Displaced Abomasum and Ketosis in Dairy Cows

Blood Profiles and Risk Factors

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Abstract

High producing dairy cows struggle to meet energy demands and handle various transitional changes in late gestation and early lactation. Negative energy balance in early lactation is inevitable and metabolic disorders may follow as a consequence of a deep negative energy balance. This thesis studies associations between blood profiles and body condition score (BCS) in dairy cows, and displaced abomasum (DA) or clinical ketosis, and investigates risk factors for the two diseases at the herd level.

In study I, blood profiles of cows with and without DA were compared. In studies II and III, blood of cows was sampled to investigate how blood profiles differed among herds with a high incidence of DA or clinical ketosis, and between cows in high-incidence and low-incidence herds, respectively. In study IV, associations between high or low incidence and factors related to housing, feeding, management and milk yield were studied to identify herd-level risk factors for DA and ketosis. The blood profiles included parameters with which to assess energy metabolism, hepatic cell damage, inflammation, and a metabolic index (RQUICKI) used in humans to assess insulin sensitivity.

The cows with DA displayed blood profiles indicating a severely altered energy metabolism (NEFA, BHB, insulin, cholesterol, RQUICKI), liver cell damage (AST, GD) and inflammatory responses (haptoglobin). At the herd level, energy markers (NEFA, insulin, glucose, cholesterol, RQUICKI) indicated altered metabolism in cows in high-incidence herds compared with cows in low-incidence herds. The markers of liver cell damage and inflammation were not different between high- and low-incidence herds. Among high-incidence herds, BCS and change in BCS, and one metabolic marker (NEFA) were found most useful to pinpoint herd problems. Large herd size, high individual milk production level, keeping all dry cows in one group, and not cleaning the feeding platform daily, were found to be risk factors for a high incidence of DA or ketosis at the herd level.

In conclusion, the studies confirm a difference in blood profiles between cows with DA and healthy herd mates as well as a difference at the herd level between cows in herds with high versus low incidence of DA and clinical ketosis.

Keywords: blood profile, dairy cow, displaced abomasum, hepatic lipidosis, herd health, ketosis, metabolic index, risk factor, RQUICKI

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Dedication

To Alice

Just a spoonful of sugar helps the medicine go down
In a most delightful way
Mary Poppins (Richard M. Sherman and Robert B. Sherman)

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Stengärde L, Holtenius K, Tråvén M, Hultgren J, Niskanen R, Emanuelson U (2010). Blood profiles in dairy cows with displaced abomasum. *Journal of Dairy Science* 93(10), 4691–4699.

II Stengärde L, Tråvén M, Emanuelson U, Holtenius K, Hultgren J, Niskanen R (2008). Metabolic profiles around calving in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. *Acta Veterinaria Scandinavica* 50:31.

III Stengärde L, Holtenius K, Emanuelson U, Hultgren J, Niskanen R, Tråvén M (2010). Blood parameters in Swedish dairy herds with high or low incidence of displaced abomasum or ketosis. (Accepted for publication in the Veterinary Journal).

IV Stengärde L, Hultgren J, Tråvén M, Holtenius K, Emanuelson U (2010) Risk factors for displaced abomasum or ketosis in Swedish dairy herds (Submitted manuscript).

Abbreviations

Ap	Antepartum
AST	Aspartate aminotransferase
AUC	Area under the curve (Receiver operator characteristics)
BCS	Body condition score
BHB	Beta-hydroxybutyrate
CI	Confidence interval
DA	Displaced abomasum
DMI	Dry matter intake
ECM	Energy-corrected milk
GD	Glutamate dehydrogenase
NADRS	Swedish national animal disease recording system
NEFA	Non-esterified fatty acids
NEB	Negative energy balance
OR	Odds ratio
Pp	Postpartum
ROC	Receiver operator characteristics
RQUICKI	*Revised* quantitative insulin sensitivity check index
SH	Swedish Holstein
SOMRS	Swedish official milk recording scheme
SR	Swedish Red
TAG	Triacylglycerol
TMR	Total mixed ration
VLDL	Very-low-density lipoprotein

1 Background

1.1 The cow

Since the early days of domestication, the great aurochs (*Bos primigenius*) have been gradually genetically transformed into today's domestic cattle (*Bos taurus*), through selecting those individuals that served people the best (Björnhag et al., 1989). During this genetic transformation, cattle have been diversified into more specialized breeds suitable for various conditions and for producing milk, meat, or both. These genetic changes have pushed the limits of the cow's metabolism, nutritional requirements and milk production. Along with genetic changes, changes in housing, feeding, and management have made it possible to reach today's high production levels.

