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The effect of solid feed intake on glucose homeostasis in heavy veal calves

Sabrina Podesta

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MSc student:	Sabrina Podesta
Registration number:	870612-658-110
Supervisor:	dr. ir. J.J.G.C. Van den Borne
Examinors:	dr. ir. J.J.G.C. Van den Borne ir. H. Berends
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Literature review

The effect of short chain fatty acids on glucose homeostasis in heavy veal calves

Introduction

Veal calves, in contrast to ruminating calves, are kept on milk replacer diets until slaughter to achieve a high energy intake, high growth rates and pale and tender meat (Doppenberg and Palmquist, 1991). Nowadays it is mandatory to additionally supply roughages to veal calves (IKB, 2008). This improves welfare and reduces abnormal oral behaviour by providing the opportunity to ruminate (Veissier et al., 1997). Rumen papillae growth is increased as a response to short chain fatty acid (SCFA) production from roughages by rumen microbes (Flatt et al., 1958). The physical structure of roughages stimulates rumen motility (McDonald et al., 2002). Besides these positive effects of roughages on health and welfare, it is uncertain how roughages influence glucose homeostasis.

Feed intake elevates plasma glucose levels and subsequently insulin release is initiated to maintain glucose homeostasis. Insulin clears glucose from the blood by inhibiting endogenous glucose production and gluconeogenesis in the liver, and by stimulating glucose uptake in muscle- and adipose tissue (Aronoff et al., 2004; Ferrannini and DeFronzo, 2003). However, milk-fed veal calves often show problems maintaining their glucose homeostasis, characterized by excessive postprandial hyperglycaemia (elevated plasma glucose level), hyperinsulinaemia (elevated plasma insulin level), glucosuria (glucose excretion via urine) (Hostettler-Allen et al., 1994; Hugi et al., 1998) and sometimes elevated preprandial plasma glucose levels (Hugi et al., 1997a). This indicates an inefficient utilization of glucose (Hugi et al., 1997a).

This disturbance in glucose metabolism is most likely caused by an impaired insulin sensitivity of body tissues (insulin resistance) (Hostettler-Allen et al., 1994; Hugi et al., 1997a). In case of insulin resistance, glycogenolysis and gluconeogenesis become resistant to the inhibitory effect of insulin, leading to elevated plasma glucose levels. As a response to the prolonged hyperglycaemia, even more insulin is produced (Ferrannini & DeFronzo, 2004; Hostettler-Allen et al., 1994). Moreover, the clearance rate of insulin from the blood by liver and kidneys could be reduced, leading to hyperinsulinaemia (Bergman et al., 2004).

Several nutritional factors could contribute to this problematic glucose metabolism. Until now, mainly the high lactose levels in milk replacer were considered to negatively affect glucose homeostasis (Hostettler-Allen et al., 1994). For example, hyperinsulinaemia in calves was more pronounced after oral administration of milk replacer than after intake of a solution of glucose or lactose and glucose at the same sugar level as the milk replacer in a study of Hostettler-Allen et al. (1994). However, only in the first 180 minutes the plasma glucose level was higher after glucose and glucose/lactose intake, whereas between 180 and 480 minutes after solution intake the plasma glucose level was markedly lower than after milk replacer intake.

Solid feed supply also seems to play an important role in glucose metabolism. The SCFAs produced from roughages by rumen microbes, which mainly consist of acetate, propionate and butyrate, have been shown to induce hyperglycaemia, hyperinsulinaemia and insulin resistance after intravenous infusion in sheep in several studies (Ash et al., 1964; Manns and Boda, 1967; Trenkle, 1970). Unfortunately, results of studies examining the effect of SCFA on glucose homeostasis are inconsistent and mostly performed on animals other than veal calves. Also, the effects of SCFA in ruminants have mostly been investigated using intravenous infusions, instead of stimulating SCFA production in the rumen by orally administering roughages. This might be a misleading indicator of the effect of roughage on glucose homeostasis, for instance because butyrate predominantly appears in blood of ruminants as β-hydroxybutyrate which does not stimulate insulin secretion in sheep (Horino et al., 1968). In contrast to studies in sheep, studies in mice suggested that SCFA increase insulin sensitivity (Gao et al., 2009), which suggests a more

efficient glucose utilization (Hugi et al., 1998). This inconsistency might be due to a difference between ruminants and monogastrics (Horino et al., 1968).

This literature study provides background information on glucose metabolism and elaborates on the possible effects of solid feed intake on glucose homeostasis in heavy veal calves. The underlying research question is:
What is the effect of solid feed intake on glucose homeostasis in heavy veal calves?

This literature review describes the effects of solid feed on:
1. Postprandial glucose, insulin, glucagon and free fatty acid (FFA) levels
2. Insulin sensitivity
3. Glucosuria
4. Triglyceride deposition in liver and muscle
5. Glycogen deposition in liver and muscle
6. Fatty acid composition in liver, muscle and perirenal fat

Information is given on the mechanisms affecting glucose homeostasis in chapter 1. Chapter 2 explains the problems heavy veal calves show with maintaining their glucose homeostasis. Chapter 3 discusses the documented effects of SCFA and other nutrients on glucose metabolism. Chapter 4 elaborates on the effect of solid feed intake on glycogen and fat content of muscle and liver. The latter chapter also discusses the effect of solid feed intake on fatty acid composition in muscle, liver and renal fat.

1. Glucose homeostasis

Postprandially, glucose in the blood is derived from feed intake. In the fasting state, endogenous glucose production secures a sufficient energy supply to body tissues (McDonald et al., 2002).
The main regulator of maintaining the right blood glucose level is the pancreas, specifically the islets of Langerhans. These produce insulin as a result of hypothalamic signalling when the blood glucose level rises beyond the hypothalamic set point due to feed intake, and glucagon when the blood glucose level drops below set point, which is the case during fasting (Aronoff et al., 2004; Peters et al., 2004). The liver also plays a role in maintaining glucose homeostasis by the production of endogenous glucose.

Although several hormones interfere with glucose homeostasis, like glucagon-like peptide-1 (GLP-1), amylin, gastric inhibitory polypeptide (GIP), epinephrine, insulin-like growth factor (IGF) and growth hormone (Aronoff et al., 2004; Vicari et al., 2008), this study focuses on the regulatory effects of insulin, and to a lesser extent glucagon.

1.1 Insulin

Pancreatic β-cells secrete insulin into the circulation when the postprandial plasma glucose level increases, until the glucose level has returned to the basal concentration. The pancreas responds to a rise in ATP/ADP ratio in the cytosol of the β-cells as a result of glucose oxidation. This closes the K^+ channels and opens the Ca^{2+} channels in the membrane. The consequential influx of Ca^{2+} activates pyruvate dehydrogenase, leading to a higher conversion of pyruvate into acetyl coenzyme A (acetyl CoA), which raises the ATP/ADP ratio further through the citric acid cycle. The concentration of plasma glucose determines the amount of insulin secreted, as β-cells measure the degree of hexokinase activation (Denton and McCormack, 1990).

The main organs containing insulin receptors are the liver, skeletal muscle and adipose tissue. Insulin stimulates the uptake of glucose, as described in paragraph 1.3. In the liver, glycogenesis is enhanced by glycogen synthase kinase-3 and gluconeogenesis and glucose excretion are inhibited (Cross et al., 1997; Khan and Pessin, 2002). In the skeletal muscle, glycogenesis is stimulated by enhancing glycogen synthetase activity (Cross et al., 1997). When the glycogen stores are saturated and glucogenic nutrient intake still exceeds the expenditure, insulin stimulates glucose storage through lipogenesis through lipoprotein lipase (Polakof et al., 2011). Insulin contributes to proteogenesis, clearing amino acids from the blood (Aiello et al., 1989; Brown and Hay, 2006). In the adipose tissue, lipogenesis is increased, which clears glucose from the blood by converting it into lipids. These actions give insulin an anabolic effect which is reinforced by its inhibition of the production and secretion of glucagon, which has a catabolic effect (see paragraph 1.2) (Aronoff et al., 2004; Khan and Pessin, 2002). A summary of the effects of insulin is shown in Table 1.1.

The production rate of insulin mainly depends on gastric emptying and feed composition, but also on hormonal and neural factors (Aronoff et al., 2004).
Preformed insulin is rapidly released as soon as feed enters the gastro-intestinal (GI)-tract, mainly as a response to elevated plasma concentrations of glucose, but also different amino acids (leucine>lysine>arginine (Milner, 1969), release of the hormones GLP-1 by intestinal L-cells and GIP by K-cells, epithelial cells that respond to nutrients entering the intestinal lumen (Aronoff et al., 2004; Croom Jr et al., 1992; Gautier et al., 2008; Lim et al., 2009) and stimulation of the vagus nerve by nutrients and gastric relaxation (Aronoff et al., 2004). Also glucagon induces insulin

secretion by stimulating the β-cells and consequently it down regulates the affinity of glucagon receptors and therefore restricts its own catabolic effect (Kawai et al., 1995).
After the initial rapid insulin release, more insulin is synthesized in the pancreas and secreted responding to the blood glucose content, after which insulin down regulates its own receptors (Hugi et al., 1998) and is broken down mainly by liver and kidney (Duckworth, 1988).

The plasma insulin level is not only determined by its production, but also by its breakdown. Insulin is taken up and degraded by all insulin sensitive tissues (Bergman et al., 2003; Duckworth, 1988), but most insulin is cleared from the blood by the liver and kidneys. The rate and degree of insulin breakdown partly determine the insulin level in the blood and seem to reduce as veal calves get older (Palmquist et al., 1992). This could be a result of recurrent hyperinsulinaemia, because excessive insulin levels may lead to liver cell damage, resulting in a reduced insulin uptake and clearance (Iwasaki et al., 1978). This does not, however, explain the hyperglycaemia.

Insulin secretion is positively influenced by the incretin effect, which describes the phenomenon that insulin secretion is enhanced when glucose is administered orally compared to intravenously, while plasma glucose levels are equal. This is accomplished by the gut hormones GLP-1 and GIP (Knop et al., 2007).

Growth hormone, cortisol and GIP, in contrast to the above mentioned, were expected to alter glucose homeostasis and insulin secretion, but could not explain any of the symptoms arising in the insulin resistant state (Hostettler-Allen et al., 1994; Hugi et al., 1997a). Epinephrine has been demonstrated to inhibit insulin secretion (Coore and Randle, 1964), whereas glucagon (Manns et al., 1967) and corticotrophin (Genuth and Lebovitz, 1965) stimulate it.

1.2 Glucagon

The plasma glucose level drops during fasting, upon which the pancreatic α-cells secrete glucagon, which has a catabolic effect, as a response to paracrine secretions of pancreatic β-cells. Glucagon has binding sites on liver, pancreas, brain, intestines, muscle- and adipose tissue (Jiang and Zhang, 2003), but its main role is stimulating glycogenolysis in liver and muscle, lipolysis in adipose tissue, muscle and liver, proteolysis in the muscle and gluconeogenesis in the liver (Campbell and Reece, 2005; Jiang and Zhang, 2003). Glucagon stimulates the production of insulin through GLP-1 receptors on β-cells, though with a much lower affinity than GLP-1 itself. In this, glucagon emphasizes on the effect of GLP-1 in inhibiting its own production through insulin production (Kawai et al., 1995). In a healthy state, glucagon also down-regulates its own receptors directly at the α-cells. In case of disturbed metabolism, for instance during prolonged fasting or in case of kidney failure, this down-regulation is redirected, leading to up-regulation of glucagon receptors (Soman and Felig, 1978).

Table 1.1: The stimulating and inhibiting effects of insulin and glucagon on different processes involved in glucose homeostasis

Process	Tissues	Insulin action	Glucagon action
Gluconeogenesis	Liver	-	+
Glycogenesis	Liver, skeletal muscle	+	-
Glycogenolysis	Liver, skeletal muscle	-	+
Lipogenesis	Adipose tissue, liver, skeletal muscle	+	-
Lipolysis	Adipose tissue, liver, skeletal muscle	-	+
Proteogenesis	Skeletal muscle	+	-
Proteolysis	Skeletal muscle	-	+
Insulin production	Pancreas	-	+
Glucagon production	Pancreas	-	-

1.3 Glucose uptake

Several cellular glucose transporters mediate glucose uptake through the cell membrane. The most important ones are discussed in this paragraph. Glucose transporter (GLUT)-1 is present on the sarcolemma of all glucose requiring tissues and stimulates glucose uptake insulin independently (Ebeling et al., 1998; Khan and Pessin, 2002). Apart from this action, glucose uptake in muscle and adipose tissue differs from glucose uptake by the liver.

1.3.1 Muscle and adipose tissue

Postprandial glucose levels are mainly decreased by GLUT-4, present in muscle cells and adipocytes. Insulin induces a translocation of GLUT-4, which is packed in intracellular vesicles, to the cell membrane surface, where it picks up glucose molecules (Derave et al., 2000; Saltiel and Kahn, 2001; Sasaki, 2002).

1.3.2 Liver

Glucose uptake in the liver is, apart from GLUT-1, stimulated by the insulin-dependent transporter GLUT-2, which is only present on liver, kidney, intestine and pancreatic β-cells (Antoine et al., 1997; Khan and Pessin, 2002; Thorens et al., 1993). However its affinity for glucose is relatively low. Its significance in glucose uptake in ruminants may also be reduced because it functions in combination with hexokinase and glucokinase, which trap the glucose in the cell by catalyzing phosphorylation (Halseth et al., 1999; Khan and Pessin, 2002). In ruminants (Ballard, 1965; Proietto et al., 1999), and possibly also in veal calves, the liver does not contain a sufficient amount of glucokinase and activity of hexokinase, and therefore cannot take up and metabolize a significant amount of glucose. Sheep livers have been shown to contain no hexokinase (Ballard and Oliver, 1964).
The liver of monogastrics responds mainly to insulin, whereas in ruminants, the amount of glucose supplied through the portal vein is more important in enhancing the glucose uptake in hepatocytes (Bergman et al., 1968; Brockman, 1982; Myers et al., 1991). The reason for this is not known, however it is known that a positive gradient of glucose between portal vein and hepatic artery stimulates glucose uptake through GLUT-2, and possibly hypothalamic signalling of the

vagus nerve may induce alterations of the hepatic glucose metabolism (Burcelin et al., 2000; Gardemann et al., 1986). As ruminants do not take up a significant amount of glucose in the blood, the hepatic glucose uptake is restrained.
The low affinity GLUT-2 receptors in the liver, together with the reduced hepatic glucose uptake as a lack of glucose uptake in the blood and reduced hexokinase and glucokinase activity compared to monogastrics, suggest a lower glucose uptake by the liver than by muscle and adipose tissue in ruminants.

1.3 Endogenous glucose production

In rest, the glucose disappears from the blood at a relatively constant rate. This loss should be complemented by endogenous glucose production, mainly because the brain, in contrast to other organs, cannot utilize fat or protein for its energy requirements (Myers et al., 1991). In ruminants, however, the brain is able to utilize β-hydroxy butyrate in case of glucose shortage (O'Neal and Koeppe, 1966).
Endogenous glucose production can be divided into glycogenolysis, the hydrolization of glycogen, and gluconeogenesis, the synthesis of glucose from especially amino acids and lactate (McDonald et al., 2002). In humans, the first 8-10 hours of a fasting period, blood glucose level is mainly increased by glycogenolysis, as a result of the stimulatory effect of glucagon. When the glycogen stores become scarce, gluconeogenesis takes over. Tissues storing glycogen, namely the liver, skeletal muscles and kidneys, are able to break down glycogen and utilize it for their energy requirements, but only the liver and kidneys contain glucose-6-phosphatase, an enzyme that enables these tissues to secrete glucose into the blood (Aronoff et al., 2004).
After feed intake, the production of endogenous glucose and its secretion into the blood is inhibited by insulin, which is transported through the portal vein to the liver. Endogenous glucose production is also inhibited by paracrine communicative actions between the α-cells and the β-cells in the pancreas, suppressing glucagon production (Aronoff et al., 2004).

2. Problems with glucose homeostasis in veal calves

The presence of hyperglycaemia, hyperinsulinaemia, glucosuria and insulin resistance in heavy veal calves is well documented (Hostettler-Allen et al., 1994; Hugi et al., 1997a; Hugi et al., 1998). This chapter explains these features and their possible causes.

2.1 Hyperglycaemia and hyperinsulinaemia

When calves are weaned, the basal plasma insulin and glucose level decrease typically (Weekes et al., 1983), but veal calves are continuously milk fed and show the opposite as they get older (Hostettler-Allen et al., 1994; Hugi and Blum, 1997). Heavy veal calves have problems maintaining their glucose homeostasis, indicating that the homeostatic control is lacking and the glucose provided with the feed is utilized inefficiently (Hugi et al., 1997b). Postprandial hyperglycaemia, an excessive plasma glucose level, and hyperinsulinaemia, an overproduction of insulin, are common in heavy veal calves (Hugi et al., 1997a).

Food intake in humans leads to increased glucose and insulin levels, but within 1.5 hours the glucose derived from the meal has been cleared from the blood and the insulin level has gone back to the preprandial level (Fukumori et al., 2000). In heavy veal calves, both glucose and insulin level rise excessively after feed intake and even after 4 hours the concentrations have often not returned to the basal level (Hugi et al., 1997a). Table 2.1 shows the degree of hyperglycaemia and hyperinsulinaemia measured in veal calves.

Problematic glucose homeostasis mostly appears in heavy veal calves that are close to the end of their fattening period. The postprandial glucose level increases with age, while this is also sometimes the case for fasting glucose levels (Hugi et al., 1997a). Basal, as well as postprandial, insulin levels are often higher as veal calves get heavier. The influence of age on glucose metabolism may partly be explained by the high lactose content in veal calf diets (Hugi et al., 1997b; Palmquist et al., 1992). Also the availability of preformed insulin increases with age (Hugi et al., 1997a). Another explanation may be derived from an experiment comparing young preruminating to older ruminating calves, showing a decrease in the ability to adapt gluconeogenesis to acute changes in insulin and glucagon levels with an increase in age. The rate of total gluconeogenesis did not change with age (Donkin and Armentano, 1995). The age effect on glucose homeostasis is also documented in humans (Arslanian, 2005; Sunehag et al., 2002).

Surprisingly, veal calves are still able to perform high growth rates, possibly due to the age-dependent increase in the anti-apoptotic, and therefore growth stimulating, hormone Insulin Like Growth Factor-1 (IGF-1) in the blood (Ballard, 1965; Brockman, 1983; Hugi et al., 1997a; Hugi et al., 1998).

2.2 Glucosuria

Glucosuria, the excretion of glucose through the urine, appears together with hyperglycaemia and also develops as veal calves get older (Hugi et al., 1997b; Palmquist et al., 1992). Glucosuria arises when the renal threshold for glucose extraction is exceeded. In veal calves, this happens when the plasma glucose concentration is more than 1.5 grams/litre, whereas the concentration should normally be approximately 0.83 grams/litre (Hostettler-Allen et al., 1994; Hugi et al., 1997b). Table 2.1 shows the urinary glucose excretion measured during different studies.

