in the periphery, which inform the central nervous system about the metabolic status of the individual. In the brain, presumably in the hypothalamus, these signals are integrated and decisions are made whether or not to eat. This thesis focuses on blood-borne factors related to feed intake in sheep. This includes the major energy providing components, metabolic hormones and gastrointestinal hormones.
The aim of this thesis is:
To gain insight in the changes in nutrient and hormone concentrations following meals of different feed qualities.
To investigate blood borne nutrients/hormones which may be involved in the regulation of feed intake.

To study this, experiments were performed in wether sheep provided with mesenteric, portal and jugular catheters.
To address the effect of feeding and feed quality on nutrients and hormones, animals were fed during 90 minutes. Before, during and after feeding, blood samples were withdrawn from jugular and portal catheters in order to identify candidates for intake regulation. Two experimental pelleted grass diets, qualified as High Quality (HQ) and Low Quality (LQ) based on crude protein and fibre contents were fed according to a cross-over design.
In chapter 2, short-term effects of feed intake on jugular and portal concentrations of metabolites such as Volatile Fatty Acids (VFA), Beta-hydroxy-butyrate (BHB) and glucose were studied. Rapid changes were observed in both jugular and portal veins due to feeding. Portal vein (PV) concentrations of acetate, propionate, butyrate, iso-butyrate and BHB were increased post-prandially in HQ-fed sheep. Due to consumption of LQ feed, bi-phasic patterns were found in acetate, propionate, butyrate levels, measured in the jugular vein (JV).
The effect of feeding on nutrient concentration may largely differ as a result of feed quality. The PV-JV difference was used to estimate the release of nutrients into the portal vein. Differences in peripheral concentration of a blood component did not necessarily result from a difference in the release of the component but could also have resulted from a changed uptake by peripheral tissues. In most cases, the observed early changes (until 30 minutes past meal start) were probably due to changes in uptake rather than alterations in release. The changes observed later then 30 minutes were likely due to changes in release.
In chapter 3, the response of metabolic and gastrointestinal hormones to feeding is described. Rapid fluctuations were shown for insulin, glucagon, Pancreatic Polypeptide (PP) and Cholecystokinin (CCK) levels in HQ-fed sheep. Sustained changes were observed for insulin, glucagon and gastrin levels in HQ-fed sheep. LQ-fed sheep showed rapid alterations in Growth Hormone (GH), gastrin, PP and