1.1.1 Changes in the transition period

The period from three weeks before to three weeks after calving is considered the transition period (Quiroz-Rocha *et al.*, 2009; Drackley, 1999; Grummer, 1995). During this time, the cow leaves pregnancy and enters lactation, undergoing numerous physiological adaptations in the process. Other changes, in housing, feeding, and management, also take place at this time, and may add to the stress from physiological changes experienced by the high-producing fresh cow.

The maximum growth-rates of the foetus and the mammary tissue coincide in the third trimester of gestation (Bauman & Currie, 1980). Negative energy balance (NEB) and suboptimal mineral levels are common in the transition period in cows (Goff, 2006a; Bell, 1995), because of higher needs. Negative energy balance is partially caused by reduced dry matter intake (DMI) around the time of calving (Grummer *et al.*, 2004). Furthermore, the cow's milk output increases faster than its feed intake capacity, which reach their maxima 4–7 weeks and 8–22 weeks after

calving, respectively (Ingvartsen & Andersen, 2000; Bell, 1995). An excessive NEB makes the cow more susceptible to metabolic disorders (Rukkwamsuk *et al.*, 1999a; Goff & Horst, 1997). To meet energy needs in the presence of NEB, adipose tissue is mobilized and the nutrient supply to the calf is ensured by a glucose-sparing state in the dam, in which peripheral tissue increases its use of ketone bodies and plasma lipids to meet energy requirements (Bell, 1995). In late gestation, decreased insulin sensitivity in peripheral tissue is part of this physiological response. Glucose is thus primarily used for the foetus during late gestation and for milk production in early lactation (Bell, 1995).

The gastrointestinal tract, as well as internal organs and body tissues (e.g., the liver and the adipose tissue), must adapt to higher energy demands in early lactation. It takes several weeks for the rumen microflora to adapt to changes in feed composition that normally occur during this period (Goff & Horst, 1997). The liver is involved in metabolism, immune functions and detoxification. In dairy cows, the swift adaptation of liver functions during the transition period is important for successful lactation. Around parturition, the dairy cow is susceptible to infectious disease, possibly because of a depression of the immune system (Ingvartsen *et al.*, 2003; Goff & Horst, 1997).

1.2 Swedish dairy herds

Dairy farming is currently undergoing rapid structural change in most industrialized countries. From 1990 to 2009, the number of Swedish dairy herds declined from 25,921 to 6020 and the number of cows declined from 427,621 to 357,194, while average herd size increased from 22 to 59 cows (Swedish Board of Agriculture, 2010). Today, one third of Swedish cows are kept in dairy farms with 100 cows or more, versus 6% of cows in 1988. Over those 20 years, milk yield has increased by 2000 kg of energy-corrected milk (ECM) to 9325 kg ECM per cow per year (Swedish Dairy Association, 2010).

The most common breeds are the Swedish Red (SR; 43%) and Swedish Holstein (SH; 50%), both highly specialized dairy breeds. In winter, dairy cows are traditionally housed in tie-stalls, although cubicle housing dominates in new operations. About half of Swedish herds are kept in cubicles and over 10% of all herds have automated milking systems. Dairy cows are generally fed forage-based rations, often along with high amounts of concentrates.

Approximately 78% of the herds are enrolled in the Swedish official milk recording scheme (SOMRS; Olsson *et al.*, 2001). The total disease incidence (number of diagnosed cases per 100 completed/interrupted lactations) in dairy herds participating in SOMRS was 32.3 in the 2007-2008 milk recording year; examples of disease incidence include mastitis (14.3), milk fever (3.3), ketosis (1.1) and DA (0.8; Swedish Dairy Association, 2009).

Sweden has a long tradition of eradicating harmful infectious livestock diseases. In 1763, Peter Hernquist was sent to the world's first veterinary school in Nantes, France, to become a veterinarian. He learned methods for disease control and eradication that he brought back to Sweden and taught at the veterinary school he founded in Skara in 1775 (Dyrendahl, 1996). Rinderpest was eradicated from the Swedish cattle population in the eighteenth century (SVA, 2010). Today, the Swedish dairy cattle population is free from tuberculosis, paratuberculosis, brucellosis, bovine herpes virus 1 and bovine leukemia virus and almost free from bovine viral diarrhoea virus (BVDV), as the ongoing programme to eradicate BVDV is nearly finished (SVA, 2009).