Table 2.1: Pre-and postprandial glucose and insulin levels and glucosuria measured in veal calves fed twice daily

Source	Feeding strategy	Body weight (kg)	Glucose level			Insulin level			Glucose in urine
			preprandial conc. (mmol/L)	max. postprandial conc. (mmol/L)	time until preprandial level (min after feed intake)	preprandial conc. (µg/L)	max. postprandial conc. (µg/L)	time until preprandial level (min after feed intake)	
Doppenberg and Palmquist 1991	MR[1], 10% fat Total MJ[2] 43-84d: 1516, >84d: 1562	85.2	7.3	n.a. AUC[8] 1536	n.a.	5.19	n.a. AUC 1134	n.a.	n.a.
		148	8.2	n.a. AUC 2800	n.a.	8.28	n.a. AUC 2061	n.a.	n.a.
	MR, 18% fat Total MJ 43-84d: 1690, >84d: 1143	92.8	8.1	n.a. AUC 1304	n.a.	2.85	n.a. AUC 939	n.a.	n.a.
		150	7.7	n.a. AUC 2693	n.a.	11.97	n.a. AUC 1943	n.a.	n.a.
	MR to 6 wk, grain and hay to 16 wk, 3% fat Total MJ 43-84d: 1775, >84d: 2157	77.4	4.1	n.a. AUC 234	n.a.	0.605	n.a. AUC169	n.a.	n.a.
		154	5.2	n.a. AUC 485	n.a.	1.424	n.a. AUC 351	n.a.	n.a.
	MR to 6 wk, grain and hay to 16 wk, 10% fat Total MJ 43-84d: 1403, >84d: 3317	69.3	4.2	n.a. AUC 178	n.a.	0.528	n.a. AUC 129	n.a.	n.a.
		157	5.1	n.a. AUC 340	n.a.	0.965	n.a. AUC 255	n.a.	n.a.
Hostettler-Allen, Tappy et al. (1994)	MR, 21% fat, 1.3-1.4 kg ADG[3]	70–176	5.4	8.3 (150min.)	480	1.138	20,475 (210min)	480	0.12 g/L
Hugi, Bruckmaier et al. (1997a)	MR, 22.7% fat, 29% lactose	76-109	4.3	7.2 (90min.)	480	0	4 (120min.)	480	0.11 g/d
		159-192	5.2	9.7 (120min.)	480	0	23 (120min.)	480	5.58 g/d
	20.7% fat, 42.3% lactose	74-109	4.4	8.8	480	0	5 (120min.)	480	0.08 g/d
		163-204	5.4	11	480	0	22 (200min.)	480	2.4 g/d
Kaufhold, Hammon et al. (2000)	whole milk, MR, premix	110-170	5.6	8.6 (120min.)	480	1	12 (120min.)	480	n.a.
Stanley, Williams et al. (2002)	10% of BW, MR 15% DM, 15% fat	38–62	4.2	4.7 (30min.)	n.a.	0.314	0,455 (30min.)	n.a.	0.054 g/L
	Once daily 10% of BW, MR 21.4% DM, 15% fat		4,3	4.9 (30min.)	n.a.	0.273	0.592 (60min.)	n.a.	0.099 g/L
Vicari, Van den Borne et al. (2008a)	MR, low feeding level	143	5.5	9.3 (90min.)	360	0	32,7 (180min.)	360	10.5
	high feeding level	175	5.5	13.5 (90min.)	360	0	90 (240min.)	360	61.8 g/d
Vicari, Van den Borne et al. (2008b)	MR	148-152,3	6.6	9.0 (90min.)	330	7.5	56,3 (150min.)	330	18 g/d
Labussiere et al. 2008	MR, <20% fat 1,132 (62 kg BW) to 2,871 (221) g DM MR/d	71	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	8 g/d
		135							78 g/d
		210							125 g/d
Labussiere et al. 2009	MR, 16.5% fat	73	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1 g/d
		152							48 g/d
		237							80 g/d
Gerrits, Van den Borne et al. (2007)	663 kJ/kg MW[4]/d[5], 0.9-2.72 g N[6]/kg MW/d, 40 g N/d	80-160	5.3	n.a.	n.a.	0.00455	n.a.	n.a.	1.8 g/d
	80 g N/d		5.5	n.a.	n.a.	0.00228	n.a.	n.a.	0 g/d
	851 kJ/kg MW/d, 0.9-2.72 g N/kg MW/d, 40 g N/d	80-160	5.3	n.a.	n.a.	0.0091	n.a.	n.a.	1.8 g/d
	80 g N/d		5.5	n.a.	n.a.	0.00455	n.a.	n.a.	0 g/d
	564 kJ/kg MW/d, 0.54-2.22 g N/kg MW/d, 40 g N/d	160-240	5.5	n.a.	n.a.	0.01	n.a.	n.a.	9 g/d

	80 g N/d		4.1	n.a.	n.a.	0.00455	n.a.	n.a.	5.4 g/d
	752 kJ/kg MW/d, 0.54-2.22 g N/kg MW/d, 40 g N/d	160-240	6.0	n.a.	n.a.	0.02184	n.a.	n.a.	54 g/d
	80 g N/d		5.0	n.a.	n.a.	0.01365	n.a.	n.a.	27 g/d
Hugi, Gut et al. 1997	MR+ whey, 14.3% fat, 51.2% lactose, 18.1% CP[7]	66.8	7.9	n.a.	n.a.	7.6	n.a.	n.a.	n.a.
	MR+ whey+ butter milk, 13.3% fat, 48.6% lactose, 22.1% CP	66.8	6.4	n.a.	n.a.	2.6	n.a.	n.a.	n.a.
	MR+ skimmed milk, 11.9% fat, 46.5% lactose, 31.8% CP	66.8	6.6	n.a.	n.a.	1.8	n.a.	n.a.	n.a.
	MR+ water, 20.8% fat, 30.2% lactose, 22.3% CP	66.8	6.5	n.a.	n.a.	1.7	n.a.	n.a.	n.a.
	MR+ whey, 13.4% fat, 54.9% lactose, 16.6% CP	181.6	7.3	n.a.	n.a.	23.8	n.a.	n.a.	n.a.
	MR+ whey+ butter milk, 14.4% fat, 48.8% lactose, 21.5% CP	181.6	7.1	n.a.	n.a.	5.9	n.a.	n.a.	n.a.
	MR+ skimmed milk, 21.1% fat, 38.5% lactose, 26.1% CP	181.6	6.3	n.a.	n.a.	3.6	n.a.	n.a.	n.a.
	MR+ water, 21.2% fat, 29% lactose, 22.5% CP	181.6	6.1	n.a.	n.a.	3.9	n.a.	n.a.	n.a.

Min.: the time between feed intake and glucose/insulin peak. [1]milk replacer, [2]mega joules, [3]average daily gain, [4]metabolic weight, [5]intake per day, [6]nitrogen, [7]crude protein, [8]area under curve, total postprandial glucose and insulin responses

2.3 Insulin resistance

Insulin is important for glucose utilization by muscle tissue, adipose tissue, liver and other vital organs and should therefore sufficiently affect these tissues, which is not the case in insulin resistance. Kahn (1978) described insulin resistance as being a condition in which normal concentrations of insulin lead to a less than normal biological response. The pronounced postprandial appearance of hyperglycaemia, hyperinsulinaemia and glucosuria, indicates that heavy veal calves suffer from insulin resistance, which has been confirmed in several studies (Hostettler-Allen et al., 1994; Hugi et al., 1998).

The causes of insulin resistance are not well documented (Ferrannini, 1998; Sternbauer and Luthman, 2002). It seems to be a multi-factorial disorder, resulting from not only nutrition, but also genetic, cellular and possibly other physiological or environmental factors (Ferrannini, 1998), although in heavy veal calves milk replacer, especially lactose, has shown to induce development of feed dependent insulin resistance (Hostettler-Allen et al., 1994; Hugi et al., 1997b).

This chapter explains the physiological consequences of insulin resistance and the method for detection of insulin resistance.

2.3.1 Physiology in the insulin resistant state

Hyperglycaemia and hyperinsulinaemia in heavy veal calves may be explained by the presence of insulin resistance. Because of the lacking response to insulin, body tissues fail to clear glucose from the blood and the hepatic glucose excretion is less inhibited, which causes a longer and more pronounced elevation of plasma glucose levels and gives an ongoing stimulus to the pancreatic β-cells, resulting in excessive plasma insulin levels (Hugi et al., 1998). A higher basal hepatic glucose production and hyperglycaemia have also been reported in insulin resistant humans (Luzi et al., 1988).

Insulin resistance is initially compensated by pancreatic overproduction of insulin to maintain euglycaemia, often followed by glucose intolerance, indicating that the glucose levels rise excessively and cannot be brought back to normal levels (Le Marchand-Brustel et al., 2003). In humans and mice this eventually leads to a lack of insulin production (Aronoff et al., 2004;

Gustavsson et al., 2008), whereas in veal calves this feature has not been reported (Hugi et al., 1997a), but possibly would have been if experiments were done at a higher age.
The organs of which the physiology is most affected by insulin resistance are the liver, skeletal muscle and adipose tissue. Figure 2.1 shows the effect of insulin resistance on these organs in comparison to the physiology of skeletal muscle, adipose tissue and liver in the insulin sensitive situation. The bold arrows show enhancements of flows, the red ones restrictions.
Insulin resistant muscle and adipose tissue fail to take up glucose after feed intake, which in humans causes the glucose to pass on to the less insulin dependent and more insulin sensitive liver, in which it is converted into mostly free fatty acids (FFA) or free cholesterol. Also proteins and lactate enhance the glucose pool in the liver through gluconeogenesis (Ballard, 1965; Zivkovic et al., 2007).
The high amount of free fatty acids in the liver is partly stored into triacylglycerol, enhancing the fat content of the liver. The free cholesterol is converted into cholesterol esters, which are secreted, together with a part of the triacylglycerol in the liver in the form of very low density lipoprotein (VLDL) (Zivkovic et al., 2007).

It is not certain to what degree this feature appears in veal calves. There could be a shunt of glucose to the liver, whereas in non-milk-fed ruminants this seems unlikely. Ruminants acquire glucose from gluconeogenesis and not by absorption from the intestines, since the diet mainly consists of roughages and glucose is metabolized by rumen microbes after feed intake (Ballard, 1965; Proietto et al., 1999). Also, it is uncertain to what extend the liver takes up glucose in ruminants, since it has a lower glucokinase and hexokinase activity (Ballard, 1965), as described in paragraph 1.3.2.

The rise in FFA and free cholesterol content in the human liver increases the fatty acid pool of the blood and the amount of fatty acids taken up by the skeletal muscle, which causes an accumulation of free fatty acids and triacylglycerol in the muscles (Zivkovic et al., 2007). This disrupts muscular functions, like oxidative phosphorylation in the mitochondria and GLUT-4 transport of insulin receptors (Guilherme et al., 2008).
Also the breakdown of glycogen in the liver and muscles is a feature of insulin resistance. The inhibitory effect of insulin on glycogenolysis is omitted. This effect is strengthened in the muscles by the need to acquire their energy from glycogenolysis, due to lack of incoming glucose.

2.3.2 Hepatic and peripheral insulin resistance

In the post-absorptive state the plasma glucose level is maintained by equal rates of endogenous glucose production and glucose disposal in peripheral tissues (DeFronzo et al., 1982). Impaired peripheral glucose uptake and impaired reduction of glucose production by the liver mainly contribute to insulin resistance (Bogardus et al., 1984). Impaired glucose uptake by the liver is reported to be unlikely as a contributor to insulin resistance in humans, as it covers only 5-6% of glucose uptake (DeFronzo et al., 1978).
Hepatic and peripheral insulin sensitivity can develop separately, even though there is some correlation between both (r^2=0.46 in humans) (Abdul-Ghani et al., 2008). The liver is generally more sensitive to insulin than peripheral tissues in humans (Campbell et al., 1988; DeFronzo et al., 1983; Kolterman et al., 1980; Rizza et al., 1981). For instance, in healthy humans, only 25 µU/min insulin is required to completely diminish a hepatic glucose production rate of 50 mg/m^2/min, whereas twice as much insulin is needed to dispose the same amount of glucose into peripheral tissues (Luzi et al., 1988).

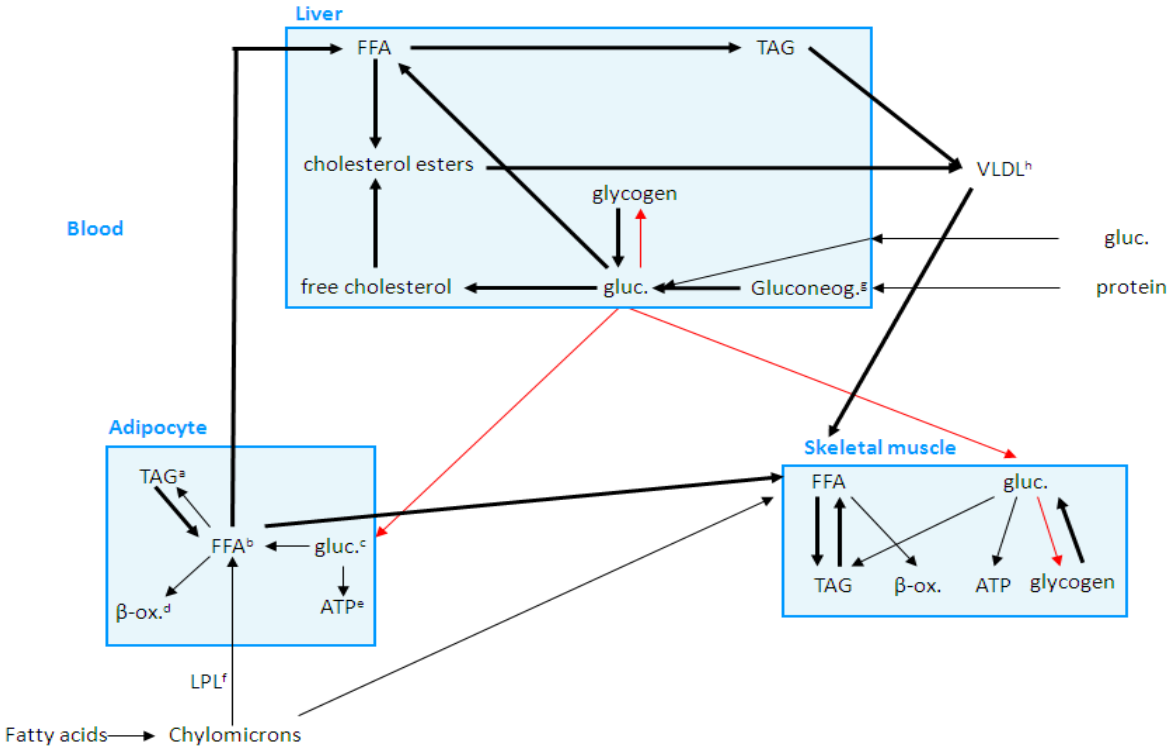

Figure 2.1: Effect of insulin resistance on the physiology of skeletal muscle, adipose tissue and liver. The inhibited (red arrows) and enhanced (bold arrows) fluxes are represented. [a] triacylglycerol, [b] free fatty acids, [c] glucose, [d] beta-oxidation, [e] adenosine triphosphate, [f] lipoprotein lipase, [g] gluconeogenesis, [h] very low density lipoprotein.

2.3.3 Possible physiological causes of insulin resistance in veal calves

It has been suggested that the number of insulin receptors on especially skeletal muscle cells, which account for about 75% of insulin dependent glucose clearance in humans and mice, reflects insulin sensitivity. The insulin receptor number on skeletal muscles has been shown to be decreased in insulin resistant calves fed high amounts of lactose (Hugi et al., 1998). However, in muscle insulin receptor knockout mice, glucose tolerance was not affected (Brüning et al., 1998).

The reduction in responsiveness of insulin sensitive tissues may, at least partly, be caused by a reduced cellular translocation of GLUT-4 protein. A study in rats has shown that expulsion of GLUT-4 activity in both muscle cells (Zisman et al., 2000) and adipocytes (Abel et al., 2001) induces intolerance of glucose and insulin resistance. In a study of Miura et al. (2001), the translocation of GLUT-4 was reduced in adipose and muscle tissue of insulin resistant mice. Conversely, insulin resistance does not alter the expression of GLUT-4 transporter, indicating that mainly GLUT-4 dysfunction contributes to insulin resistance (Gelardi et al., 1999). The concentration of GLUT-4 within the cells is not related to insulin resistance (Schalin-Jäntti et al., 1994).

Hyperglucagonaemia may also be a cause of insulin resistance, as glucagon and insulin have antagonistic effects on glucose flow (Sherwin et al., 1978). The same effect could be obtained when glucagon stimulates instead of down-regulates its own receptors, which is the case in for instance kidney failure and prolonged fasting (Soman and Felig, 1978).

Ruminants are known to be less sensitive to insulin than non-ruminants (Sternbauer and Luthman, 2002), mainly because of their hexokinase deficient livers, which are not able to take up a significant amount of glucose as a response to insulin (Ballard, 1965), and because the liver

mainly responds to the flow of glucogenic nutrients through the portal vein (Peters et al., 2004), rather than to insulin. Therefore the liver cannot switch between glucose uptake and glucose production like in monogastrics (Ballard, 1965; Brockman, 1983; Peters et al., 2004).

Once insulin resistance is present, this state persists in several ways. Hyperinsulinaemia leads to a reduced affinity of insulin receptors on muscle cells (Le Marchand-Brustel and Freychet, 1979). Also the high free fatty acid levels are shown to not only be a result of, but also cause insulin resistance (Boden, 1999; Guilherme et al., 2008), as shown in paragraph 4.2.4. Normally, in the fasting state, an equilibrium between the release of adipose tissue bound fatty acids into the circulation and the uptake and β-oxidation exists. Up to a certain fatty acid load from nutrition, this equilibrium is maintained, but an overload leads to hypertrophy of adipocytes. Also adipose tissue starts secreting proteins like monocyte chemotactic protein-1 (MCP-1) and therefore attracts macrophages, which brings the adipose tissue into a pro inflammatory state. The macrophages secrete tumor necrosis factor-α (TNF-α), leading to a chronic inflammation, which impairs the deposition of triglycerides and enhances lipolysis. Therefore the free fatty acid level in the blood is increased and even more fat is deposited into non-adipose tissue (Guilherme et al., 2008).

Glucose and lipids are the main energy supplies for veal calves. Because of the close interaction in respiration, oxidation of fatty acids reduces the effect of insulin and therefore the respiration of glucose. In insulin resistance, the high amount of FFAs may enhance fatty acid oxidation as peripheral tissues fail to efficiently take up glucose, leading to a further reduction of insulin effectivity (Randle et al., 1988), which aggravates the insulin resistance. The interaction between fatty acids and glucose will further be discussed in Chapter 3.

2.3.4 Measuring insulin resistance

The most exact method for the measurement of whole body insulin sensitivity is the hyperinsulinaemic euglycaemic clamp method (Abdul-Ghani et al., 2007; DeFronzo et al., 1979; Hermans et al., 1999). This method is implemented after an overnight fasting period of at least 10 hours, to achieve a constant glucose turnover (Abdul-Ghani et al., 2007; Hugi et al., 1998) and to exclude disturbances of pancreatic insulin production and plasma glucose level elevation by feed intake. By infusion, the plasma insulin concentration is rapidly increased, which inhibits pancreatic production, and kept constant by maintaining the same infusion rate. This insulin infusion causes a drop in plasma glucose level, which is then lifted again by infusing glucose. By measuring the glucose level regularly, the glucose infusion rate (GIR) can be adjusted to reach a steady state. In calves a near-steady state condition is reached within 3 hours, however the GIR keeps rising slightly (Hostettler-Allen et al., 1994; Hugi et al., 1998). The GIR gives an indication of the whole body insulin sensitivity. The higher the amount of glucose needed to compensate for the clearance from the blood due to insulin activity, the more sensitive the body tissues are to insulin (DeFronzo et al., 1979).