CCK levels. Sustained changes were observed for insulin, GH, gastrin, PP and CCK levels.
The rapid changes in hormone concentration may be due to decreased parasympathetic activity and/or increased sympathetic activity. More sustained changes were likely nutrient induced. Feed quality mainly affected the magnitude of the meal induced changes in hormone levels, with the HQ-fed sheep showing more pronounced differences.
In chapter 4, the hypothesis that propionate is a short-term feed intake regulating agent is discussed. In the first experiment, sheep were infused over 20 min with Na-propionate into the mesenteric vein, while monitoring feed intake and feeding pattern over 1.5 hours. Feed intake was reduced by infusions at 2 mmol/min which were associated with marked increases in jugular as well as portal concentrations of insulin, glucose and propionate.
In a second experiment, animals were infused with 2 mmol/min Na-propionate into the portal vein. No decrease in feed intake was observed, though similar increases in insulin, glucose and propionate as found in mesenteric vein infused animals were observed. It was concluded that mesenteric propionate in high doses acted as a satiety factor. Possible explanations for the difference between site of infusion may be a different distribution of the infusate over the liver, and/or the presence of propionate sensitive receptors in the mesenteric/portal vein region.
In a more extensive experiment, described in chapter 5, sheep were infused via the mesenteric catheter with 0, 1.5 or 6 mmol/min Na-propionate for 20 minutes. Infusion of 6 mmol/min Na-propionate decreased feed intake but also induced discomfort. Portal levels of propionate, glucose and insulin were increased while decreased levels of butyrate, BHB, gastrin, PP and CCK were observed. Jugular levels generally showed similar patterns as portal levels except for butyrate. Jugular butyrate was immediately increased after start of the meal, presumably due to a smaller liver uptake.
Infusion of 1.5 mmol/min Na-propionate resulted in elevated levels of propionate and insulin while gastrin and PP concentrations were decreased.
It was concluded that propionate is not a major factor influencing intake, since infusion of a physiological dose did not affect meal size. It is possible that effects found during and after a meal on insulin, gastrin, and PP can be attributed to propionate.
Since propionate levels, which affected intake were rather high, and VFA's are reported to stimulate release of insulin, an experiment was performed in which sheep received an infusion with insulin. This study described in chapter 6, was designed to investigate the effect of insulin infusion on feed intake, feeding pattern and blood concentrations of metabolites and hormones related to feeding and feed quality. During a 90 minutes feeding period, sheep provided with jugular, portal and mesenteric catheters were infused via the mesenteric catheter with 6.7 mU/min insulin or saline for 20 minutes. Blood was frequently sampled from jugular and portal veins. The study was performed on two diets differing in feed quality.
Infusion of insulin did not decrease feed intake but decreased feeding time. Portal insulin levels of sheep receiving an insulin infusion were increased in animals fed a low quality diet but not in animals fed a high quality diet. Insulin levels in the jugular vein were not influenced by infusion of insulin compared to saline infusion. No differences due to infusion of insulin were shown on glucose, glucagon, gastrin, and PP levels. Effects of diet composition were reflected by glucagon levels but not by other hormones.

It was concluded that insulin might be a factor involved in satiety, but not by regulation of meal size. It was also postulated that regulation of endogenous insulin release might be more sensible in animals fed a higher feed quality.
In chapters 7 and 8, the results are presented of a combined infusion of CCK (two dosages) and Na-propionate. In chapter 7, the effect of the 20 minutes infusion with 0, 1.2 or 2.4 nmol/min CCK-8 is described. Infusion of CCK-8 increased levels of CCK-8 in the portal vein but not in the jugular vein. A very accurate clearance of CCK-8 from the liver may have attributed to the absence of increased jugular levels. Infusion of both 1.2 and 2.4 nmol/min CCK-8 decreased portal and jugular CCK-33 levels, suggesting a decreased endogenous release of CCK. Portal PP levels were decreased as a result of 2.4 nmol/min CCK-8 infusion. This may be due to a decrease in release or an enhanced portal blood flow.
Cortisol concentrations, as an indicator of stress, were decreased during infusion of saline but increased as a result of CCK-8 infusion. It was concluded that CCK-8 might have induced some discomfort. Despite the increased portal CCK-8 levels and the increased cortisol levels no effect was found on feed intake.
In chapter 8, the effect of an infusion with 0, or 2.4 nmol/min CCK-8 or 0.5 mmol/min propionate or a combination of CCK and propionate are described.
Feed intake was only reduced by combined infusion of CCK-8 and propionate but not by separate infusion of CCK-8 or propionate. Increased levels of propionate and insulin were observed following the propionate infusion. Infusion of CCK decreased propionate, acetate, butyrate, and glucose levels while insulin levels were initially increased followed by decreased levels. Combined infusion of CCK and propionate induced similar blood concentration as infusion of CCK solely on acetate, butyrate, glucose, and insulin, while propionate levels were decreased compared to propionate infused animals but increased compared to CCK infused sheep.
It was postulated that decreased levels of VFA's and insulin may be due to increased portal flow and that mechanisms of induction of insulin secretion by propionate and CCK may be different.
In chapter 9, some of the observations made in the preceding chapters are analysed using a conceptual model. The model proved very suitable in explaining profiles of hormones and metabolites following feed intake. It also proved useful in interpreting the effects the infusion studies. One of the major observations was that release and uptake are usually very tightly matched even during infusion of large amounts of propionate. It also showed that changed plasma concentration may arise from changed uptake (in case of the early peak in jugular propionate following feeding) or changed release (in case of insulin increase following feeding).
Finally, in the general discussion (chapter 10) some remarks are made concerning the possible role of hormones and metabolites in the regulation of feed intake.
In conclusion, as a result of feeding, sheep showed both rapid and more sustained changes in plasma concentration of several metabolites and hormones. Furthermore, differences in feed quality may result in differences in hormone and metabolite concentration but also in differences in plasma profiles.
The infusion studies with propionate, insulin and CCK indicated that the regulation of intake must be regarded as a multifactorial process. It is therefore necessary to study intake regulation as a multifactorial system bearing in mind that gross manipulations may influence other systems involved in intake regulation. Experiments with low (physiological) dosages of combinations of hormones and metabolites should be performed to elucidate the concerted role of these substances.