1.3 Herd health

Herd health is an important veterinary field that aims to ensure good animal health and welfare and high herd productivity. Herd health programmes are based on preventive healthcare and should include a written herd health plan stating the health goals of the farm. In such programmes, the incidence of certain diseases, such as metabolic diseases, mastitis and claw disorders, is monitored and specific farm recommendations are based on the recorded findings. Further monitoring tools found useful in these programmes are milk yield data, clinical examination of animals, biomarkers such as blood parameters, and body condition score (BCS).

There are large between-country differences in dairy production. The Swedish situation, in terms of housing of dairy cattle, feed composition, high milk yield, low disease incidence and freedom from certain contagious diseases, differs in many respects from that of countries where studies of biomarkers and risk factors for metabolic disorders have been carried out. This makes it highly relevant to carry out national-level research to use as a basis for preventive herd health programs.

1.4 Metabolic disorders related to energy metabolism

1.4.1 Displaced abomasum

The abomasum is normally located in the right ventral part of the abdominal cavity in cattle. It is widely accepted that decreased abomasum motility, followed by disturbed abomasal emptying and gas accumulation, are prerequisites for dilatation and subsequent displacement (Doll *et al.*, 2009; Geishauser, 1995). Abomasal displacement may be left-sided or right-sided with or without torsion of the abomasum. Most DA are left-sided (Karatzias & Panousis, 2003; Stengärde & Pehrson, 2002; Coppock, 1974), but a DA can shift from a left-sided to a right-sided displacement and vice versa (Geishauser, 1995); the different forms are considered to have a similar multi-factorial aetiology (Van Winden & Kuiper, 2003; Cameron *et al.*, 1998; Geishauser, 1995).

One of the first described cases of DA was a Swedish right-sided DA found in a 15 year-old cow described by the farmer as having good appetite and production (Lagerlöf, 1925b). The dislocation was assumed to be congenital. Left-sided DA was first described in 1950 (Begg, 1950). Since then, DA has become a significant disease in dairy cows.

Lactational incidence rates of DA have been reviewed (Doll *et al.*, 2009; Ingvartsen *et al.*, 2003) and found to range from 0.6% to 5% in the included studies. In the USA, incidence has increased, and in 2001, approximately 3.5% of dairy cows were diagnosed with DA (Goff, 2006b). A similar trend has been observed in Swedish dairy cows, and in 2008, there were 0.8 diagnosed cases of DA per 100 lactations versus 0.3 cases in 1993 (Swedish Dairy Association, 2009; Swedish Dairy Association, 1994).

Symptoms of DA in cows include inappetence, depressed general condition, decreased milk production, faecal alterations, dehydration, recumbency, shock, and death (Radostits *et al.*, 2007a). Diagnosis is based on symptoms, high-pitched tympanitic ping sounds along with splashing or tinkling sounds heard at auscultation and percussion of the abdomen, and rectal palpation. Most cases of DA in dairy cows are diagnosed within the first month postpartum (pp; Stengärde & Pehrson, 2002; Erb *et al.*, 1984) However, DA occurs in all lactational stages and occasionally in calves and steers.

Cows with DA are more likely to have had twins, a stillborn calf, endometritis, or metritis and puerperal paresis (Stengärde & Pehrson, 2002; Rohrbach *et al.*, 1999) than cows that do not develop DA. In addition, concurrent disease (Stengärde & Pehrson, 2002; Rohrbach *et al.*, 1999) and

hepatic lipidosis is found in many cows with DA (Rehage *et al.*, 1996; Muylle *et al.*, 1990).

Economic aspects of DA include treatment costs, reduced milk production (Detilleux *et al.*, 1997) and a shorter life expectancy (Gröhn *et al.*, 1998; Milian-Suazo *et al.*, 1988) in diseased cows.

1.4.2 Ketosis

Ketosis is relatively common in high-producing dairy cows and lactational incidence rates of 1.1–10% have been reported (Ingvartsen *et al.*, 2003). In Sweden, veterinary-treated ketosis incidence is decreasing, reaching 1.1 cases of ketosis per 100 lactations as of 2008 (Swedish Dairy Association, 2009).