3. Feed intake and glucose homeostasis

The actual causes of the earlier mentioned homeostatic disturbances are not completely certain. Milk replacer contains a high lactose level, which clearly enhances the postprandial glucose and insulin level (Blum and Hammon, 1999; Hostettler-Allen et al., 1994). However, this is unlikely to be the only factor leading to these problems, since feed supply leads to insulin release disproportionate to the glucose and lactose contents of the diet in veal calves and sheep (Hugi et al., 1997a; Manns et al., 1967). After milk replacer intake, hyperinsulinaemia in calves (Hugi et al., 1997a) is markedly greater than after oral administration of lactose or glucose. In the study of Hugi, Bruckmaier et al. (1997a), preprandially induced hyperglycaemia in the control group exceeded postprandial hyperglycaemia responding to milk replacer intake, however the postprandial insulin response was markedly greater and the insulin/glucose ratio was 14 times higher.

This chapter describes the effect of the most important nutrients in veal calf diets on glucose homeostasis.

3.1 Effect of macronutrients on glucose homeostasis

Veal calves are fed a combination of mainly glucose/lactose, fat, protein and fibre. Because the nutrients present in milk replacer are easily digestible and the feeding levels are high, rapid and excessive elevations of plasma glucose and insulin may be triggered. In veal calves, high feeding levels or low feeding frequencies lead to problems in maintaining glucose homeostasis (Vicari et al., 2008). In a study on sheep, the amount of feed also seemed to affect insulin sensitivity, with ad libitum fed sheep being less sensitive to insulin than restrictively fed sheep (Sano et al., 1999).

As shown in Figure 3.1, glucose, fat and protein interfere with glucose metabolism. When energy is needed, glucose, in ruminants mainly derived from glucogenic nutrients, is converted into acetyl CoA, after which it enters the citric acid cycle (CLA) to generate ATP. This is also the case for lipids, amino acids and SCFA (McDonald et al., 2002). It is not clear how a combination of these nutrients and roughages influence glucose utilization and homeostasis.

3.1.1 Glucose and lactose

Glucose is known as the primary stimulus for insulin release (Ximenes et al., 2007). Also other hexoses have this effect on the pancreas (Boda, 1964; Manns et al., 1967). Lactose is the main glucogenic nutrient obtained from milk replacer. It is converted into glucose in the intestine (Palmquist et al., 1992).

Hugi et al. (1997a) have examined the effect of a milk replacer diet with additional lactose on glucose tolerance in veal calves. The additional lactose diet did not affect the basal plasma glucose or insulin level. In the lactose group, the postprandial glucose peak was achieved 60 min. later than in the control group (150 min. vs. 90 min.) and especially at the end of the fattening period the total postprandial plasma glucose elevation was larger and persisted longer in the lactose group (±11.5 vs. 10 mmol/L). There were no group differences in postprandial insulin level (±23 µg/L) or insulin peak moment (150 min.).

Hugi et al. (1997b) examined the effect of milk replacers with different lactose, total sugar, fat and protein contents but the same amount of energy on the glucose and insulin profiles of veal calves. The high lactose and sugar diets increased the glucose level more than the high fat or protein diet. The high sugar diet led to the highest insulin levels, which were even more than

three times higher than in calves fed the other diets. Milk replacer supplemented with lactose also gives a higher and longer elevation of the plasma glucose level (Hugi et al., 1997a). This is confirmed by Wijayasinghe et al. (1984) showing that calves fed a diet of high-lactose and low-fat milk replacer develop hyperglycaemia and glucosuria.

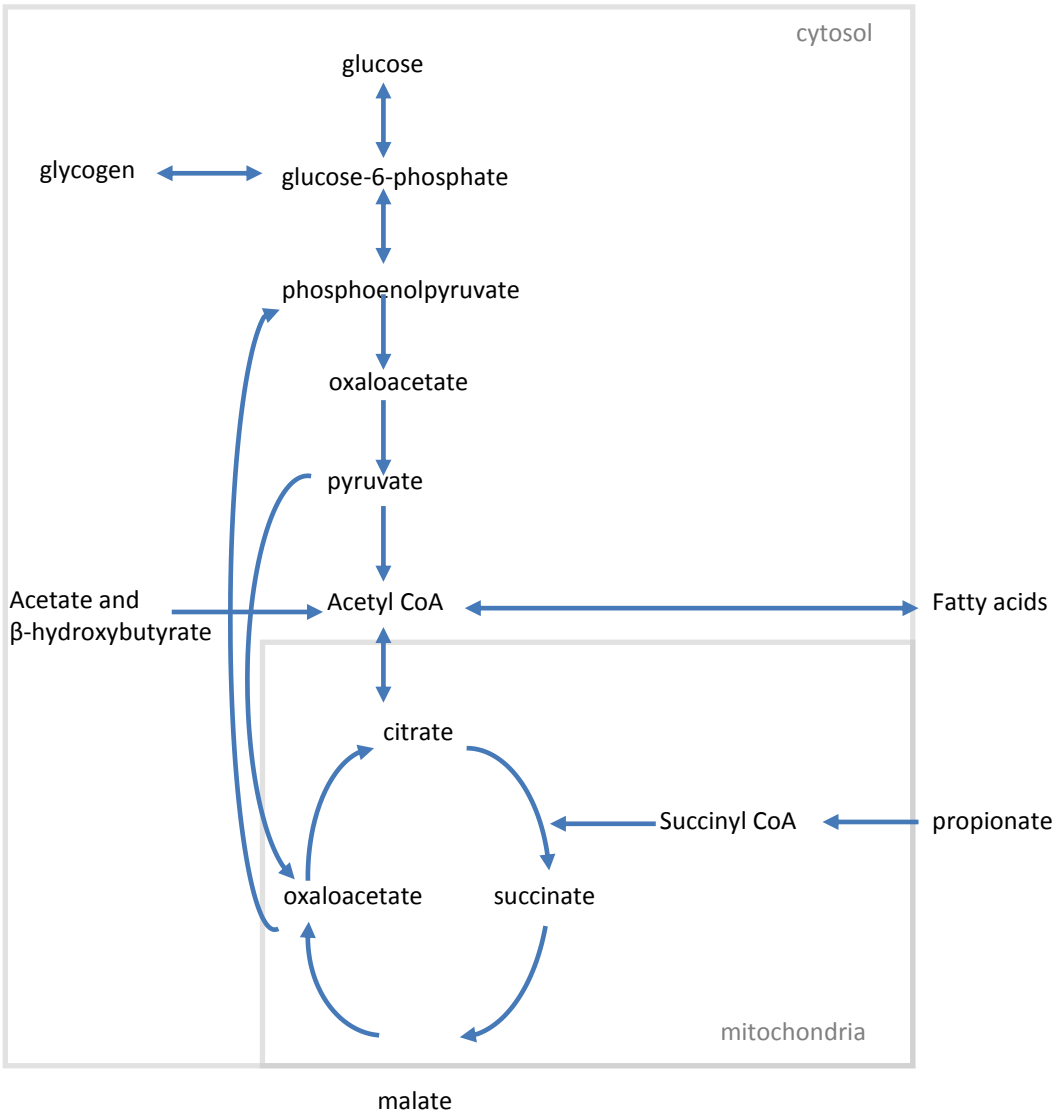

Figure 3.1: The interference of other nutrients with the metabolic pathways of glucose.

3.1.2 Fatty acids

Fatty acids and glucose have an inhibitory effect on each other's oxidation and utilization. Boden et al. (1991a) found that long chain fatty acid (LCFA) infusion in humans inhibited glucose oxidation already in the first hour after infusion. After 2-4 hours also glucose uptake and storage were inhibited. Also in people suffering from hyperglycaemia, intravenous LCFA infusion reduced the insulin stimulated glucose uptake, which is compensated by hyperinsulinaemia (Boden and Chen, 1995; Boden et al., 1995). Conversely, during artificially induced hyperglycaemia in clamp studies, the glucose elevation may completely diminish the plasma FFA level (Kelley et al., 1993). A study of Bevilacqua et al. (1987) in humans did not shown a reduced glucose utilization after LCFA infusion, although hepatic insulin sensitivity was reduced. Also the effect on

gluconeogenesis is debatable, since contradictory results have been achieved by LCFA infusions (Bevilacqua et al., 1987; Saloranta et al., 1991; Sindelar et al., 1997; Wolfe and Shaw, 1984).

The interaction between fatty acids and glucose may partly be explained by the glucose fatty acid cycle, as described by Randle et al. (1963). This cycle tries to maintain a constant plasma glucose level independently of regulatory hormones, although insulin does modify the functioning of this cycle by its lowering effect on plasma glucose levels. A glucose load stimulates glucose oxidation and storage of both glucose and fatty acids, but inhibits FFA oxidation. Conversely, a FFA load stimulates FFA oxidation, FFA storage and glucose storage, but inhibits glucose oxidation, which increases intracellular glucose concentrations and reduces cellular glucose uptake from plasma. In this way, a high dietary fat content may lead to increased plasma glucose levels and reduced insulin stimulated glucose uptake.

The main mechanism controlling this includes an increase in the mitochondrial acetyl CoA/CoA ratio following FFA oxidation. This inhibits pyruvate dehydrogenase activity, which decreases glucose oxidation (Randle, 1998; Zhou and Grill, 1995). Also citrate levels increase by FFA oxidation, which inhibits phosphofructokinase activity which in turn inhibits hexokinase activity and therefore glucose phosphorylation (Garland et al., 1962; Newsholme et al., 1962; Randle, 1998).

There may be some other ways in which lipids alter glucose metabolism.

In humans, saturated fatty acids are thought to reduce insulin sensitivity by reduction of GLUT-4 signalling. Phospholipids in muscles carrying saturated fatty acids make the membranes of the muscle cells less fluid than when transporting mono- and polyunsaturated fatty acids, which decreases insulin signalling through the membrane (Frayn et al., 2010; Marshall et al., 1997). Polyunsaturated fatty acids (PUFAs) are associated with increased insulin sensitivity. This effect of PUFAs may be caused by the inhibitory effect on glucose-6-phosphatase in the liver, which reduces the glucose excretion by the liver. PUFAs also prevent the decrease of GLUT-4 signalling (Delarue et al., 2004).

The effect of FFAs on glucose transport is not restricted to the cellular response to insulin. Insulin secretion itself may also be altered by fatty acids. Elks (1994) found that arachidonic acid (C20:4 ω-6) stimulates glucose induced insulin secretion, possibly by stimulating intracellular release of Ca^{2+} (Metz, 1988). Palmitic acid (16:0) stimulates insulin secretion in rat pancreatic cells by enhancing the Ca^{2+} influx, but through mechanisms other than blocking the K^{+} channels as described in paragraph 1.1 (Warnotte et al., 1994). These fatty acids therefore may stimulate hyperinsulinaemia and insulin resistance.

Palmquist et al. (1992) were not able to show differences in basal plasma glucose and insulin levels in ruminating veal calves (Table 2.1). Postprandial insulin and glucose responses were decreased by higher fat intake. Veal calves fed high fat diets had an increased insulin sensitivity. Palmquist et al. (1992) state that this might have been caused by the higher total volatile fatty acid (VFA) concentration in the rumen of calves fed the high fat diet. It was suggested that these veal calves were more able to cope with glucose challenges, being used to high nutrient loads. This seems unlikely based on the findings mentioned in paragraph 3.2. A problematic glucose homeostasis would possibly have been detected if experiments were done at a higher age. It should be taken into account that even the high fat milk replacer did not contain high fat levels compared to conventional milk replacers.

In conclusion, LCFAs reduce insulin sensitivity and glucose utilization, whereas PUFAs might increase insulin sensitivity. Additional fat intake in veal calves has been shown to increase insulin sensitivity (Palmquist et al., 1992), however it is improper to draw conclusions about the effect of fat intake on insulin sensitivity in veal calves as studies examining this are scarce.

3.1.3 Proteins

Competition between amino acids and glucose exists for respiration (Randle, 1998). Amino acid levels, especially alanine and to a lesser extent leucine, isoleucine, valine, tyrosine, methionine, phenylananine, cystine and threonine, were elevated in the insulin resistant human (Felig et al., 1969; Felig et al., 1974), probably because of the enhanced proteolysis of insulin resistant muscles. Alanine is the main amino acid released during proteolysis, as it accounts for 30% of the amino acids released into the blood in humans, while it comprises only 7-10% of the muscle protein, possibly because alanine in muscle tissue is synthesized de novo from pyruvate. As alanine is also the main amino acid utilized for gluconeogenesis and the hepatic uptake of precursors for gluconeogenesis is increased in insulin resistance, this may lead to higher glucose excretion by the liver and consequently higher insulin levels in the blood, when liver glycogen stores are depleted (Felig, 1975).
Conversely, intravenous amino acid infusions have led to insulin resistance in human and rat adipose tissue and skeletal muscle, elevations of the plasma glucose level and insulin resistance. The exact mechanisms involved are not certain, however it is thought that elevated amino acid levels in muscles reduce glucose oxidation in the same way as FFAs, by elevating the acetyl CoA/CoA ratio and therefore reducing pyruvate dehydrogenase (Krebs et al., 2002).
The substrate competition between amino acids and glucose is not always demonstrated, for instance experimental settings in the ovine fetus have shown that a shift in plasma glucose level does not alter amino acid oxidation (Brown and Hay, 2006).

The effect of protein on glucose homeostasis has been examined in veal calves by Gerrits et al. (2007) and demonstrated the opposite from the findings mentioned above. The dietary treatments consisted of 6 different protein levels and 2 protein free energy levels and were fed twice daily. At bodyweights (BW) of 80-160 kg, calves received diets containing 663 kJ/kg MW/d and 5.6-13.3 g/kg MW protein/day or 851 kJ/kg MW/d and 7.2-17.7 g/kg MW protein/day. At BW 160-240 kg, calves received 564 kJ/kg MW/d and 3.4-10.4 g/kg MW protein/day or 752 kJ/kg MW/d and 4.5-13.9 g/kg MW protein/day. Blood samples were taken every 14 days 5-6 hours after morning feeding. A higher protein intake lead to lower glucose and insulin levels and less glucose excretion through urine. In this study it was suggested that the problems with glucose homeostasis following a low dietary protein intake could be caused by the reduced apolipoprotein B production by the liver, impeding fatty acid transport as VLDL. This leads to an accumulation of fat in the liver, which in humans causes insulin resistance (Kelley et al., 2003). Also, amino acids have been demonstrated to be important in insulin signalling and glucose transport (Meijer and Sauerwein, 1999). This may correspond with a study of Vicari et al. (2008b), examining the effect of separation of lactose and protein intake across meals. Separation of protein and lactose over different meals reduced insulin secretion following lactose intake.

It seems that protein may influence insulin sensitivity in several contrasting ways. It is not certain which of these opposite effects of protein on plasma glucose and insulin levels are more pronounced in veal calves, although the study of Gerrits et al. (2007) suggests that protein intake may be beneficial for glucose homeostasis. Comparing this study to other studies is erroneous, since studies examining the effect of additional oral protein intake are scarce.

3.1.4 Fibre

In monogastrics, dietary fibres are associated with improvements in glucose homeostasis and insulin sensitivity (Chandalia et al., 2000; Rave et al., 2007; Salmeron et al., 1997; Ylönen et al., 2003) and reduced postprandial glucose levels in experimental settings (Jenkins et al., 1976). The

beneficial effect of fibre on glucose homeostasis may result from reduced feed intake or lower gastric emptying rate. In the colon, short chain fatty acids (SCFA) are produced from fibre by microbes, whereas in ruminants this occurs in the rumen. Therefore, the effect of fibre on glucose homeostasis in ruminants is expected to be influenced by SCFAs, however in monogastrics the effect of SCFAs is expected to be less pronounced.

In sheep, a diet with a high roughage content significantly lowered the insulin response compared to a concentrate based diet (Sano et al., 1992). This may also be due to the carbohydrate, fat and protein in the concentrates. These studies suggest a positive effect of fibre intake on glucose metabolism, although it should be taken into account that the above mentioned studies examined the effect of different iso-energetic diets, therefore reducing the carbohydrate and/or fat content of high fibre diets.

In veal calves, the differences in glucose metabolism between ruminating and non-ruminating veal calves have been measured by Palmquist (1992) (Table 2.1). Basal glucose and insulin levels were higher in milk-fed calves, although this was mainly due to the lactose in milk replacer. Also the average postprandial glucose excretion in urine was increased in milk-fed calves compared to ruminating calves (54 vs. <5 mg/dl).

3.2 Effect of short chain fatty acids on glucose metabolism

SCFAs interfere with glucose metabolism in ruminants, as shown in Figure 3.1. In this way, veal calves obtain energy from glucose as well as VFAs. Per mole of nutrient, glucose yields 38 moles of ATP, acetate 10, propionate 17 and butyrate 25 (McDonald et al., 2002).

The effect of roughage intake on glucose homeostasis in veal calves is hard to predict based on studies in ruminants as well as monogastrics (including humans), since the metabolism of veal calves may differ from both groups. In the past, veal calves only received milk replacer, mostly consisting of glucogenic nutrients, which did not induce rumen development and therefore lead to a more 'nonruminant' way of digesting feed (Hostettler-Allen et al., 1994). Nowadays, it is mandatory to supply a small amount of roughages to increase rumen activity, improve welfare and reduce abnormal oral behaviour (Veissier et al., 1998). Solid feed mainly consists of polysaccharides, which can only be broken down by microbial cultures in the rumen, which convert them predominantly into the short chain fatty acids acetate (C2), propionate (C3) and butyrate (C4) (McDonald et al., 2002). The effect of additional solid feed intake on glucose metabolism has not been studied in veal calves yet, whereas the effects of the end products of fermentation of roughages have, as described in this paragraph. To quantify some effects of SCFAs on glucose and insulin levels, in Table 3.1 the results of several studies in sheep and cows are summarized, since no studies testing the effect of SCFAs infusions on glucose metabolism have been done in veal calves. For comparison also results of glucose infusions have been added.

3.2.1 Acetate

Acetate, mainly produced from lipogenic nutrients, passes through the rumen wall and the liver unaffected and is then released into the blood, from where it is transported to muscle and adipose tissue, in which it yields energy (ATP) through the citric acid cycle or is stored by lipogenesis (McDonald et al., 2002).

Intravenous acetate infusions have been shown to induce a slight hyperglycaemia in rats (Jauhonen and Hassinen, 1978). In sheep, acetate does not cause a marked rise in plasma glucose level, and only a slight increase in insulin level after intravenous infusion, except when infusions

at rates far higher than physiological production rates are administered (Ambo et al., 1973; Ash et al., 1964; Manns and Boda, 1967). Intraruminal administration of acetate in beef heifers induced no changes in glucose and insulin levels (DiCostanzo et al., 1999) (Table 3.1). However, the amounts infused were lower than the amounts administered in the previously mentioned studies, although closer to physiological production levels. The glucose and insulin responses to acetate infusion are summarized in Table 3.2.