SAMENVATTING

Bij de zoektocht naar factoren die betrokken zijn bij de regulatie van voeropname zijn er vele experimenten uitgevoerd in een grote verscheidenheid aan diersoorten. Over de fysiologische achtergrond van de regulatie van voeropname in herkauwers is echter zeer weinig bekend. In vroegere experimenten is er veel aandacht besteed aan het concept van fysische regulatie. Opname zou beperkt zijn vanwege de beperkte capaciteit van het maagdarmstelsel.
Het concept van fysiologische regulatie werd geïntroduceerd vanwege de observatie dat herkauwers hun energiebehoefte effectief kunnen dekken onder zeer variërende omstandigheden en voedersoorten. Fysiologische of metabole regulatie kan gedefinieerd worden als een systeem van perifere terugkoppelingssignalen die het centrale zenuwstelsel informeren omtrent de metabole status van een individu. In de hersenen, waarschijnlijk de hypothalamus, worden deze signalen geïntegreerd en wordt de beslissing om al dan niet te eten genomen. Dit proefschrift zal zich richten op factoren aanwezig in het bloed die gerelateerd zijn aan voeropname in schapen. Dit behelst ondermeer de belangrijkste energieverstrekkende componenten en metabole en gastro-intestinale hormonen.

Het doel van dit proefschrift is:

1. Het verschaffen van inzicht in de veranderingen in bloedconcentraties van nutriënten en hormonen na maaltijden van verschillende kwaliteiten

2. Het onderzoeken van nutriënten en hormonen in het bloed die mogelijk betrokken zijn bij de regulatie van de voeropname

Om dit adequaat te bestuderen is gebruik gemaakt van hamels voorzien van catheters in de vena jugularis (VJ) , vena porta (VP) en vena mesenterica (VM).
Om het effect van eten en voederkwaliteit op de concentratie van nutriënten en hormonen in het bloed te onderzoeken werden de dieren gedurende 90 minuten voorzien van voer. Voor, tijdens en na de voerperiode werd bloed afgenomen via de porta en jugularis catheter. Dit had mede als doel kandidaten te selecteren die betrokken zouden kunnen zijn bij de regulatie van de voeropname. Twee proefvoeders gekwalificeerd als High Quality (HQ) en Low Quality (LQ) op basis van hun ruw eiwit en vezel inhoud werden in een cross-over design aan de dieren gevoederd.

In hoofdstuk 2 werden de korte termijn effecten van voeropname op de bloedgehalten aan metabolieten zoals Vluchtige Vetzuren (VFA=Volatile Fatty Acid), Beta-hydroxy boterzuur (BHB) en glucose bestudeerd. Zowel in de VJ als in de VP werden snelle veranderingen waargenomen. In de VP van de HQ gevoederde dieren, stegen de gehaltes aan azijnzuur, propionzuur, boterzuur, iso-boterzuur en BHB na een maaltijd. Consumptie van het LQ-voer leidde tot een tweetoppig patroon in de VJ van azijnzuur, propionzuur en boterzuur.
Het effect van eten op het nutriëntenhalte in het bloed kan in grote mate fluctueren als gevolg van de voederkwaliteit. Als maat voor de afgifte van nutriënten in de VP, werd het verschil tussen de gehalten in de VP en VJ gebruikt. Een verschil in gehalte in de perifere circulatie hoeft niet perse te resulteren uit een verschil in afgifte maar kan ook veroorzaakt worden door een verschil in opname door de