No or only slight effects of acetate on glucose and insulin response are expected in veal calves. Acetate is probably mainly utilized for fat deposition, as most energy requirements are fulfilled by lactose and fat. Acetate is therefore unlikely to interfere with the utilization and oxidation of other nutrients like lactose, which probably leaves the plasma glucose and insulin levels unaltered. Also, several studies have shown that acetate does not alter glucose homeostasis in sheep (Ash et al., 1964; Manns and Boda, 1967).

3.2.2 Propionate

Propionate is to a large extent converted into glucose by gluconeogenesis in the liver (Figure 3.1). The obtained glucose-6-phosphate can be used for ATP production through the glycolytic pathway and the citric acid cycle, stored into glycogen or incorporated into triacylglycerol or cholesterol esters (Zivkovic et al., 2007). The glucose that leaves the liver without being utilized, can supply energy to the muscle or be used for glycogenesis in muscle tissue or lipogenesis in muscle and adipose tissue (McDonald et al., 2002).

In isolated rat pancreatic islets, propionate reduces the insulin secretion responding to glucose (Ximenes et al., 2007). An in vivo experiment in rats has shown that orally administered propionate lowers fasting blood glucose levels and glucose excretion from the liver (Berggren et al., 1996). Boillot et al. (1995) examined the long term effect of additional propionate diets on glucose metabolism and insulin sensitivity of rats. Preprandial glucose levels were lower in the propionate receiving rats, but insulin levels were not altered by the diet. Gluconeogenesis and glucose utilization were also not changed.

It has been shown that propionate injection has a more rapid, but lower elevating effect on the plasma glucose and insulin level than glucose injection in sheep (Horino et al., 1968; Manns and Boda, 1967). Another study in sheep (Ambo et al., 1973) showed that propionate markedly increased the insulin response compared to equimolar amounts of glucose, even though the glucose level after infusion of propionate did not rise significantly (Ambo et al., 1973). This suggests that SCFAs interfere with glucose homeostasis, and that propionic acid not only stimulates insulin secretion due to its glycogenic properties (Ambo et al., 1973; Ash et al., 1964), but possibly also has a direct stimulatory effect on the pancreas (Manns et al., 1967).

In beef heifers, propionate infusion into the rumen slightly elevated the glucose response but gave no insulin response. The infusion rates administered were lower than in the studies mentioned above, however the absence of an insulin response may also result from the route of administration, due to lower uptake into the blood and clearance by the liver. Therefore the insulin response may partly be a result of enhanced gluconeogenesis.

Some results of studies on the effect of propionate on insulin and glucose responses are summarized in Table 3.1. Table 3.2 shows the average effect of propionate on glucose and insulin responses in ruminants. The cause of the difference between monogastrics and ruminants is unclear. In combination with feed intake, propionate may reduce feed digestion by inhibiting amylase activity, therefore reducing carbohydrate uptake (Todesco et al., 1991).

It is expected that the plasma glucose levels slightly rise responding to propionate uptake in veal calves, probably due to enhanced gluconeogenesis. The effect of propionate on insulin is uncertain.

3.2.3 Butyrate

Butyrate is mostly utilized by the rumen and intestinal wall. A certain amount of butyrate is converted into β-hydroxy butyrate, which passes the liver and, when secreted into the blood, supplies muscle and adipose tissue with energy or can be stored through lipogenesis (McDonald et al., 2002). At these sites, there is competition between β-hydroxy butyrate and glucose for respiratory purposes (Randle, 1998).

Not many studies have been done on the effect of butyrate infusion on glucose and insulin responses in monogastrics. In a study in mice by Gao et al. (2009), basal insulin levels seemed to be lower and insulin sensitivity improved when fed a diet supplemented with butyrate.

Several studies have been done on the effect of butyrate on the plasma glucose level in ruminants. Butyrate has been shown to induce an elevation of the plasma glucose level in sheep (Ash et al., 1964; Phillips et al., 1969), even though butyrate is a ketogenic nutrient. In the study of Sano et al. (1995b) in sheep, the plasma glucose level did rise, but only at higher butyrate infusion rates, and the glucose level dropped below basal value after infusion.

Ambo et al. (1973) suggested that butyrate may induce glucose release from the liver due to glucagon secretion as a result of stimulation of the vagus nerve, which is normally stimulated by mainly carbohydrates and protein in the gut (Croom Jr et al., 1992). Obara et al. (1971) have indeed shown an abolishment of the hyperglycaemia responding to intraruminal butyrate injections in vagectomized sheep, whereas in the non-vagectomized sheep, hyperglycaemia did appear. It should be noted that ruminal administration of butyrate in non-vagectomized ruminants not always leads to elevated glucose levels (Armstrong and Blaxter, 1957).

In the isotope study of Ash et al. (1964), the plasma glucose level in sheep rose after butyrate infusion and only a small part of this glucose appeared to originate from butyrate itself, indicating that an increase in plasma glucose level is not provoked by gluconeogenesis from butyrate and confirming that the remaining glucose production may be induced by enhanced glycogenolysis. However, glucose release from isolated liver cells, which was also measured in this study, was not induced by butyrate. This suggests that any effect of butyrate on glycogenolysis may only be obtained indirectly, for instance by stimulation of glucagon action.
In a study of Phillips et al. (1969), sheep were pancreatectomized. Intravenous injections of butyrate did not elevate the plasma glucose level, suggesting that production of glucagon may indeed be the factor enhancing the plasma glucose level after butyrate infusion, rather than conversion of butyrate itself. Also the report of Phillips and Black (1966) stated that butyrate itself is not converted into glucose.

The effects of butyrate on insulin production are in general more pronounced than the effects on plasma glucose levels.

Manns and Boda (1967) have shown a higher insulin response after intravenous butyrate infusion than after glucose infusion, when the same degree of hyperglycaemia was induced. Even when the glucose response after butyrate infusion is lower than after glucose administration in sheep (Ambo et al., 1973), the insulin response to butyrate was higher. This shows that the effect of butyrate on insulin release cannot purely be a result of a rise in plasma glucose level originating from the butyrate itself.

Intravenous injection of butyrate leads to even higher and more rapid insulin production in sheep as propionate does at the same degree of hyperglycaemia (Ash et al., 1964; Manns and Boda, 1967; Mineo et al., 1994; Sano et al., 1995b). Also Sano et al. (1995b) found clear insulin level elevations after intravenous butyrate infusions, even though the plasma glucose level only rose slightly at high infusion rates. It seems that fatty acids containing 3 to 8 C-atoms give a higher insulin response with increasing chain length, only partly explained by the hyperglycaemia, and all give a markedly higher insulin response than fatty acids containing more or less C-atoms (Ambo et al., 1973; Ash et al., 1964).

The exact mechanism behind the insulin stimulating effect of butyrate is unclear. In vitro studies have validated that butyrate has a direct effect on pancreatic secretion of insulin (Jordan and Phillips, 1978; Manns et al., 1967). Phillips et al. (1969) suggested that the elevating effect of butyrate on the insulin level may also be caused by butyrate induced glucagon excretion from the pancreas and therefore glycogenolysis in the liver. An elevated glucagon production is demonstrated in sheep after butyrate infusion, but at much higher infusion rates than those needed to achieve a significant plasma insulin increase (Sano et al., 1995b), suggesting that the stimulatory effect of butyrate on glucagon release is of minor importance.

A contribution of the liver to pancreatic stimulation is not excluded. The liver extracts butyrate from the portal blood and therefore determines how much butyrate pursues to the pancreas (Manns et al., 1967). Manns et al. (1967) tested the effect of butyrate on liver metabolite secretion by infusing butyrate into the jugular and portal vein. Administration into the jugular vein lead to explicitly higher butyrate and insulin concentrations in the blood. This suggests that the effect of butyrate on insulin release may not be due to liver metabolites.

Most studies conclude that in ruminants butyrate leads to higher insulin responses than glucose and propionate. However, after being metabolized by rumen epithelial cells following roughage intake, most butyrate appears in blood of ruminants as β-hydroxybutyrate, which does not stimulate insulin secretion in sheep (Horino et al., 1968; Jordan and Phillips, 1978; Stern et al., 1970). It is therefore expected that the effect of butyrate on insulin secretion may be less pronounced after intake of roughages than after butyrate infusion. The effect of VFA (volatile fatty acid) passage through the GI-tract on insulin and glucose levels has been examined in the study of Istasse et al. (1987). The results of this study suggest that SCFAs produced in the rumen indeed have a less pronounced effect on glucose and insulin levels than suggested by intravenous infusion studies. This effect may be reduced due to the slower rate of absorption into the blood and due to passage through and absorption by the rumen wall and liver prior to passing the pancreas.

The results of several studies mentioned above are summarized in Table 3.1. Table 3.2 gives a general overview of the effect of butyrate on glucose and insulin levels in ruminants.

Table 3.1: Plasma glucose and insulin responses to injections and infusions of glucose and SCFAs

Source	Treatment	Glucose peak (g/L)	Insulin peak (µU/mL)
Manns and Boda 1967	glucose, 1.25 mmol/kg BW[1] injection (sheep)	0.6, 0.6, 0.7 and 0.8 (5-10 min) at 2, 6, 12 and 24 wk	750, 300, 350, 400 (5-10 min) at 2, 6, 12 and 24 wk
	acetate, 2.5 mmol/kg BW injection	±0.2 (15 min)	not present
	propionate, 2.5 mmol/kg BW injection	0.3, 0.4, 0.3 and 0.35 (10-15 min) at 2, 6, 12 and 24 wk	400, 300, 400 and 600 at 2, 6, 12 and 24 wk
	butyrate, 2.5 mmol/kg BW injection	0.5, 0.4, 0.2, 0.2 (10-15 min) at 2, 6, 12 and 24 wk	850, 800, 850 and 600 (10-15 min) at 2, 6, 12 and 24 wk
Ambo et al. 1973	glucose, 1.25 mmol/kg BW in 2 min (sheep)	0.14 (15 min)	40 (60 min)
	acetate, 1,25 mmol/kg BW in 2 min	0.05 (15 min)	50 (10 min)
	propionate, 1,25 mmol/kg BW in 2 min	0.7 (20 min)	60 (10 min)
	butyrate, 1,25 mmol/kg BW in 2 min	0.75 (30 min)	110 (20 min)
Sano et al. 1995	16 mmol/kg BW/min butyrate infused for 30 min (sheep)	0.7	255
	32 mmol/kg BW/min infused for 30 min	1.0	455
	64 mmol/kg BW/min infused for 30 min	1.2	496
Sasaki et al. 1984	Grass hay, 59.2-67.6 g DM/kg BW (sheep)	0.68 (420 min)	12.6 (150 min)
Istasse et al. 1987	0.45 MJ/kg MW 24 h rumen infusion, 65% acetate, 17.5% propionate, 17.5% butyrate (non lactating cows)	0.65 (24 h)	0.025 (24 h)
	0.45 MJ/kg MW 3 h rumen infusion, 74% acetate, 6% propionate, 20% butyrate	0.63 (180 min)	0.09 (180 min)
DiCostanzo et al. 1999	75 µmol/kg BW/min, 6 h rumen infusion, acetate	5.4 (360 min)	0
	42 µmol/kg BW/min, 6 h rumen infusion, propionate	5.6 (360 min)	0

[1] Body weight

Table 3.2: The average glucose and insulin responses to SCFAs in ruminants

	plasma glucose	plasma insulin
acetate	+/-	+/-
propionate	+	+
butyrate	+	++

4. Glycogen and fat deposition in liver and muscle

Glycogen and fat deposition, as well as the fatty acid composition, partly depend on the diet. The effect of roughage supply is explained in this chapter. Also alterations in glycogen and fat deposition due to insulin resistance are described.

4.1 Glycogen deposition

Glycogen deposition is the result of glycogen formation by glycogen synthase and glycogen breakdown by glycogen phosphorylase (Taylor et al., 2006). Glucose is deposited as glycogen in liver and muscle if the oxidative capacities of glucose utilizing tissues are exceeded, which is a result of the intake of high carbohydrate quantities. In the postabsorptive state, the glucose from glycogen may be released and utilized or stored as fat. Glycogen deposition is therefore only temporary (Jacot et al., 1982). As glycogen stores fill up, glycogen synthase activity is inhibited and therefore glycogen stores reach their limit (Boden et al., 1991b).

4.1.1 Effect of macronutrients on glycogen deposition

As glycogen formation is a condensation reaction between uridine diphosphate glucose molecules and earlier formed glycogen, glycogen can be derived from all nutrients that can be converted to glucose through gluconeogenesis, like amino acids and lactate, and carbohydrates (McDonald et al., 2002).

Because of the interaction between lipids and glucose metabolism, lipids influence glycogen storage. Increased lipid availability is strongly associated with reduced glycogen synthesis (Clarke, 2000). The decreased glucose transport and glucose phosphorylation (necessary for glycogen synthesis), described in paragraph 3.1.2., may partly cause this reduction in glycogen storage. Also, FFA are known to reduce glycogen synthase activity (Boden et al., 1991b; Kelley et al., 1993). This is partly caused by a relocalization of glycogen synthase. Because it shifts to an insulin insensitive pool, FFAs reduce the response of glycogen synthase to insulin (Taylor et al., 2006). The reduction in glycogen synthesis may partly be restricted by intake of PUFAs. These fatty acids stimulate oxidation of fatty acids, reduce glucose oxidation and stimulate glycogen formation. This is done by up-regulation of the expression of genes coding for proteins regulating fatty acid oxidation and down-regulating the expression of genes coding proteins that stimulate lipogenesis (Clarke, 2000).

When fat is already stored, it might stimulate glycogen formation. Adding leptin, formed by adipocytes, to isolated rat hepatocytes has been shown to increase glucose incorporation into glycogen (Aiston and Agius, 1999). The amount of fat deposited may therefore restrict the inhibitory effects of FFA on glycogen synthesis described above.

4.1.2 Effect of short chain fatty acids on glycogen deposition

A study on isolated rat diaphragms (Parnes and Wertheimer, 1950) has demonstrated the effect of acetate and propionate on glycogen synthesis. Acetate raised glycogen synthesis slightly, but the presence of this effect is dose dependent. The combination of glucose and acetate lead to a higher glycogenesis than both substrates could achieve separately. The mechanism behind this is not certain. Acetate also lowers glucose oxidation. In contrast, propionate did not increase glycogen deposition, even though this is a glycogenic nutrient. Another study in mice did not find any effects of intravenous acetate infusion (Jauhonen and Hassinen, 1978). Donkin and

Armentano (1995) have shown that propionate may even reduce the glycogen content in hepatocytes in certain situations. The combination of propionate and glycerol, probably due to propionate (Chan and Freedland, 1972; Donkin and Armentano, 1995), has been demonstrated to decrease gluconeogenesis from lactate, which is an important precursor for gluconeogenesis in ruminants.
An in vivo experiment on muscle and liver glycogen content confirmed the higher glycogen content in acetate fed rats, whereas propionate supplements did not alter the amount of glycogen in muscle and liver (Parnes and Wertheimer, 1950).

The effect of intravenous butyrate administration to sheep on glycogen deposition was examined by Phillips and Black (1966). Hepatic phosphorylation (the uncoupling of one glucose molecule) was increased and the glycogen content decreased.

The above mentioned studies are not consentient about the effect of SCFAs on glycogen deposition. No marked alterations of glycogen stores in veal calves are expected following roughage supply. Differences in glycogen deposition are more likely caused by differences in diet composition, feed intake level and insulin sensitivity.

4.1.4 Relation between glycogen stores and insulin resistance

Insulin-mediated storage of glucose into glycogen mainly depends on glucose transport, rather than glycogen synthase activity or glucose phosphorylation (Cline et al., 1999). In muscles of insulin resistant humans, the transport of GLUT-4 from intracellular sites to the cell membrane responding to insulin is impaired (Zierath et al., 1996). Because of the higher glycogenolysis in the insulin resistant state, glycogen stores are expected to be lower, which was confirmed in a study on humans, showing that low glycogen levels in skeletal muscles are correlated with a low insulin sensitivity and plasma hyperglycaemia (He and Kelley, 2004).
It has also been shown in rats that high muscle glycogen contents are associated with a high insulin sensitivity (Taylor et al., 2006). Conversely, high muscle glycogen stores influence GLUT-4 transport and therefore stimulate insulin sensitivity, although in a study of Derave et al. (2000) low glycogen contents in muscles were more associated with a higher insulin sensitivity than high glycogen contents.
In insulin resistant veal calves, glycogen stores are expected to be reduced, like in insulin resistant humans and rats.

4.2 Fat deposition

The most important steps in fat deposition take place in the cytosol, involving palmitate production from acetyl CoA or butyryl CoA. Acetyl CoA is formed in the mitochondria by decarboxylation of pyruvate and by degradation of amino acids and fatty acids. In ruminants, mostly acetate is used for this matter. Acetyl CoA is converted into citrate to enable transportation into the cytosol, following regeneration into acetyl CoA. Acetyl CoA is converted into malonyl CoA. Both acetyl CoA (C2) and malonyl CoA (C3) are attached to an acyl carrier protein (ACP), after which a reaction between both forms acetoacetyl-ACP (C4), β-hydroxy butyryl-ACP (C4), crotonyl-ACP and finally butyryl-ACP. Butyryl-ACP (C4) reacts with malonyl CoA, forming palmitate (C16) (Bender, 2008; McDonald et al., 2002).
Stearate and oleate in fat depots are synthesized from palmitate by elongation with malonyl CoA, which occurs mainly in the endoplasmic reticulum and to a small extent in the mitochondria. In the endoplasmic reticulum, preformed fatty acids may be desaturated by a desaturase (McDonald et al., 2002).
This paragraph describes the nutrients influencing fat deposition.

4.2.1 Effect of macronutrients on fat deposition

Mainly lipids and carbohydrates contribute to fat deposition in monogastrics (McDonald et al., 2002). Especially postprandially, lipogenesis of carbohydrates is increased. The carbon skeleton of protein may also be deposited as fat. In general, a high protein diet, with the same energy content as control diets, is associated with a lower fat deposition (Pichon et al., 2006). In ruminants body fat is mainly synthesized from acetate (Hanson and Ballard, 1967).

Mechanisms of fat deposition in veal calves are more complicated. In heavy veal calves, the incorporation of glucose into lipid stores is negligible (Van den Borne et al., 2007b). A possible cause could be the high fat intake through milk replacer. Calves therefore mainly synthesize fat from lipid intake, although amino acids may also be incorporated into fat.

Since veal calves are fed above maintenance level, dietary lipids are most likely utilized for fat deposition. Tikofsky et al. (2001) examined the effect of three iso-energetic and iso-nitrogenous diets with 14.8%, 21.6% and 30.6% fat on veal calves. The diets resulted in the same daily weight gain. The percentage fat from total gain increased with increasing dietary fat levels. The study concluded that above 15% fat content in the diet, lipids were utilized for additional fat deposition.

The effect of supplemental protein on fat deposition has been examined in veal calves by Gerrits et al. (1996). Calves were assigned to 6 different protein levels at 2 protein-free energy levels, as mentioned in paragraph 3.1.3. Calves fed the high protein level had a higher empty body weight gain than calves fed the low protein levels, namely 184 g/d more at 80-160 kg BW, from which 7.6% was accounted for by protein, and 265 g/d more at 160-240 kg BW, 12.8% accounted for by protein. Also fat deposition increased at the higher protein levels. Fat accounted for about 50% of the additional weight gain. This suggests that protein may contribute to fat deposition in veal calves.

It should be taken into account that the amount and combination of nutrients fed are important in determining the metabolic fate. A shortage of glycogenic nutrients will increase amino acid oxidation and reduce fat deposition. In veal calves, most fat deposition is probably derived from dietary fat and acetate, however a contribution of dietary protein is expected.

4.2.2 Effect of short chain fatty acids on fat deposition

In rodents, additional resistant starch, which is fermented in the colon where it causes the production of SCFAs, decreases the fat deposition in adipose tissue. The same effect is obtained when SCFAs are added to the feed (Zheng et al., 2010). The effect on veal calves could be different, since acetate is an important precursor for fat synthesis in ruminants.

Hanson and Ballard (1967) compared the fat deposition in rats fed standard pellets, pigs fed alfalfa hay and mixed grain, and cows grazing on mixed grass and fed mixed grain. When glucose intake was absent, cows and sheep utilized ten times more acetate for fat deposition in adipose tissue than rats. The rate of lipogenesis in the liver from acetate was comparable between the species. Adding both glucose and insulin, led to a significantly higher incorporation of acetate into lipids (Hanson and Ballard, 1967). This could mean that in veal calves, as they obtain both glucose and acetate from their diet, the rate of lipogenesis from acetate is relatively high compared to other ruminants, which might restrict the stimulating effect of insulin on glucose incorporation into lipids, possibly enhancing the plasma glucose and tissue glycogen level.

High forage diets have been shown to reduce the intramuscular fat deposition in Holstein bulls (Aharoni et al., 2004) in a study comparing iso-energetic high and low fibre diets. The higher

fat deposition in low fibre diets could also be caused by a higher fat content of the diet. An elevated carbohydrate content is unlikely to increase intramuscular fat deposition, since glucose is hardly utilized for lipogenesis in veal calves (Smet et al., 2000; Van den Borne et al., 2007b).

Zheng et al. (2010) showed a decrease of body fat up to 80 per cent after additional acetate, propionate and butyrate supply to nematodes, which are a common animal model. This remarkable finding is caused by enhanced energy expenditure or lower feed intake.

From these results, it is hard to predict the effect of additional acetate on fat deposition in veal calves. Based on the studies in ruminants, it is expected that fat deposition may increase following additional acetate uptake.

4.2.4 Relation between fat storage and insulin resistance

In obese but not in healthy humans and rats, not only circulating free fatty acid levels are associated with insulin resistance, as described in paragraph 2.3, but also the deposition of fatty acids into non-adipose tissues. Both induce insulin resistance more than body fat itself (Oakes et al., 1997; Perseghin et al., 1999). Body fat has even been shown to stimulate insulin sensitivity, since a shortage of body fat due to lacking storage sites leads to higher circulating free fatty acid levels and a higher fat deposition in muscle tissue. Adipose tissue is also required for the production of adipokines, for instance leptin and adiponectin, which play a role in regulating feeding behaviour and metabolism (Guilherme et al., 2008) and therefore help controlling glucose homeostasis.

4.3 Fatty acid composition

This chapter gives results of studies examining the effect of some macronutrients and SCFAs on the fatty acid composition of muscle and adipose tissue.

4.3.1 Effect of macronutrients on fatty acid composition

Smet et al. (2000) did research on the effect of different energy (7.38 MJ ME/kg DM and 8.03 MJ ME/kg DM) and crude protein (CP) (127, 153 and 172 g CP/kg DM) levels on fatty acid composition in muscles (m. longissimus thoracis) of Belgian blue bulls. Energy level did not influence the saturated fat deposition. Monounsaturated fatty acid (MUFA) deposition was increased, whereas polyunsaturated fat deposition was decreased at higher energy levels. However, this could partly be due to the added beef tallow, which was used to increase the energy level of the diet. The higher energy level led to increased C14:0, C16:0, C16:1 and C18:1 concentrations and decreased C18:2 and C20:4 levels. The fatty acid composition also depended on the level of fatness, with increased fatness being correlated to a higher saturated and monounsaturated fat content. No conclusions could be drawn about the effect of protein level on fatty acid composition.

In monogastrics, fatty acids obtained from the diet are readily deposited into fat depots. In ruminants, rumen microbes hydrogenate fatty acids which results in an increased production of stearic acid (Hoflund et al., 1956). Without this intervening process, more unsaturated fatty acids would be readily incorporated into fat depots in ruminants (Tove and Smith, 1960). Because of the lower unsaturated fatty acid deposition into lipid stores, more saturated fatty acids are utilized for fat deposition (Tove and Smith, 1960). Wrenn et al. (1973) have obtained results confirming this. They tested the effect of supplemental C18:2 in veal calves fed the same amount of cow milk (2.5 vs. 14.2% C18:2) and ad libitum grain and alfalfa hay till 10 weeks of age. The

supplemented milk was obtained from cows fed supplements of safflower oil protected from dehydrogenation by microbes. From 11-18 weeks, calves received either protected or unprotected safflower (13% of concentrate). The largest C18:2 concentrations in supplemented milk lead to a 4 times higher C18:2 deposition in the tailhead (the root of the tail) and omentum (a fold in the peritoneum) than unsupplemented milk. The C18:2 concentration in intramuscular fat and perirenal fat was nearly doubled. As C18:2 contents increased, C16:0 and C14:0 concentrations dropped. Feeding protected, compared to unprotected, safflower also increased the C18:2 deposition in the tailhead 3 times in the group that had not received supplemented milk.

Veal calves are expected to deposit more MUFAs than fattening bulls or other ruminants as the rations contain high energy levels. Increased energy intake due to additional fat intake has led to increased MUFA deposition according to Smet et al. (2000). Also, the deposited fatty acids are expected to be mainly saturated, since veal calves probably hydrogenate at least part of the unsaturated fatty acids.

4.3.2 Effect of short chain fatty acids on fatty acid composition

The fatty acid profile of muscle and body fat differs among species, mainly due to the diet. Enser et al. (1996) analyzed the fatty acid profile of beef, lamb and pork muscle and adipose tissue in retail samples. The contents of the main fatty acids and the fatty acids discussed further in this paragraph are shown in Table 4.1. The main fatty acids in all three species were palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1 n-9), although pork also showed high concentrations of C18:2 n-6 (linoleic acid). Beef generally contained less C20 and C22 poly unsaturated fatty acids than lamb and pork.

The total intramuscular fatty acid content was 38.4, 49.3 and 22.6 g/kg muscle and the total fatty acid content in adipose tissue was 699.7, 705.7 and 653.4 g/kg adipose tissue in beef, lamb and pork respectively. Increased concentrations of palmitic and oleic acid reflected the higher total fat concentrations. Other fatty acid concentrations resulted from difference in species and diet.

Table 4.1: Fatty acid profile of beef, lamb and pork muscle and adipose tissue

Fatty acid	Muscle (g/kg)			Adipose tissue (g/kg)		
	Beef	Lamb	Pork	Beef	Lamb	Pork
C12:0	0.03	0.1	0.03	7.0	2.5	9.7
C14:0	1.0	1.6	0.3	26.2	28.5	10.2
C16:0	9.6	11.0	5.3	182.7	155.3	156.1
C18:0	5.1	9.0	2.8	85.4	159.6	83.5
C18:1 n-9	14.0	16.3	7.6	246.3	203.3	235.5
C18:2 n-6	0.9	1.3	3.0	7.7	9.2	92.6
C18:3 n-3	0.3	0.7	0.2	3.4	6.7	9.3
C20:4 n-6	0.2	0.3	0.5	ND	ND	1.1

ND: not detected

Xiccato et al. (2002) tested the effect of maize supplementation on fatty acid composition of muscle (m. longissimus thoracis) in veal calves fed twice a day. All calves received the same amount of milk replacer. The main fatty acids as percentages of the total fatty acids are shown in Table 4.2.

There was a significant increase in palmitic acid with increasing maize supplementation. Linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4 n-6), EPA (C20:5) and total polyunsaturated fatty acids were significantly decreased with increasing maize intake.

Table 4.2: Fatty acids as percentages of total fatty acids in veal calf muscle

Fatty acid	Milk replacer	Maize
C12:0	0.43	0.41
C14:0	4.57	4.51
C14:1	0.64	0.69
C15:1	0.15	0.16
C16:0	23.2*	23.8
C16:1	3.22	3.21
C17:0	0.64	0.66
C17:1	0.31	0.34
C18:0	15.04	14.97
C18:1 n-9	37.79	38.63
C18:2 n-6	10.26*	9.36
C18:3 n-3	0.46*	0.44
C20:1 n-9	0.2	0.2
C20:2 n-6	0.09	0.09

* $P<0.05$

Kelley et al. (2004) documented the percentages of some fatty acids in the lipid fraction of the mouse liver (Table 4.3). The amount of palmitic acid is comparable to the amount found in muscles in the above mentioned studies. The amount of stearic acid was markedly lower. Oleic acid was by far the most common fatty acid in mouse liver.

Table 4.3: Fatty acids as percentages of total fatty acids in mouse liver

Fatty acid	% of fatty acids
C16:0	23.3
C16:1	4.9
C18:0	2.3
C18:1 n-9	46.6
C18:2 n-6	13.2

The effect of increasing amounts of maize in iso-nitrogen, calcium and phosphor diets on the fatty acid composition of kidney and subcutaneous fat in Boer goats was examined by Casey and Van Niekerk (1984). There was a positive correlation between maize level and C18:0 concentration in subcutaneous fat. Also C18:1 was increased in both depots for increased amount of maize. C16:0 and C14:0 levels in subcutaneous fat showed a decrease with increasing maize diets, but not significantly. However, it is hard to compare the results of Boer goats with other ruminants because of their species specific metabolism.

In a study on Holstein bulls comparing iso-energetic diets, high and low fibre contents consisting of maize, barley, soybean(meal), vetch hay and wheat silage, induced no differences in fatty acid composition of intramuscular or subcutaneous fat (Aharoni et al., 2004).

In sheep, diets containing a high amount of concentrates not supplemented with fat lead to lower C18:0, C16:0, C14:0 and C18:3 and higher C18:1 and C18:2 levels in adipose and muscle tissue than diets containing only roughages. Supplementing diets with vegetable fat resulted in higher levels of monounsaturated and polyunsaturated fatty acids in adipose and muscle tissue (Bas et al., 2001). Conclusions based on studies in sheep should be drawn carefully, as sheep contain higher concentrations of C18:1 than cows.

The fatty acid composition in beef alters with age. Higher amounts of C17:0, C18:1 and C18:2 are associated with a higher fat deposition in lambs (Crouse et al., 1982). Melton et al. (1982) also reported higher C18:1 levels in finishing steer muscle and subcutaneous fat at the end of the fattening period.

Based on these studies, it is expected that veal calves will show higher C18:0, C18:1 and C18:2 levels and lower C14:0 and C16:0 levels as they receive more roughages, especially near the end of the fattening period.

5. Conclusions

Glucose homeostasis of veal calves is not comparable to either monogastrics or ruminants. Glucose and insulin levels in heavy veal calves respond excessively to feed intake due to insulin resistance. The causes are uncertain, but there are several factors that might induce insulin resistance, from which the diet is thought to be an important one because of the high feeding levels and intake of lactose and fat. Feeding roughage and fat to veal calves may worsen insulin resistance, especially the production of butyrate in the rumen can lead to excessive insulin levels. However, PUFAs seem to increase insulin sensitivity, at least in other species. Fat deposition would be expected to decrease when roughages are fed, but in case of insulin resistance, fat deposition is normally increased. As a result of roughage intake, this fat is expected to consist of higher concentrations of C18:0, C18:1 and C18:2 and lower levels of C16:0 and C14:0. The effect of roughages on glycogen deposition is uncertain since studies give varying results. In case of insulin resistance, enhanced glycogen breakdown is expected, but this has not been confirmed in veal calves.

Article

The effect of solid feed intake on glucose homeostasis in heavy veal calves

The effect of solid feed intake on glucose homeostasis in heavy veal calves

S. Podesta

Division of Animal Nutrition, Animal Sciences Group, Wageningen University and Research Centre, Wageningen, The Netherlands

Abstract

Veal calves often develop excessive postprandial hyperglycaemia and hyperinsulinaemia, glucosuria and insulin resistance. Fasting plasma glucose levels may also be elevated. The development of these features could be affected by short chain fatty acids, produced during fermentation of roughages and concentrates in the rumen. To examine the effect of solid feed intake on glucose homeostasis in heavy veal calves, 48 Holstein Friesian bull calves were fed a milk replacer diet supplemented with 0, 9, 18 or 27 g DM solid feed per kg metabolic body weight. The solid feed consisted of 25% chopped wheat straw, 25% maize silage and 50% concentrate based on DM weight. Glucosuria was measured at 107.9 ± 1.1 and 163.9 ± 1.6 kg BW. At ±165 kg BW, a hyperinsulinaemic euglycaemic clamp (HEC) study was performed to measure whole body insulin sensitivity. At slaughter, when calves weighed ±185 kg, liver, muscle and perirenal fat tissues were collected pending determination of triglyceride and glycogen concentrations in liver and muscle as well as fatty acid profile in liver, muscle and perirenal fat.

Solid feed intake increased ($P<0.001$) fasting glucose levels and decreased glucose infusion rates GIR_{60-240} and $GIR_{170-240}$ during the HEC. In urine, glucosuria was detected in all calves and developed with age ($P<0.05$), but effects of solid feed intake were absent. Solid feed intake did not influence glycogen concentration in liver and muscle. Also triglyceride contents in liver and muscle were not influenced by solid feed intake, although roughage intake tended ($P<0.10$) to increase triglyceride concentration in muscle. In the lipid fraction of the liver, solid feed intake decreased ($P<0.001$) the concentration of C12:0, but increased ($P<0.05$) the concentration of C17:0, C18:0 and C20:3n6. In the lipid fraction of the muscle, solid feed intake decreased ($P<0.05$) the concentration of C17:1. In perirenal fat, solid feed intake decreased the concentration of C12:0. Solid feed tended ($P<0.10$) to decrease the concentration of C14:0 and the total PUFA concentration. In addition, insulin sensitivity was correlated negatively ($P<0.01$) with muscle triglyceride concentration, but positively ($P<0.05$) with muscle glycogen concentration.

In conclusion, solid feed intake seemed to negatively affect glucose homeostasis, but glycogen and triglyceride concentrations were not substantially affected. The fatty acid profile of liver, muscle and perirenal fat was altered by solid feed intake, which may be due to an increased fatty acid synthesis from acetate.

Keywords: Veal calves, short chain fatty acids, glucose homeostasis, insulin, milk-fed, triglyceride, glycogen, liver, muscle, perirenal fat

Abbreviations: DM, dry matter; BW, body weight; MW, metabolic weight ($BW^{0.75}$); SCFA, short chain fatty acid(s); MR, milk replacer; HEC, hyperinsulinaemic euglycaemic clamp; SEM, standard error of the mean; KOH, potassium hydroxide; $HClO_4$, perchloric acid; FAME, fatty acid methyl ester; $CaCl_2$, calcium chloride; I_2, iodine; FFA, free fatty acid(s); PUFA, poly-unsaturated fatty acid(s); MUFA, mono-unsaturated fatty acid(s); SFA, saturated fatty acid(s); FFA, free fatty acids

Introduction

Veal calves are kept on milk replacer diets until slaughter to achieve a high energy intake, high growth rates and meat which is characterized by its paleness and tenderness (Doppenberg and Palmquist, 1991). It is nowadays mandatory to supply roughages to veal calves (IKB, 2008). This improves welfare and reduces abnormal oral behaviour by providing the opportunity to ruminate (Veissier et al., 1997). Rumen papillae growth is increased as a response to short chain fatty acid (SCFA) production from roughages by rumen microbes (Flatt et al., 1958). The structure of roughages stimulates rumen motility and rumination (McDonald et al., 2002). Despite these positive effects of roughages, it is expected that roughages negatively affect glucose homeostasis.

Feed intake normally elevates the plasma glucose level, which initiates insulin release to maintain glucose homeostasis. Insulin clears glucose from the blood by inhibiting endogenous glucose production (incl. gluconeogenesis and glycogenolysis) in the liver, and by stimulating glucose uptake in muscle- and adipose tissues. The formation of glycogen in muscle and liver, and the formation of lipids in adipose tissue and muscle are stimulated, while glycogenolysis and lipogenesis are inhibited (Aronoff et al., 2004; Ferrannini and DeFronzo, 2003). Despite this delicate regulation of glucose homeostasis, heavy veal calves often express problems characterized by postprandial hyperglycaemia, hyperinsulinaemia and glucosuria (Hostettler-Allen et al., 1994; Hugi et al., 1998), indicating inefficient utilization of glucose (Hugi et al., 1997a).

This disturbance in glucose metabolism is most likely caused by an impaired insulin sensitivity of liver, muscle and adipose tissue (Hostettler-Allen et al., 1994; Hugi et al., 1997a). In case of insulin resistance, glycogenolysis and gluconeogenesis become resistant to the inhibitory effect of insulin, leading to higher plasma glucose levels. Together with the failed glucose uptake by body tissues, this causes an ongoing hyperglycaemia (Ferrannini & DeFronzo, 2004; Hostettler-Allen et al., 1994).

Several nutritional factors could contribute to an impaired glucose homeostasis. Until now, mainly the high lactose levels in milk replacer and the high feeding levels were considered to negatively affect glucose homeostasis (Hostettler-Allen et al., 1994). However, solid feed supply may alter glucose metabolism as well. SCFA (mainly acetate, propionate and butyrate), produced during degradation of roughages by rumen microbes, have been shown to induce hyperglycaemia, hyperinsulinaemia and insulin resistance after intravenous infusions in several studies in sheep (Ash et al., 1964; Manns and Boda, 1967; Trenkle, 1970). However, results of studies examining the effect of SCFA on glucose homeostasis are inconsistent and do not regard veal calves. Moreover, the effects of SCFA in ruminants have mostly been achieved using intravenous infusions, instead of stimulating SCFA production in the rumen by orally administering roughages. This might be a misleading indicator of the effect of roughage on glucose homeostasis, for instance because butyrate appears in blood of ruminants as β-hydroxybutyrate, which does not stimulate insulin secretion in sheep (Horino et al., 1968). In contrast to studies in sheep, SCFA in mice increase insulin sensitivity (Gao et al., 2009), which implies enhanced glucose utilization efficiency (Hugi et al., 1998; Horino et al., 1968). Overall, it is expected that additional roughage supply enhances the development of insulin resistance.

This study examined the effect of additional roughage supply on glucose homeostasis in heavy veal calves.

Materials and methods

Experimental design

To examine the effect of roughage intake on glucose metabolism, 48 Holstein-Friesian bull calves at 54.7 ± 2.1 kg BW were allocated to one of four dietary treatments (MR supplemented with 0, 9, 18 or 27 g DM solid feed/kg MW/day) after arrival at experimental accommodation "De Haar" of Wageningen University and Research Centre. Calves were on average ±7 weeks old and there was an age difference of 22 days between the oldest and the youngest calf. Within each treatment, groups of three calves were assigned to one of four time-blocks, representing different moments at which experiments took place. The blocks corrected for differences in age between the calves to achieve similar ages at the glucosuria and hyperinsulinaemic euglycaemic clamp experiments.