perifere weefsels. In de meeste gevallen kunnen de snelle veranderingen (tot 30 minuten na het begin van de maaltijd), eerder toegeschreven worden aan een veranderde opname dan aan een veranderde afgifte. Een veranderde afgifte lag vaak ten grondslag aan de langdurigere veranderingen.
In hoofdstuk 3 werd de respons van metabole en gastro-intestinale hormonen op een maaltijd beschreven. Snelle veranderingen in bloedgehalten van insuline, glucagon, pancreatic polypeptide (PP) en cholecystokinine (CCK) werden gezien bij schapen die het HQ-voer aten. Meer langduriger veranderingen in insuline-, glucagon- en gastrinegehalten werden tevens beschreven. De LQ-gevoerde dieren vertoonden snelle stijgingen in groeihormoon- (GH), gastrine-, PP- en CCK-gehalten. Langdurigere veranderingen werden geobserveerd in plasmaconcentratie van insuline, GH, gastrine, PP en CCK.
De snelle veranderingen worden mogelijk geïnduceerd door een verhoogde parasympathische activiteit en/of een verlaagde sympathische activiteit. De meer langdurige effecten zijn mogelijk veroorzaakt door de verandering in nutriëntengehalten. Voederkwaliteit beïnvloedde voornamelijk de grootte van de veranderingen in bloedgehalten, waarbij de HQ-gevoederde dieren de grootste veranderingen vertoonden.
De hypothese dat propionzuur betrokken is bij de regulatie van voeropname werd in hoofdstuk 4 bestudeerd. Tijdens de in het betreffende hoofdstuk beschreven experimenten kregen de schapen gedurende een periode van 20 minuten een infuus met een Na-propionaat oplossing. Ondertussen werd de het eetgedrag geregistreerd gedurende de 90 minuten durende voederperiode en het totale opgenomen voer gewogen. Als gevolg van een infuus van 2 mmol/min in de VM werd een reductie in voeropname gesignaleerd. Tevens werden sterk verhoogde concentraties aan insuline, glucose en propionzuur waargenomen in de VJ en VP.
In een daaropvolgend experiment werden de dieren via de VP geïnfundeerd met een 2 mmol/min Na-propionaat oplossing. Nu werd er geen effect op de totale voeropname waargenomen terwijl de toenamen in plasmaconcentraties van insuline, glucose en propionzuur vergelijkbaar waren met de resultaten uit het eerdere experiment. Conclusie van deze experimenten was dat hoge concentraties propionzuur in de MV verzadiging kan induceren. Als mogelijke verklaring voor de verschillende resultaten op het gebied van de voeropname tussen de twee experimenten werd een veranderde verdeling van de infuusoplossing over de lever gegeven. Als alternatief werd de aanwezigheid van propionzuurgevoelige receptoren in het gebied van de VM of VP geopperd.
In een meer uitgebreide studie, beschreven in hoofdstuk 5, werden de dieren via de MV geïnfundeerd met 0, 1.5 of 6 mmol/min Na-propionaat gedurende 20 minuten. Toediening van propionaat met 6 mmol/min verlaagde de voeropname maar veroorzaakte ook ongemak voor het dier. Portale plasmagehaltes van propionzuur, glucose en insuline stegen als gevolg van de infuus met 6 mmol/min Na-propionaat, terwijl gehaltes van boterzuur, BHB, gastrine, PP en CCK daalden. In de VJ werden dezelfde observaties gedaan als in de VP.
Toediening van 1.5 mmol/min Na-propionaat resulteerde in verhoogde concentraties van propionzuur en insuline terwijl gastrine- en PP- concentraties verminderden. Op basis van de uitgevoerde experimenten werd de conclusie getrokken dat propionzuur geen belangrijke factor in de regulatie van voeropname is. De effecten van een maaltijd op insuline, gastrine en PP zouden mogelijk toegeschreven kunnen worden aan propionzuur.