Diets and feeding

Calves in group A received only MR (Alpuro, Uddel, The Netherlands), whereas calves of group B, C and D received an ascending amount of solid feed. Calves were fed twice daily at 7.00 and 16.00. During the first experimental period (107.3 ± 7.5 kg BW), calves received 37.28 g DM MR/kg MW. During the second experimental period (160.9 ± 10.2 kg BW), calves received 40.66 g DM MR/kg MW. Between experimental periods, MR provision was adjusted to achieve similar BWs between treatments and blocks at the start of the experimental periods. From two weeks before the experiments until the end of the experiments, all calves received the same amount of MR to minimize block effects. MR was fed at a temperature of 40-42˚C and supplied in buckets. The ingredient and nutrient composition of the MR are shown in Table 1 and 2 respectively.

Calves of groups B, C and D received 9, 18 and 27 g DM/kg MW respectively, consisting of 25% chopped wheat straw (Gedizo Trading, Staverden, The Netherlands), 25% maize silage (Gedizo Trading, Staverden, The Netherlands) and 50% concentrate (Denkavit, Voorthuizen, The Netherlands) based on DM weight. The roughages were supplied in troughs of stainless steel directly after MR supply. The nutrient composition of the solid feed components is shown in Table 3 and the ingredient composition of the concentrate in Table 4. Nutrient compositions of the premixes in MR and concentrate are shown in Table 5.

Feed intake was registered daily by weighing roughage residuals of the previous feeding and milk residuals 30 minutes (min) after feeding.

Water was continuously available through drinking niples, attached to tuns containing bar lines to indicate the volume of water in the tun. Water supply was monitored twice daily after feeding by registering the decrease in volume.

Table 1. Analyzed ingredient composition of milk replacer

Ingredient	%
Whey protein concentrate	20.0
Delactosed whey protein	13.3
Whey	35.7
Starch	2.0
Solubilized wheat protein	4.0
Soy protein concentrate	2.5
Lard	6.0
Coconut oil	4.6
Tallow	6.0
Palm oil	2.0
Soy lecithine	1.4
Premix	0.25

Table 2. Analyzed nutrient composition of milk replacer

Nutrient	Content
Dry matter (g/kg)	967.7
Crude ash (g/kg)	76.0
Crude protein (g/kg)	212.1
Crude fat (g/kg)	192.2
Starch (g/kg)	19.4
NDF[1] (g/kg)	6.7
GE[2] (MJ/kg)	21.7

[1]NDF = neutral detergent fiber
[2]GE= gross energy
All nutrient contents measured in the dry matter fraction

Table 3: Analyzed nutrient composition of the solid feed components

Nutrient	Concentrate (g/kg)	Maize silage (g/kg)	Wheat straw (g/kg)
DM	883.1	496.7	923.4
Crude protein	127.6	76.7	21.9
Crude fat	35.7	28.3	8.7
Crude ash	66.2	36.3	66.2
Starch	449.8	356.0	11.0
GE (MJ/kg)	17.7	18.7	18.0
NDF	150.8	399.5	813.9
NSP[1]	320.7	502.7	892.2

[1]NSP = non-starch polysaccharides
All nutrient contents measured in the dry matter fraction

Table 4: Analyzed ingredient composition of concentrate

Ingredient	g/kg
Barley	300
Carob	122
Lupins, crude protein<335 g/kg	220
Maize	300
Premix	10
$MgSO_4$	5.6
NaCl	7.7
$CaCO_3$	21.5
KH_2PO_4	13.5

Table 5: Nutrient composition of premix in milk replacer and concentrate

Nutrient	Content (DM/kg concentrate)	
	Milk replacer	Concentrate
Ca (g/kg)	8,6	
P (g/kg)	6,1	
Mg (g/kg)	1,3	20
K (g/kg)	17,2	
Lysine (g/kg)	3,5	
Methionine (g/kg)	2	
Fe (ppm)	8	
Zn (mg/kg)	50	25
Cu (mg/kg)	8	15
Se (mg/kg)		0.15
I (mg/kg)		0.8
Retinol (IU/kg)	25000	4000
Calcipherol (IU/kg)	4000	500
Tocopherol (IU/kg)	149	100

Housing and catheterization

Between experimental periods, the groups of three calves were housed in group pens.

During experiments, calves were housed individually in metabolic cages of 0.87X1.85 m., attached to the fence with a halter and chain. Both individual and group housing systems contained wooden grids and fences of stainless steel. Stables were lit from 6.30 till 23.00.

Glucosuria measurements were performed during two periods. The hyperinsulinaemic euglycaemic clamp study was performed only during the second period.

Four days before the experiments, calves were adapted to individual housing and harnessed. A bag for feces collection was attached to the harness, to enable separation of urine from feces for the glucosuria measurements. Thereafter, semi-permanent catheters (Careflow, Becton Dickinson, Alphen aan den Rijn, The Netherlands) were inserted in the left and right jugular vein. To suture the catheter to the skin, Vicryl suturing (Ethicon, Sumerville, NJ) was used. The catheter was lengthened with a three-layer extension tube (Vygon, Valkenswaard, NL) of 150 cm. The tubes were secured to the harness with tape. Directly after insertion and a day before performing the meal response test, catheters were flushed with a solution of saline and heparin.

Following the adaptation period, glucosuria measurements were performed during four days. After the glucosuria measurements of the first experimental period, calves were directly

placed back into the group pens. One to three days after the glucosuria measurements of the second period, a HEC was performed. Two to four days after the HEC, calves were placed back into the group pens.

Welfare and health

The experiments were approved by the Ethical Committee. During the study, health status of the calves was analyzed daily by monitoring water- and feed intake, general behaviour and other health indicating features (nose, eyes, pulmonary sounds, feces). Calves were weighed once weekly. Body temperature was measured in case illness was suspected, and daily when calves were catheterized. Antibiotics or electrolytes were administered when necessary according to general veterinary practise.

Calves were withdrawn from the experiments when total roughage residuals exceeded 10% during the period in the respiration chambers, or 20% the first two feedings in the respiration chambers. This was also the case when at least 10% of MR provision was refused during the first two feedings or in total during the last three days in the respiration chambers. A minimal daily growth rate retardation of 30%, compared to the calf with the average lowest growth rate in the same group of three, during the last two weeks before glucosuria measurements also led to exclusion from the experiments. The final exclusion criteria were fever (body temperature > 39.5˚C) and a history of substantial medication.

Glucosuria measurements

Directly after the adaptation period, glucosuria measurements were performed (in 44 calves at 107.9 ± 1.1 and 40 calves at 163.9 ± 1.6 kg BW). Urine was collected quantitatively daily by leading it through funnels with filters into buckets containing 15 mL of 25% H_2SO_4. Afterwards, a homogenous 6 mL sample was collected and stored at -20˚C pending further analyses. Glucose concentrations in urine were analysed using the hexokinase method Glucose LiquiUV (Human, Wiesbaden, Germany).

Hyperinsulinaemic euglycaemic clamp

To measure whole body insulin sensitivity, a HEC was performed on 27 calves at 166.1 ± 1.8 kg BW. Morning feeding was omitted to achieve a steady glucose turnover rate.

At 7.00 am, a continuous infusion of [^{2}H]-glucose was initiated. From 9.30 till 10.10, blood samples were taken every 10 min to measure the basal plasma glucose level, which was the target glucose level during the clamp study.

At 10.15 am (time=0), an insulin (Actrapid 100 IE/mL, Novo Nordisk, Denmark) priming dose of 2.1 mU/kg BW/min was infused into the left catheter, followed by insulin infusion at a continuous rate of 1 mU/kg BW/min, which was maintained for four hours. The insulin infusion was adjusted to the BW, which had been measured two days before the HEC, directly after the urine collections.

At four min after starting the insulin infusion, glucose (20%, Braun, Germany) infusion started at a rate of 5.5 µmol/kg BW/min.

The plasma glucose level was measured every 5 min in the first hour and every 10 min in the remaining three hours using Precision Xtra Plus test strips in combination with the Precision Exceed Sensor (Abbott, Weesp, The Netherlands). Responding to the changes in plasma glucose level, the glucose infusion rate (GIR) was adjusted to maintain a constant, basal plasma glucose level. The posture of the calf (standing or lying) was registered every 5 min in the first hour and every 10 min in the last three hours.

Two syringe pumps (NE-1600, New Era Pump Systems, New York) were used to control the infusion rates of glucose and insulin separately.

Blood samples were taken from the right catheter every 10 min. Blood was collected in 10 mL plasma heparin tubes and directly cooled on ice. The same day, samples were centrifuged (15 min, 1000g) and plasma was collected and stored at -20˚C for later determination of insulin and glucose concentrations. Tracer data, for determining endogenous glucose production, are not included in this report.

The effect of solid feed intake was analysed on three average GIRs, namely $GIR_{60\text{-}240}$ (average GIR from 60 to 240 min), $GIR_{80\text{-}140}$ (average GIR from 80 to 140 min) and $GIR_{170\text{-}240}$ (average GIR from 170 to 240 min). The period from 0 to 60 min was considered the GIR stabilization phase. $GIR_{60\text{-}240}$ was considered the average GIR during the clamp. $GIR_{80\text{-}140}$ was chosen for its relative stability and $GIR_{170\text{-}240}$ was chosen because the GIR was on average not affected by time and because, in this stage, a stable GIR should have been reached (Hostettler-Allen et al., 1994; Hugi et al., 1998).

Slaughter and tissue collection

At 185.5 ± 2.0 kg BW the calves were culled. Thirteen calves were culled at the experimental accommodation using T61 and the remaining calves at a slaughterhouse using a captive bolt gun. Muscle (m. quadriceps femoris), liver and perirenal tissue samples were collected and stored at -20°C. One calf (treatment C) was not included in the collection of tissues, because it was culled during the study for practising tissue isolation.

Laboratory tissue analyses

Triglyceride concentration was determined in 250 mg muscle and 150 mg liver slices using an enzymatic colorimetric method (GPO-PAP). Triacylglycerols were saponified to triglycerides in a 25% KOH solution according to the method of Eggstein and Kreutz (1966) and ground using a homogenizer. Triglycerides were converted to glycerol with 2.5 mol/L $HClO_4$ and coloured using a Liquicolour triglyceride kit (Human, Wiesbaden, Germany). The glycerol concentration was spectrometrically measured at 500 nm.

Glycogen content was determined in liver and muscle samples of 1500 mg under continuous cooling on ice. For each mg of tissue, 1 µL of 7% $HClO_4$ was added, after which the tissues were ground using a homogenizer. Samples were centrifuged and the supernatant was washed with petroleum ether. After adding a colour reagent consisting of a iodine solution (0.026% I_2 and 0.26% KI_2) added to a 10% $CaCl_2$ solution (13:1000), the glucose content was measured in an ELISA plate reader at 450 nm. Hepatic bovine glycogen (Type IX, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard.

Fatty acid composition was determined in liver and muscle samples of 400 mg. Lipids were extracted using Folch's method (Folch et al., 1957). FAME content was measured by gas chromatography, using a split ratio of 1:25. Tridecanoic acid (C13:0) was used as internal standard.

Statistical analyses

Data were expressed as mean ± SEM. Values were considered significant for confidence levels exceeding 0.95. For all statistical analyses, SAS (SAS 9.1 for Windows, 2003) was used. Normality of residuals of the models were checked using the univariate procedure and if normality assumptions were not confirmed, a log transformation was applied on the data.

For glucosuria, urine excretion, water supply and feces excretion, the Mixed procedure was used with the regression model $Y_{ijkl} = \mu + roughage_i + period_j + roughage_i*period_j + block_k + e_{ijkl}$, where

μ = mean glucose level, $roughage_i$ = one of four roughage levels set as continuous variable, $period_j$ = one of two experimental periods, set as repeated measure, $roughage_i$*$period_j$ = interaction between the amount of roughage and experimental period, block = random effect of time-block and e_{ijk} = residual error. In case of significant period effects, the model $Y_{ijk}= \mu + roughage_i + block_j + e_{ijk}$ was analysed for each period with the GLM procedure.

From the HEC, three periods were determined for analyses; the average GIR during the clamp (GIR_{60-240}), the period from 80 to 140 min after initial insulin infusion (GIR_{80-140}) and the period from 170 till 240 min ($GIR_{170-240}$). The latter two periods were chosen for their relatively stable GIR. The effect of roughage on $GIR_{60-240,}$ GIR_{80-140} and $GIR_{170-240}$ was analyzed with the model $Y_{ijkl} = \mu + roughage_i + time_j + roughage_i*time_j + block_k + e_{ijkl}$ with the Mixed procedure, $time_j$ being the time points at which blood samples were taken. The effects of the total time standing and the amount of changes to another posture on GIR have been analyzed statistically with the models $Y_{ijkl}= \mu + standingtime_i + roughage_j + block_k + e_{ijkl}$ and $Y_{ijkl}= \mu + changes_i + roughage_j + block_k + e_{ijkl}$, where $standingtime_i$= time standing during the clamp (0-240 min) in min and $changes_i$= number of changes from sitting to standing or vice versa during the clamp (0-240 min).

For analyzing roughage effects on triglyceride and glycogen concentrations in liver and muscle, the model $Y_{ijk}= \mu + roughage_i + block_j + e_{ijk}$ was used. The time between the last feed intake and slaughter and the location at which the calves were slaughtered were initially added as covariables to the model. After concluding that these factors did not affect the outcomes, they were excluded from the model.

The effect of roughage on fatty acid profile in liver, muscle and perirenal fat was analyzed with the model $Y_{ijk}= \mu + roughage_i + serie_j + e_{ijk}$. Serie represented the day during which the laboratory analysis took place, therefore accounting for inconsequence during the laboratory analysis.

Pearson correlations were calculated with the Correlation procedure between urinary glucose excretion, urine excretion and water supply. Additionally, the correlation between moisture excretion in feces and urine excretion was tested, after concluding that solid feed intake reduced urine excretion. Correlations were also calculated between urinary glucose excretion, fasting glucose level, GIR_{60-240}, GIR_{80-140}, $GIR_{170-240}$, liver triglyceride concentrations, muscle triglyceride concentrations, liver glycogen concentrations and muscle glycogen concentrations. Additionally, the correlation was calculated between C16:0 (palmitic acid), which has been suggested to reduce insulin sensitivity (Warnotte et al., 1994), and GIR_{60-240}, GIR_{80-140} and $GIR_{170-240}$.

Results

Hyperinsulinaemic euglycaemic clamp

Average fasting plasma glucose levels, measured before the start of the HEC study, were 5.3±0.0, 5.7±0.1, 5.8±0.1 and 5.9±0.1 mmol/L for treatment A, B, C and D respectively. Each g DM/kg MW solid feed in the diet increased average basal glucose level with 0.0033 mmol/L ($P<0.001$).

In general, the GIR increased with time during the clamp ($P<0.001$; Figure 1). GIR was not related to fasting plasma glucose level. Solid feed intake decreased GIR_{60-240} and $GIR_{170-240}$. Table 6 shows the GIR_{60-240}, GIR_{80-160} and $GIR_{170-240}$ per solid feed intake level. Effects of the total time standing and the amount of changes to another posture on the GIRs were absent.

Table 6: Effects of solid feed intake (chopped wheat straw, maize and concentrate) on glucose infusion rate during the hyperinsulinaemic euglycaemic clamp study in veal calves.

Solid feed (g DM/kg MW)	n	GIR_{60-240} (g/min)	GIR_{80-140} (g/min)	$GIR_{170-240}$ (g/min)
0[1]	8	0.68 ± 0.02	0.56 ± 0.04	0.81 ± 0.03
9[2]	8	0.64 ± 0.02	0.53 ± 0.03	0.77 ± 0.03
18[3]	6	0.64 ± 0.04	0.56 ± 0.08	0.71 ± 0.06
27[4]	5	0.62 ± 0.02	0.52 ± 0.03	0.74 ± 0.02
Slope (per g DM/kg MW)		-0.005	-0.003	-0.007
P-value		<0.001	0.085	0.001

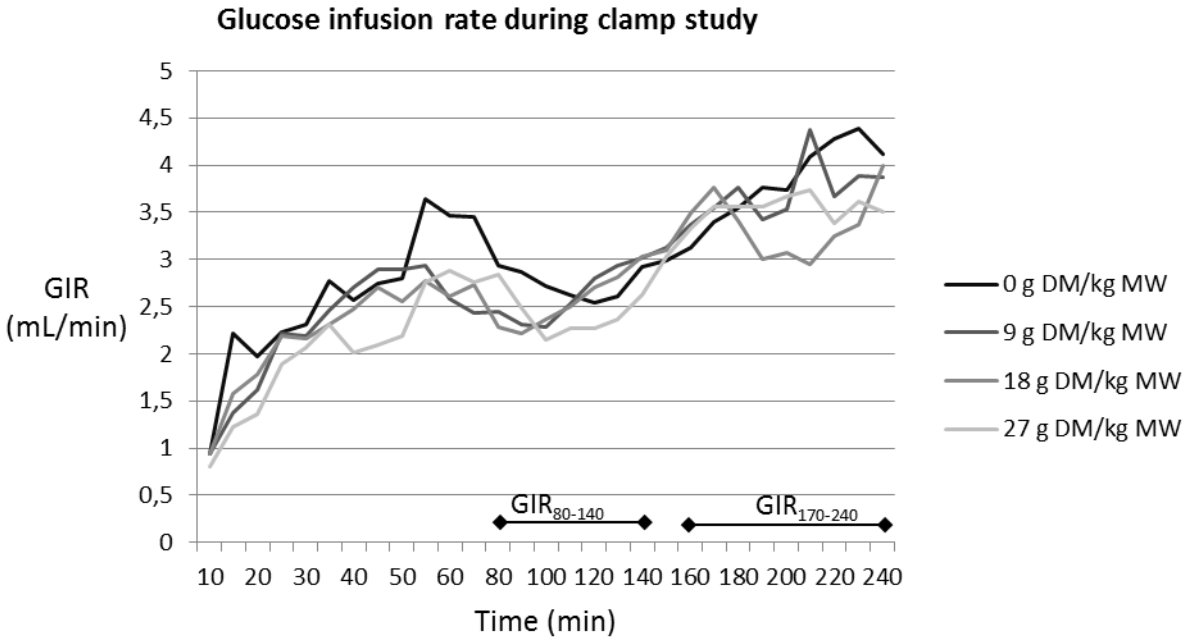

Figure 1: Effect of solid feed (chopped wheat straw, maize and concentrate) intake on glucose infusion rates during the hyperinsulinaemic euglycaemic clamp in veal calves.

Glucosuria, urine excretion, water supply and feces excretion

Solid feed intake had no effect on urinary glucose excretion. Urinary glucose excretion was higher ($P<0.05$, Table 7) in period 2 than in period 1. The volume of urine excreted was reduced by solid feed intake ($P<0.01$). Urine excretion did not differ between periods. Daily water supply was higher ($P=0.01$) in period 2 than in period 1, but was not altered by solid feed intake.

Since glucose excretion and water supply differed between periods and urinary volume differed between treatments, average values are given per treatment and period (Table 7). Treatment and period did not interact.

Pearson correlation coefficients were calculated between urine excretion, urinary glucose excretion and water supply (Table 10 Appendix). Urine excretion and water supply were positively correlated ($P<0.001$, $r=0.41$). Urinary glucose excretion was not correlated with urine excretion or water supply.

Feces production in period 1 was on average 309±31, 676±60, 1395±85 and 1806±101 g/day for calves that had received 0, 9, 18 and 27 g DM/kg MW solid feed per day, respectively. Feces production in period 2 was on average 405±67, 551±71, 1012±55 and 1337±18 g/day for calves that had received 0, 9, 18 and 27 g DM/kg MW solid feed per day. Feces production was

higher in period 2 that in period 1 P<0.001). Solid feed intake was positively correlated with feces production (r=0.91; P<0.001 in period 1, r=0.86; P<0.001 in period 2). The amount of water secreted through feces was measured in period 2 and was on average 0.36±0.07, 0.50±0.05, 0.81±0.05 and 1.18±0.10 L/day for calves that had received 0, 9, 18 and 27 g DM/kg MW solid feed per day, respectively. Solid feed intake was positively correlated with the amount of water secreted through feces per day (P<0.001, r=0.83). Urine production was negatively correlated with the total amount of water excreted in the feces per day (P<0.001, r=0.47).

Table 7: Effect of solid feed intake (chopped wheat straw, maize silage and concentrate) on urinary volume, urinary glucose excretion and water supply in veal calves

Solid feed intake (g DM/kg MW)	n	Urine excretion (L/d)	Glucose excretion (g/d)	Water supply (L/d)
Period 1 (107.9 ± 1.1 kg BW)				
0[1]	10	7.95 ± 0.45	6.22 ± 3.18	2.37 ± 0.49
9[2]	12	6.97 ± 0.21	6.35 ± 2.69	1.68 ± 0.56
18[3]	12	6.44 ± 0.24	7.26 ± 2.47	1.41 ± 0.29
27[4]	10	5.96 ± 0.25	7.91 ± 3.28	3.04 ± 0.74
P-value		<0.001	0.612	0.545
Slope (per g DM/kg MW)		-0.073	0.073	0.018
Period 2 (163.9 ± 1.6 kg BW)				
0[5]	9	8.19 ± 0.63	17.47 ± 8.49	4.94 ± 1.50
9[6]	10	7.59 ± 0.31	18.91 ± 6.21	3.23 ± 0.93
18[7]	12	6.83 ± 0.27	24.34 ± 5.22	2.96 ± 1.10
27[8]	9	6.56 ± 0.47	21.59 ± 6.43	2.85 ± 0.47
P-value		0.007	0.536	0.078
Slope (per g DM/kg MW)		-0.064	0.209	-0.095

[1]n=10, [2]n=12, [3]n=12, [4]n=10, [5]n=9, [6]n=10, [7]n=12, [8]n=9. An effect of block was found on water supply in both periods and an effect of period was found on glucose excretion and water supply (P<0.05).

Glycogen and triglyceride concentrations in liver and muscle

Solid feed intake tended to increase (P<0.10) triglyceride concentrations in muscle (Table 8). Effects of solid feed on glycogen levels were absent in both liver and muscle. Triglyceride concentrations in liver were not influenced by solid feed intake.

Liver triglyceride concentration was negatively correlated (r=-0.36; P<0.01) with liver glycogen concentration. Muscle triglyceride concentration tended to be positively correlated (r=0.19; P<0.10) with liver glycogen concentration.

Table 8: Effect of solid feed (chopped wheat straw, maize and concentrate) intake on glycogen and triglyceride concentration in liver and muscle (m. quadriceps femoris) tissue of heavy veal calves

		Liver		Muscle	
Solid feed (g DM/kg MW)	n	Glycogen (g/kg)	Triglycerides (g/kg)	Glycogen (g/kg)	Triglycerides (g/kg)
0[1]	12	44.61 ± 4.15	18.93 ± 1.32	0.55 ± 0.13	4.81 ± 0.83
9[2]	12	52.08 ± 3.34	16.75 ± 0.95	0.94 ± 0.30	6.95 ± 1.13

18^3	10	46.81 ± 4.87	19.02 ± 1.00	0.66 ± 0.36	7.00 ± 1.63
27^4	12	50.44 ± 3.63	17.64 ± 0.84	1.21 ± 0.42	6.20 ± 0.62
P-value		0.324	0.405	0.436	0.079
Slope		0.141	-0.030	1.142	1.009

Block effects were present on liver and muscle triglyceride and glycogen concentrations.

Correlations between glucosuria, insulin sensitivity and triglyceride and glycogen concentrations in liver and muscle

$GIR_{170\text{-}240}$ was negatively correlated with muscle triglyceride concentration ($P<0.01$, $r=-0.51$). $GIR_{80\text{-}140}$ was positively correlated with muscle glycogen concentration ($P<0.05$, $r=0.46$). Fasting glucose level was positively correlated with muscle triglyceride concentration ($P<0.01$, $r=0.53$). Other correlations between glucosuria, insulin sensitivity, triglyceride concentrations in liver and in muscle and glycogen concentrations in liver and in muscle were not found.

Fatty acid composition of liver, muscle and perirenal fat

Concentrations of fatty acids in liver, muscle and perirenal fat are shown in Table 11 of the Appendix. The liver mostly contained C16:0, C18:0 (stearic acid) and C18:1n9c (cis-oleic acid). The main fatty acids in the muscle were C16:0, C18:1n9c and C18:2n6c (cis-linoleic acid). Perirenal fat mostly contained C16:0, C18:0 and C18:1n9c.

The fatty acids as percentages of the lipid fraction in liver and muscle and as percentages of perirenal fat are given in Table 9. The effect of solid feed intake on the percentages of fatty acids in the lipid fraction of liver and muscle and on the percentage of fatty acids in perirenal fat tissue are also shown in Table 8. A correlation between C16:0 and insulin sensitivity was not found.

In the lipid fraction of the liver, solid feed intake decreased ($P<0.001$) the concentration of C12:0 (lauric acid), but increased ($P<0.05$) the amount of C17:0 (margaric acid), C18:0 and C20:3n6 (dihomo-gamma-linolenic acid). Total SFA, MUFA and PUFA concentrations were not influenced by solid feed intake.

In the lipid fraction of the muscle, the amount of C17:1 (heptadecenoic acid) was decreased ($P<0.05$) by solid feed intake. Solid feed intake tended to decrease ($P<0.10$) the C18:2n6c concentration, whereas solid feed intake increased ($P<0.01$) the C20:3n6 concentration. Total SFA, MUFA and PUFA concentrations were not influenced by solid feed intake.

In perirenal fat, roughage decreased the concentration of C12:0 ($P<0.05$). Roughage tended to decrease ($P<0.10$) the concentration of C14:0 (myristic acid) and C18:2n6c and the total PUFA concentration.

Table 9: The effect of solid feed (chopped wheat straw, maize and concentrate) intake on the fatty acids as percentages of the lipid fractions in liver and muscle and of perirenal fat tissue in heavy veal calves (n=46)

	Liver				Muscle				Perirenal fat			
Fatty acid	% of lipid fraction	SE	Slope	P-value	% of lipid fraction	SE	Slope	P-value	% of lipid fraction	SE	Slope	P-value
C12:0	0.051	0.012	-0.004	<0.001	0.239	0.089	-0.074	0.409	0.790	0.070	-0.138	0.037
C14:0	0.824	0.06	-0.008	0.117	2.093	0.539	0.001	0.980	3.483	0.284	-0.433	0.085
C14:1	0.01	0.005	-0.001	0.141	0.203	0.112	0.007	0.551	0.158	0.014	-0.011	0.398
C15:0	0.069	0.009	0.000	0.604	0.028	0.02	0.001	0.618	0.111	0.009	-0.005	0.536
C16:0	12.165	0.489	-0.045	0.279	26.223	2.778	-0.195	0.482	11.963	0.996	-1.319	0.130
C16:1	0.486	0.041	-0.004	0.253	1.338	0.392	0.015	0.714	0.819	0.071	-0.075	0.244
C17:0	0.546	0.027	0.006	0.026	0.15	0.05	0.001	0.917	0.273	0.024	-0.009	0.687
C17:1	0.066	0.011	0.000	0.647	0.761	0.123	-0.021	0.020	0.113	0.010	-0.011	0.235
C18:0	21.233	0.838	0.152	0.048	14.445	1.442	-0.136	0.331	8.092	0.678	-0.240	0.670
C18:1n9t	0.055	0.009	0.000	0.706	0.016	0.016	0.001	0.664	0.080	0.021	-0.006	0.742
C18:1n9c	9.098	0.391	-0.009	0.803	20.854	3.509	-0.019	0.957	12.695	1.220	-0.854	0.497
C18:2n6t	0.062	0.011	0.001	0.206	0.006	0.006	0.000	0.664	0.026	0.003	-0.004	0.114
C18:2n6c	17.861	0.758	-0.090	0.157	30.445	2.645	-0.432	0.089	3.245	0.286	-0.434	0.092
C20:0	0.186	0.027	0.001	0.149	0.061	0.016	ND	ND	0.061	0.012	-0.003	0.533
C20:1	0.035	0.005	0.000	0.962	0.005	0.005	-0.001	0.481	0.064	0.006	-0.004	0.472
C18:3n3	0.826	0.057	-0.006	0.166	0.692	0.089	-0.007	0.400	0.305	0.027	-0.039	0.130
C20:2	0.43	0.02	-0.001	0.719	0.255	0.029	-0.004	0.115	0.035	0.003	-0.005	0.136
C22:0	0.025	0.007	0.001	0.180	0.051	0.019	0.002	0.252	0.019	0.012	0.018	0.152
C20:3n6	2.171	0.166	0.054	<0.001	1.501	0.126	0.001	0.002	0.008	0.001	0.001	0.404
SFA[1]	34.949	1.304	0.102	0.381	43.233	4.773	-0.333	0.483	24.795	2.037	-2.129	0.225
MUFA[2]	9.714	0.434	-0.012	0.740	23.172	4.067	-0.018	0.966	13.864	1.277	-0.958	0.466
PUFA[3]	21.350	0.899	-0.042	0.584	32.900	2.852	-0.450	0.101	3.619	0.317	-0.480	0.096

[1]Saturated fatty acids, [2]Mono-unsaturated fatty acids, [3]Poly-unsaturated fatty acids.

N=12, 12, 10 and 12 for the 0, 9, 18 and 27 g DM/kg MW solid feed receiving groups, respectively.

Discussion

Insulin sensitivity

Solid feed intake was expected to reduce insulin sensitivity.

In general, GIR increased with time. This has also been shown in other studies (Hostettler-Allen et al., 1993; Hostettler-Allen et al., 1994; Hugi et al., 1998). The rise in GIR may partly be caused by incomplete insulin-induced inhibition of gluconeogenesis responding to the steady-state insulin infusion, as demonstrated by Hostettler-Allen et al. (1993).

Between 80 and 140 min GIR was stable, which was also the case in the study of Hostettler-Allen et al. (1994). The stability in GIR results from a steady state between glucose entering and being cleared from the blood, and therefore a more predictable GIR. Also, the finetuning of the GIR improved. The stability of the GIR cannot be ascribed to the posture and activity of the veal calves.

After 140 min the GIR rose again. Since this moment coincided with the afternoon, calves may have experienced more stress because of the notice that the afternoon feeding came near, which could have increased glucose requirements.

From 170 to 240 min, GIR was again relatively stable and comparable to GIRs of other studies (Hostettler-Allen et al., 1993; Hostettler-Allen et al., 1994). Average GIR, $GIR_{60\text{-}240}$, was measured from 60 to 240 min, since previous studies have stated that after 60 min a stability in GIR should be observed. $GIR_{60\text{-}240}$ was comparable to GIRs found in other studies on veal calves (Hostettler-Allen et al., 1993; Hostettler-Allen et al., 1994; Hugi et al., 1998). These studies concluded that insulin resistance had developed. Hostettler-Allen et al. (1994) considered the only 82% suppression of hepatic glucose production as a response to the hyperinsulinaemic steady-state to represent insulin resistance.

Solid feed intake decreased $GIR_{60\text{-}240}$ and $GIR_{170\text{-}240}$ and tended to decrease $GIR_{80\text{-}140}$. This corresponds with previous studies, in which infusions of propionate and butyrate have shown to induce hyperglycaemia and hyperinsulinaemia in ruminants (Ambo et al., 1973; Ash et al., 1964; Manns et al., 1967; Sano et al., 1995a). This might induce insulin resistance and therefore reduce the ability of mainly muscle, adipose tissue and liver to clear glucose from the blood (Abdul-Ghani et al., 2008; Aronoff et al., 2004; Groop et al., 1989). Besides, it has been suggested that SCFA stimulate glucagon secretion directly at the pancreas, and therefore enhance glycogenolysis and gluconeogenesis from precursors of glucose (Phillips and Black, 1966; Phillips et al., 1969). This causes hyperglycaemia which may lead to hyperinsulinaemia and insulin resistance.

Solid feed intake indeed induced insulin resistance in our study, probably through SCFA production in the rumen.

Fasting plasma glucose levels

Solid feed was hypothesized to elevate fasting plasma glucose levels through reduction of insulin sensitivity by SCFA.

Average fasting plasma glucose levels were comparable to levels found in other studies on veal calves (Gerrits et al., 2007; Hostettler-Allen et al., 1994; Kaufhold et al., 2000; Vicari et al., 2008a, 2008b). Doppenberg and Palmquist (1991) and Palmquist et al. (1992) found higher fasting plasma glucose concentrations in veal calves fed milk replacer diets (6.3 and 6.2 mmol/L at already 8 weeks of age (Doppenberg and Palmquist, 1991) and 8.2 and 7.7 at 150 kg BW (Palmquist et al., 1992), for 10 or 18% fat diets, respectively). This may be caused by the severity of the insulin resistance these calves had developed. Possibly, the relatively low fat content of the diets in that study led to an increase in feed intake and therefore increased the lactose load, reduced insulin sensitivity and enhanced the plasma glucose response. Besides, feeding levels were relatively low in our study (on average 1,401 kg DM MR/day during experimental period 2). Also, the blood

samples were taken four hours after feeding and considered fasting levels, whereas four hours after feeding plasma glucose levels in veal calves have often not returned to fasting values yet (Hugi et al., 1997a; Vicari et al., 2008a).

Solid feed intake increased fasting plasma glucose levels, probably by reduced insulin sensitivity as described in the previous paragraph.

Some studies (Donkin and Armentano, 1995; Hugi et al., 1997a), but not all (Hostettler-Allen et al., 1994), found an increase in fasting glucose levels with age in veal calves, whereas plasma glucose levels decline with age in calves on dry diets (Doppenberg and Palmquist, 1991). The age effect on fasting glucose concentration was not examined in our study, although it is expected that fasting plasma glucose levels would have been lower when experiments were done in younger calves. Plasma glucose levels were analyzed at an average BW of 166.1 ± 10.7 kg. Hugi et al. (1997a) found similar plasma glucose levels at this weight (5.2 and 5.4 mmol/L for low and high lactose intakes), whereas at lower body weights (76-109 kg for low lactose intake and 74-109 kg for high lactose intake) lower glucose levels were found (4.3 and 4.4 mmol/L). However, Palmquist et al. (1992) found high basal glucose levels (7.3 mmol/L for 10% fat diet, 8.1 mmol/L for 18% fat diet) already at 85.2 and 92.8 kg BW. These calves were already intensively fed from three days of age onwards, which might have accelerated the development of insulin resistance.

Water supply, urine excretion and glucosuria

In veal calves, not many studies have measured water supply and urine production, but glucosuria has been well documented (Gerrits et al., 2007; Hostettler-Allen et al., 1994; Hugi et al., 1997a; Stanley et al., 2002; Vicari et al., 2008a, 2008b). Solid feed intake was expected to increase urinary glucose secretion and water supply. Urinary glucose secretion, water supply and urine were hypothesized to be higher in period 2 compared to period 1.

Water supply

Daily water supply was higher in period 2 than in period 1. Water supply rises with age (Andersson and Lindgren, 1987; Praktijkonderzoek Veehouderij, 2002). Solid feed intake tended to decrease water supply in the second period. Dry matter intake increased water consumption in goats and ewes (Andersson and Lindgren, 1987; Forbes, 1968) due to the water binding capacity. Apparently the MR provided in the water requirements.

Urine excretion

Urine excretion did not differ between periods. The increase of water supply with age was expected to increase urine excretion. The increased concentrate intake at higher ages could have increased water requirements, causing the water supply to rise and the urine excretion to be maintained.

Solid feed intake negatively affected urine excretion. Calves receiving solid feed produced more feces and although the feces had a higher DM content as calves received more roughages, more water was excreted via feces instead of via urine for solid feed receiving calves in our study.

Glucosuria

Urinary glucose excretion was expected to be increased by solid feed intake and to be higher in period 2 than in period 1.

Urinary glucose excretion was higher in period 2 than in period 1, since glucosuria develops with age (Hugi et al., 1997a).

In the first experimental period, our study showed higher urinary glucose concentrations than the study of Hugi et al. (0.11 g/L at 109 kg BW) (1997a). The control group had a relatively low lactose intake (29% of MR), which might have been beneficial for glucose homeostasis. Labussiere et al.

(2008) found slightly higher daily glucose excretions already at 71 kg BW (8 g/d). In that study, crude protein and energy levels were on average higher than in our study (no roughages were provided). High energy and lactose levels have been shown to induce hyperglycaemia in veal calves (Hugi et al., 1997a; Hugi et al., 1997b) which leads to glucosuria. Protein intake has been shown to decrease plasma glucose levels in veal calves (Gerrits et al., 2007), whereas in monogastrics protein intake had the opposite effect (Felig, 1975; Felig et al., 1969; Felig et al., 1974; Krebs et al., 2002).

Glucosuria levels found in the study of Hostettler-Allen et al. (1994) were probably comparable to levels found during the second period in our study (0.12 g/L before and 4.7 g/L after feed intake at 148 kg BW)(1994), although this study did not measure urinary glucose over a three-day period but only before and after feed intake. Vicari et al. (2008b) found higher glucosuria levels (200 mmol/d at on average >148 kg BW for calves receiving 50% of daily protein and 50% of daily lactose intake per meal). Labussiere et al. (Labussiere et al., 2008) found urinary glucose excretion levels of 78 g/d at 136 kg BW and 125 g/d at 212 kg BW (averaged over different protein intake levels). This difference might be caused by the relatively low feeding levels in our study (±1,401 compared to ±2,300 (Labussiere et al., 2008) g DM MR/day at BW comparable to the BW during the second experimental period in our study). Hugi et al. (1997a) found lower (5.7 mmol/L at 182 kg BW) glucose excretions than we did in the second experimental period (11.9 mmol/L for groups receiving solely MR). The difference with the non-solid feed receiving groups in our study, however, was only slight. Therefore it is expected that supplying solid feed compared to not supplying solid feed might increase glucosuria levels due to reduced insulin sensitivity or due to concentrate supplementation.