Vanwege de grote hoeveelheid propionzuur die nodig was om een effect op voeropname te bewerkstelligen en de observatie dat VFA's de afgifte van insuline bevorderen werd een experiment met insuline uitgevoerd. Gedurende dit experiment, beschreven in hoofdstuk 6, werden de effecten van een infuus met insuline op eetgedrag, totale voeropname en plasmagehaltes aan metabolieten en hormonen bestudeerd. Gedurende de eerste 20 minuten van de maaltijdperiode (90 minuten) werden de dieren geïnfundeerd met 6.7 mU/min insuline of fysiologisch zout. Frequente bloedafname vond plaats uit de VP en VJ. De experimenten werden uitgevoerd met twee voederkwaliteiten.
Een infuus met insuline resulteerde niet in een veranderde voeropname maar wel in een verkorte eettijd onafhankelijk van de voerkwaliteit. Dieren die het LQ-voer consumeerden hadden tijdens de het infuus met insuline een verhoogd insulinegehalte in de PV terwijl de dieren die het HQ-voer verstrekt kregen geen veranderde insulinegehalten lieten zien. Er werden geen effecten gezien op glucose-, glucagon-, gastrine- of PP-gehalten. Alleen het glucagongehalte werd beïnvloed door de voederkwaliteit.
Conclusies uit de bovenstaande studie waren dat insuline mogelijk betrokken is bij het proces van verzadiging door beïnvloeding van het gedrag en dat dieren die met een betere voederkwaliteit gevoederd worden een gevoeliger insuline regulatie vertonen.
In de hoofdstukken 7 en 8 werden de resultaten van een gecombineerd infuus van CCK (twee doseringen) en Na-propionaat gepresenteerd. In hoofdstuk 7 werden de effecten van 0, 1.2 of 2.4 nmol/min CCK-8 beschreven. Een infuus met CCK-8 resulteerde in een verhoogde CCK-8-spiegel in de VP maar niet in de VJ, hetgeen wijst op een zeer adequate klaring van CCK door de lever. Een infuus van 1.2 en 2.4 nmol/min verlaagde de gehaltes aan CCK-33 in de VP en VJ hetgeen een indicatie van een verlaagde endogene afgifte van CCK is. Ook het PP gehalte in de VP was verlaagd bij het 2.4 nmol/min infuus hetgeen verklaard werd door een verhoogde bloedflow in de VP.
Cortisolgehalten die bij een fysiologisch zout infuus daalden, stegen als gevolg van een CCK-8 infuus. Dit impliceert dat het CCK-8 infuus ongemak induceerde. Ondanks de toename in portale CCK-8 en cortisol gehalten werd er geen effect gezien op de voeropname.
In hoofdstuk 8 werd het effect van een infuus van 0 of 2.4 nmol/min CCK-8 al dan niet gecombineerd met een 0.5 mmol/min Na-propionaat bestudeerd. Voeropname werd door een gecombineerd infuus van CCK-8 en Na-propionaat verlaagd. Toename in gehaltes van propionzuur en insuline werden gesignaleerd bij een infuus van 0.5 mmol/min Na-propionaat. Het infuus van CCK resulteerde in verlaagde propionzuur-, azijnzuur-, boterzuur- en glucosespiegels terwijl de insulinespiegels een initiële stijging lieten zien eveneens gevolgd door een daling. Een gecombineerd infuus van CCK en Na-propionaat leverde vergelijkbare resultaten op als de enkelvoudige infuzen op de gemeten parameters. Uitzondering was het propionzuurgehalte dat bij een gecombineerd infuus lager was dan bij een Na-propionaat infuus maar hoger dan bij een CCK infuus.
De geobserveerde dalingen in VFA's en insuline kunnen mogelijk toegeschreven worden aan een verhoging van de portale bloeddoorstroming. Verder werd geconcludeerd dat het mechanisme van insuline-afgifte door propionzuur en CCK mogelijk verschillend zijn.
In hoofdstuk 9 werden enkele observaties uit vorige hoofdstukken geanalyseerd met behulp van een model. Het model bleek uitermate geschikt voor het verklaren van

de gevonden hormoon- en metabolietcurven als gevolg van de opname van voer. Het model kon ook gebruikt worden om de effecten van de infuusstudies te verklaren. Een van de belangrijkste conclusies was dat afgifte en opname vrijwel altijd zeer sterk gekoppeld zijn zelfs gedurende de toediening van grote hoeveelheden propionzuur. Een andere belangrijke conclusie was dat een verandering in plasmaconcentratie voort kan komen uit een veranderde opname (zoals de snelle propionzuurpiek in de VJ na een maaltijd) of een veranderde afgifte (zoals de insuline toename na een maaltijd).
Tenslotte werd in hoofdstuk 10 (discussie) enkele opmerkingen gemaakt over de mogelijke rol van andere hormonen en metabolieten in de regulatie van voeropname.
De belangrijkste conclusies uit dit proefschrift zijn dat als gevolg van een maaltijd er zowel snelle als ook langduriger veranderingen waar te nemen zijn in het bloed van schapen. Verschillen in voederkwaliteit kunnen niet alleen leiden tot verschillen in hormoon- en metabolietconcentraties maar ook tot verschillen in plasmaprofielen.
De infuusstudies met propionzuur, insuline en CCK geven aan dat regulatie van voeropname een multi-factorieel proces is. Het is daardoor noodzakelijk om bij het bestuderen van dit proces rekening te houden met de mogelijkheid dat ingrijpende manipulaties andere systemen kunnen beïnvloeden. Het uitvoeren van experimenten met lage, fysiologische doseringen van (gecombineerde) infuzen met hormonen en metabolieten kan uitsluitsel geven over de mate van betrokkenheid van deze factoren bij de regulatie van de voeropname.

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Curriculum Vitae

Hendrik Gerrit Derk (Henri) Leuvenink is geboren op 19 juli 1966 te 's Heerenbroek (IJsselmuiden). Na de lagere schooltijd doorgebracht te hebben in Hengelo (O) en Diemen is hij in Stadskanaal (Ubbo Emmius Lyceum) begonnen aan de HAVO. In 1984 behaalde hij zijn HAVO diploma aan het Christelijk Lyceum te Almelo. In 1986 werd het VWO diploma behaald en vervulde hij zijn militaire dienstplicht. In 1987 startte hij met de studie Biologie aan de Rijksuniverstiteit te Groningen. In 1992 studeerde hij af in de richting Medische Biologie (Cum Laude). Vanaf oktober 1992 tot april 1997 is hij werkzaam geweest als Assistent in Opleiding (AIO) bij de vakgroep Fysiologie van Mens en Dier aan de Landbouwuniverstiteit te Wageningen. Het onderzoek dat hij verrichtte heeft geleid tot dit proefschrift. Tijdens zijn aanstelling als AIO is hij lid geweest van het vakgroepsbestuur en het WAPS (WIAS associated PhD Students).
Na zijn aanstelling als AIO heeft hij gedurende korte tijd als cursusleider opleidingen verzorgt bij 't WEB IT&C te Hoogeveen.
Sinds september 1997 is hij als onderzoeker werkzaam bij Numico research in de afdeling bioactive components te Wageningen.

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