However, statistical effects of solid feed intake on glucosuria were absent. Solid feed intake did increase basal plasma glucose levels and therefore glucosuria might seem a logical consequence. Hyperglycaemia is known to increase urinary glucose excretion when the renal threshold for glucose uptake is exceeded, which is the case at plasma glucose levels of >1.5 g/L (8.3 mmol/L) (Hostettler-Allen et al., 1994; Hugi et al., 1997b). However, the fasting glucose levels did not exceed this level. The postprandial glucose responses, which probably mainly determine the amount of glucose excreted (Hugi et al., 1997a), remain unknown.

Concluding, calves in our study showed glucosuria indicating reduced glucose homeostasis, but a linear relation with solid feed intake was absent.

Correlations between water supply, urine excretion and glucosuria

Correlations between water supply, urine excretion and urinary glucose excretion were expected.

Glucose excretion was not correlated with water supply or urine excretion. In humans, hyperglycaemia may lead to glucosuria and an elevated water intake and urine excretion (Arima and Oiso, 2010; Baylis and Cheetham, 1998). Compared to the study of Hugi et al. (1997a), calves in our study produced more urine. This might have diluted the glucose concentration in the urine. Regulated by the hypothalamus, water intake in humans increases as a result of urine excretion, which was also found in our study. In hyperglycaemic humans, water intake increases as an attempt to dilute the blood to compensate for the high plasma glucose levels (Baylis and Cheetham, 1998), which also may have been the case in veal calves of our study. As described previously, water intake increased with age in our study, whereas the increase in urine excretion from period 1 to period 2 was not significant. The positive correlation between both suggests that urine excretion rises slightly with age, but less than water intake.

Arginine vasopressin deficiency, or a lack of kidney response to this hormone, often occurs in insulin resistant humans. This reduces renal reabsorption of solute-free water and therefore further increases urine production (Arima and Oiso, 2010; Baylis and Cheetham, 1998). In our study, insulin sensitivity, as described in the next paragraph, was not related with urine excretion. Therefore, this cannot explain the amounts of urine excreted.

Triglyceride concentrations in muscle and liver

Solid feed intake, insulin resistance and fasting plasma glucose levels were expected to increase triglyceride concentrations in muscle and liver.

Muscle triglyceride concentrations were quite similar to concentrations measured in other studies. Marounek et al. (2008) found an average triglyceride concentration of 5.2 g/kg in the muscle (m. longissimus dorsi) of veal calves. Fernandez et al. (1996) found triglyceride levels of 8 g/kg in the muscle (m. longissimus lumborum). Fat deposition may vary slightly between different muscles in the pig (D'Souza et al., 2004), which is also expected to be the case in veal calves.

Solid feed intake tended to increase muscle triglyceride concentrations, probably because of the higher energy level and/or acetate production in the rumen. To achieve comparable growth rates between treatments during the inter-experimental periods, solid feed intake was compensated by a reduced MR intake. This might have compensated for the increased energy intake during the experiments, causing the effect of solid feed on triglyceride concentrations not to be clearly significant.

Liver triglyceride concentrations were lower than in the study of Gerrits et al. (2007), who reported liver triglyceride concentrations of 25.5 and 32.7 g/kg in veal calves of 160 and 240 kg BW, respectively. Gerrits et al. (2007) mentioned that protein intake reduced liver fat content. Since the protein intake level was comparable to protein intake in our study, this factor probably has not contributed to the lower fat levels in the liver. Energy levels were on average possibly higher in our study (on average 43 MJ gross energy per day in our study compared to 663 and 851 kJ/kg MW/d digestible protein free energy intake at 120 kg BW and 564 and 752 kJ/kg MW/d at 200 kg BW), which was expected to increase energy deposition in the form of fat.

Solid feed intake did not alter triglyceride concentrations in the liver. The reason for the lack of effects of solid feed intake on the muscle triglyceride content mentioned above, may also cause the absence of effects of solid feed intake on the triglyceride content in the liver.

$GIR_{170\text{-}240}$ was negatively correlated with muscle triglyceride concentrations, meaning insulin resistance increases triglyceride concentrations in the muscle. In humans, insulin resistant muscle and adipose tissue fail to take up glucose after feed intake, which causes the glucose to pass on to the less insulin dependent and more insulin sensitive liver, in which it is converted into mostly FFA or free cholesterol, increasing the liver triglyceride pool. FFA are secreted into the blood and taken up by the muscle, increasing also the muscle triglyceride pool (Ballard, 1965; Zivkovic et al., 2007). A correlation between insulin sensitivity and triglyceride concentration in liver was not found. Because in humans the liver is a more important fat store than in ruminants (Van Soest, 1982), it might have been incorrect to expect an effect of insulin sensitivity on liver triglyceride concentration based on studies in humans.

Fasting plasma glucose level was positively correlated with muscle triglyceride concentration (probably through the development of insulin resistance), but not with liver triglyceride concentration.

A correlation between the triglyceride content of liver and muscle was not present. However, at a higher dietary fat content this correlation might have evolved. The liver would have possibly been more saturated with lipids and as a result more FFAs would have been excreted by the liver and taken up by the muscle. Shunting of FFA from liver to muscle has been demonstrated in insulin resistant humans (Zivkovic et al., 2007).

Concluding, solid feed intake and insulin resistance increased triglyceride concentration in muscle as expected.

Glycogen concentrations in muscle and liver

Glycogen concentrations in liver and muscle were expected to be enhanced by solid feed intake (because of the starch in concentrate) and reduced by insulin resistance and, through development of insulin resistance, by fasting plasma glucose levels. These effects could compensate in insulin resistant, solid feed receiving calves.

Muscle glycogen concentrations were lower than found by Dreiling et al. (1987) in the longissimus dorsi (back) muscle (2.98 g/kg in fresh meat and 3.21 g/kg after storage at -20°C) and sternomandibularis (neck) muscle (6.64 g/kg in fresh meat and 2.28 g/kg after storage at -20°C) of bulls and bull calves. Unfortunately, details on the diet were not included. Besides, the animals studied by Dreiling et al. (1987) might have been generally older than the calves in our study. Therefore glycogen stores possibly were depleted less quickly than in our study, since relatively less energy was required for growth. The time between the last meal and slaughter was expected to be negatively associated with the glycogen content. This effect was not significant in our study. Also, even though tissues were cooled, glycogen may have been broken down during the laboratory analyses. The effect of the time tissues were stored frozen on glycogen content varies between muscles. Dreiling et al. (1987) found clear effects on the m. sternomandibularis, but not on the m. longissimus dorsi.

Glycogen concentration in liver was higher and glycogen concentration in muscle was lower in our study than in the study of Garssen et al. (1995), who found glycogen concentrations in liver and muscle (m. longissimus thoracis) of 31.0 and 8.4 g/kg, respectively. Due to the lack of information on the diet applied, comparison with this study is dubious.

Effects of solid feed on glycogen levels were absent in both liver and muscle. Calves received the same amount of milk replacer and therefore the additional starch in the solid feed was probably a minor contributor to glycogen levels. Also, glycogen concentrations are, according to Van Den Borne et al. (2007a), not much influenced by glucose/lactose intake. In the study of Van den Borne et al. (2007a), calves were assigned to one of two feeding levels (1.5 and 2.5 times metabolizable energy requirements for maintenance) and one of three feeding frequencies (1, 2 or 4 times a day). Unregardless of feeding level and feeding frequency, ingested glucose/lactose was mainly oxidized, rather than stored into glycogen or fat.

Although studies on glycogen concentrations in muscle and especially liver of veal calves are scarce, it seems in our study a shift in glycogen storage from muscle to liver might have taken place. Possibly, muscles were already somewhat insulin resistant, whereas the liver is less insulin dependent and more insulin sensitive than muscle tissue (Campbell et al., 1988; DeFronzo et al., 1983; Kolterman et al., 1980; Rizza et al., 1981). Therefore glucose uptake and glycogen storage are more likely to occur in the liver. Besides, the inhibitory effect of insulin on glycogenolysis might persue in the liver, while this inhibitory effect of insulin is impaired in insulin resistant muscle. Also, more glucose in the blood might be shunted to the liver when the muscles are more insulin resistant, instead of entering the muscles.

$GIR_{80\text{-}140}$ was positively correlated with muscle glycogen concentrations. This probably results from failed inhibition of glycogenolysis and reduced glycogen uptake in insulin resistant muscle and liver.

Correlation between fasting plasma glucose levels and glycogen concentrations in liver or muscle were absent. Although elevated fasting glucose levels and insulin sensitivity are often associated with each other (Hostettler-Allen et al., 1994; Hugi et al., 1997b), insulin resistance does not necessarily lead to elevated basal glucose levels (Hugi et al., 1997a). Also, these features may develop consecutively. Besides, while insulin resistance and glucosuria develop with age, fasting glucose levels are not always increased with age (Hugi et al., 1997a).

In our study, glycogen concentrations were generally more affected by insulin sensitivity than by solid feed intake.

Correlations between triglyceride and glycogen levels in liver and muscle

In insulin sensitive veal calves, triglyceride concentrations were expected to be positively correlated with glycogen concentrations, but in insulin resistant veal calves these were expected to be negatively correlated.

Muscle triglyceride concentration tended to be positively correlated with liver glycogen concentration. Stored fat might have been positively correlated with glycogen formation as a result of leptin production by adipocytes. Adding leptin to isolated rat hepatocytes has been shown to increase glucose incorporation into glycogen (Aiston and Agius, 1999). Also the ration might have been sufficient to stimulate triglyceride storage and provide enough energy for oxidation to spare glycogen stores.

Liver triglyceride concentration was negatively correlated with liver glycogen concentration. Insulin resistance was present in the veal calves and may be characterized by failed inhibition of glycogenolysis by insulin, leading to reduced glycogen stores. The lipid content of liver and muscle increases in insulin resistant humans (Ballard, 1965; Zivkovic et al., 2007), as described earlier. The positive effect of insulin resistance on glycogenolysis and lipid accumulation might explain the negative correlation between liver triglyceride and liver glycogen concentrations. Also, competition between glucose and FFA oxidation might favour either glycogen or lipid storage, leading to a negative correlation between both. The underlying mechanism may be explained by the glucose fatty acid cycle, as described by Randle et al. (1963). This cycle suggests maintenance of a constant plasma glucose level independently of regulatory hormones, although insulin does modify the functioning of this cycle by its lowering effect on plasma glucose levels. A glucose load stimulates glucose oxidation and storage of both glucose and fatty acids, but inhibits FFA oxidation. Conversely, a FFA load stimulates FFA oxidation, FFA storage and glucose storage, but inhibits glucose oxidation, which increases intracellular glucose concentrations and reduces glucose uptake from plasma.

It is suggested that in our study, correlations between muscle triglyceride and glycogen concentrations were mostly a result of feed intake and correlations between liver triglyceride and glycogen concentrations of insulin sensitivity.

Fatty acid composition of liver, muscle and perirenal fat

Fatty acid composition of solid feed intake was expected to be reflected in the fatty acid composition of liver, muscle and perirenal fat.

The liver and perirenal fat mostly contained C16:0 (12.17 and 11.96%, respectively), C18:0 (21.23 and 8.09%, respectively) and C18:1n9c (9.10 and 12.70%, respectively). Kelley et al. (2004) found that C18:1n9 had the highest concentration in mouse liver (46.6%), whereas C18:0 formed a relatively low part of the total fatty acid fraction (2.3%). Diet, as well as the difference between monogastric and ruminating animals, are considered factors inducing this difference. In monogastrics, fatty acids obtained from the diet are mostly readily deposited into fat depots. In ruminants, rumen microbes hydrogenate and dehydrogenate fatty acids which results in the production of stearic acid (Doreau and Chilliard, 1997; Hoflund et al., 1956). Also, ruminants ingest more roughages, which lead to C18:0 and C18:1 production from acetate (McDonald et al., 2002; Rule et al., 1976). Because the concentration of internal standard was elevated during FAME analysis on perirenal fat, obtained values may slightly differ from reality. However, this is not expected since the elevation was constant among samples.

The main fatty acids in the muscle were C16:0, C18:1n9c and C18:2n6c. Other studies also found C16:0 and C18:1n9c to be main fatty acids (Enser et al., 1996; Xiccato et al., 2002). Concentrations of C16:0 were comparable to those found in muscle (m. longissimus) tissue of milk-fed calves (23.2%) and calves receiving maize supplementation (23.8%) of 30 g/calf/day in week 1 to 550 g/calf/day from week 14 in the study of Xiccato et al. (2002) and to those found in beef (m.

longissimus dorsi; 25.0%) in the study of Enser et al. (1996). Xiccato et al. (2002) found 37.8% and 38.6% C18:1n9 (incl. trans-form), and 10.3% and 9.4% C18:2n6 (incl. trans-form) in milk-fed and maize-supplement fed veal calves, respectively.

Compared to an average MR fatty acid profile (CVB, 1989)(11% C12:0, 6% C14:0, 23% C16:0, 2% C16:1, 12% C18:0, 32% C18:1, 6% C18:2, 0.7% C18:3), liver tissue in our study contained higher C18:0, C18:2 and C18:3 and lower C12:0, C14:0, C16:0, C16:1 and C18:1 concentrations than MR, muscle tissue higher C16:0, C18:0 and C18:2, lower C12:0, C14:0, C16:1, C18:1 and comparable C18:3 concentrations and fatty acid concentrations (excl. fatty acids with a retention time above that of C18:3) in perirenal fat were all lower. The increase in C18:0 in liver and muscle suggests de novo fat syntheses from acetate (Rule et al., 1976).

The lower C18:1n9 and higher C18:2n6 concentration in muscle tissue in our study compared to the study of Xiccato et al. (Xiccato et al., 2002) may be explained by a lower MR intake and higher roughage intake, since MR as described by CVB (1989) contains a high percentage of C18:1n9 and a low percentage of C18:2n6.

Solid feed intake decreased the amount of C12:0 acid and increased the amount of C17:0, C18:0 and C20:3n6 in the liver triglyceride fraction. In the muscle, solid feed intake decreased C17:1 concentrations and tended to decrease C18:2n6c concentrations. Solid feed intake tended to increase C20:3n6 concentrations in the muscle. In perirenal fat, solid feed decreased the concentration of C12:0 and tended to decrease the concentration of C14:0, C18:2n6c and total PUFA concentration.

Xiccato et al. (2002) found an increase in C16:0 with increasing maize supplementation in veal calves. C18:2, C18:3, C20:4 n-6, C20:5 (EPA) and total PUFAs were significantly decreased with increasing maize intake, possibly due to competition with milk replacer for fat deposition.

That study, as well as the study of Enser et al. (1996) found higher concentrations of C18:0 than C18:2n6c. In the study of Enser et al. (1996), details are not given on the diets the animals from which beef was gathered had received, but beef cattle normally receive more roughages than veal calves. This can explain the higher C18:0-C18:2n6 ratio. C18:2n6 cannot be synthesized from acetate and is taken up with the feed (Rule et al., 1976).

The calves in the study of Xiccato et al. (2002) receiving maize were expected to have a less developed rumen than the calves in our study, which received roughages as well as concentrates. Microbial interference with fatty acids (Doreau and Chilliard, 1997; Hoflund et al., 1956) in the rumens of the calves in our study, together with a possibly higher competition of solid feed with milk replacer for fat deposition, might have caused the higher C18:2n6c-C18:0 ratio, since this fatty acid cannot be synthesized de novo (Rule et al., 1976). Also, more de novo fat synthesis might have taken place, leading to higher C18:0 synthesis (Rule et al., 1976). It should be taken into account that fatty acid profiles in different muscles may vary and that the fatty acid profile in the concentrate was unknown.

Some fatty acids have been shown to alter insulin sensitivity. Elks (1994) found that C20:4n6 (arachidonic acid) stimulates glucose induced insulin secretion (Metz, 1988). The relation between C20:4n6 and insulin sensitivity would have been interesting to examine. However, fatty acids with a longer retention time than C20:3n6 could not be identified in our study. C16:0 has been demonstrated to stimulate insulin secretion in rat pancreatic cells (Warnotte et al., 1994) and was therefore expected to do the same in veal calves. C16:0 was not related to insulin sensitivity in our study. A stimulation of insulin secretion could have been present, but this does not necessarily mean that insulin resistance will develop.

Concluding, solid feed intake alters fatty acid composition in liver, muscle and perirenal fat, predominantly by increasing C18:0 concentrations.

Conclusions

Solid feed intake reduced glucose homeostasis; it elevated fasting glucose levels and insulin sensitivity. Glucosuria was found in all calves, although effects of solid feed intake on glucosuria were not detected. Solid feed intake did not influence glycogen storage in liver and muscle. Roughage intake tended to increase the triglyceride concentration in muscle, but the triglyceride concentration in the liver was unaffected.

Fatty acid composition in the lipid fraction of liver and muscle and in perirenal fat was altered by roughage intake, mainly by elevated C18:0 concentrations.

Concluding, solid feed intake negatively influenced glucose homeostasis and altered fatty acid profile in liver, muscle and perirenal fat.

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Appendix

Table 10: Correlations between urinary volume, glucose excretion and water supply

Variable	Urinary volume (L/d)	Glucose excretion (g/d)	Water supply (L/d)
Period 1			
Water supply			
P-value	<0.0001	0.397	
Slope	0.410	-0.742	
Urinary volume			
P-value		0.259	<0.0001
Slope		-1.753	1.194
Glucose excretion			
P-value	0.259		0.409
Slope	-0.019		-0.026
Period 2			
Water supply			
P-value	0.130	0.130	
Slope	0.131	-1.840	
Urinary volume			
P-value		0.133	0.130
Slope		3.788	0.609
Glucose excretion			
P-value	0.133		0.124
Slope	0.017		-0.04

Relations are corrected for roughage and block effects. Effects are calculated by setting all variables as dependent (above) as well as independent (left) variables, since it is assumed that all factors may be causal as well as consequential.

Table 11: Fatty acid concentrations in liver and muscle (m. quadriceps femoris) of heavy veal calves

	Liver		Muscle		Perirenal fat	
Fatty acid	(g/kg)	SE	(g/kg)	SE	(g/kg)	SE
C12:0	0.010	0.002	0.012	0.005	7.903	0.703
C14:0	0.148	0.012	0.112	0.033	34.832	2.838
C14:1	0.002	0.001	0.012	0.007	1.582	0.140
C15:0	0.012	0.002	0.002	0.001	1.109	0.094
C16:0	2.147	0.089	1.319	0.140	119.634	9.962
C16:1	0.088	0.008	0.074	0.025	8.189	0.713
C17:0	0.095	0.004	0.008	0.003	2.728	0.240
C17:1	0.012	0.002	0.036	0.005	1.128	0.098
C18:0	3.691	0.106	0.711	0.058	80.921	6.779
C18:1n9t	0.010	0.002	0.001	0.001	0.799	0.212
C18:1n9c	1.606	0.073	1.081	0.209	126.951	12.199
C18:2n6t	0.011	0.002	0.000	0.000	0.264	0.026
C18:2n6c	3.134	0.119	1.474	0.056	32.449	2.862
C20:1	0.031	0.004	0.003	0.000	0.605	0.116
C20:0	0.006	0.001	ND	ND	0.643	0.060
C18:3n3	0.146	0.010	0.034	0.004	3.047	0.269
C20:2	0.076	0.003	0.013	0.001	0.352	0.034
C22:0	0.004	0.001	0.002	0.001	0.191	0.123
C20:3n6	0.372	0.026	0.073	0.003	0.078	0.013

ND= not detected