Ketosis occurs when adipose tissue is used to meet energy demands and non-esterified fatty acids (NEFA) are incompletely oxidized into ketone bodies. There are two principal forms of ketosis, type I and type II (Holtenius & Holtenius, 1996b). Primary, or type I, ketosis occurs 3–6 weeks pp and affected cows have hyperketonemia and low concentrations of both glucose and insulin in plasma (Holtenius & Holtenius, 1996b). This classic form of ketosis was first reported in Denmark in 1886 (Lagerlöf, 1925a). Andersen called the disease chronic indigestion, and described the cows as exhibiting anorexia, weight loss, dry faeces, and decreased milk production. The cows at that time developed ketosis because of poor feeding; the traditional Swedish name for the condition, *småbrukaresjuka,* means "crofter's disease", and refers to the poor feeding sometimes practiced by these small land holders. Primary ketosis is less common today but may occur in early lactation in high-producing cows in a serious NEB. Symptoms in dairy cows affected by type I ketosis are inappetence, depressed general condition, and reduced milk production. Central nervous system symptoms are present in some cases, for example, muscle fibrillation, incoordination, excessive licking or chewing, aggression, or bellowing (Radostits *et al.*, 2007b).

Secondary, or type II, ketosis occurs earlier in lactation than does type I ketosis and is concurrent with other diseases (Holtenius & Holtenius, 1996b). In type II ketosis, cows have hyperketonemia and varying serum concentrations of glucose and insulin. Affected cows are often overconditioned, and the traditional Swedish word describing the condition, *herrgårdssjuka,* refers to rich feed rations on estate farms. The symptoms of type II ketosis are the same as those of the other concurrent diseases.

Alimentary ketosis has been attributed to intake of fermented feed high in ketogenic precursors (Kronfeld, 1982). Subclinical ketosis is considered

present in dairy cows with an elevated concentration of ketone bodies in blood but without clinical symptoms of ketosis (Andersson, 1988). The diagnosis of ketosis is based on symptoms, time in lactation, and tests to detect ketone bodies in milk, urine, or blood.

1.4.3 Hepatic lipidosis

Hepatic lipidosis, an increased amount of triacylglycerol (TAG) in the hepatocyte cytosol, is common in cows in the first weeks of lactation (Jorritsma *et al.*, 2001; Grummer, 1993; Emery *et al.*, 1992). Hepatic lipidosis occurs when the influx of NEFA to the liver exceeds its oxidation and secretion capacity, causing TAG to accumulate in the hepatocytes (Bobe *et al.*, 2004; Grum *et al.*, 1996). In addition, the hormonal status of cows in the transition period and stress that causes release of cortisol or catecholamines can induce hepatic lipidosis (Goff & Horst, 1997; Holtenius & Holtenius, 1996a; Luthman & Holtenius, 1972). Hepatic lipidosis can develop rapidly, even within 24 hours (Holtenius & Holtenius, 1996a; Luthman & Holtenius, 1972). Hepatic lipidosis has been associated with other diseases, such as DA, ketosis, and various infectious diseases (Goff & Horst, 1997; Rehage *et al.*, 1996). In the transition period, overconditioned cows may develop fat cow syndrome, a condition caused by hepatic lipidosis (Rukkwamsuk *et al.*, 1999a; Morrow, 1976). Cows with fat cow syndrome will be predisposed to a combination of metabolic, digestive, infectious, and reproductive conditions, but will respond unfavourably to treatment.

1.5 Risk factors for DA and ketosis

Both DA and clinical ketosis occur mainly the first month pp (Erb *et al.*, 1984), and may indicate energy-related metabolic disorders in the dairy herd. The association between DA and ketosis has been identified as bidirectional (Gröhn *et al.*, 1989; Curtis *et al.*, 1985), and several traits have been found to predispose cows to both diseases (Constable *et al.*, 1992; Correa *et al.*, 1990; Gröhn *et al.*, 1989). At the cow level, early lactation (Constable *et al.*, 1992), higher parities (Rasmussen *et al.*, 1999), a high body condition score at calving (Rasmussen *et al.*, 1999; Rukkwamsuk *et al.*, 1999a), and other diseases (Correa *et al.*, 1990) have been associated with DA and ketosis. Results concerning whether high milk yield at the individual or herd levels is associated with DA or ketosis are inconclusive (Fleischer *et al.*, 2001; Rajala & Gröhn, 1998; Gröhn *et al.*, 1995; Andersson & Emanuelson, 1985; Coppock, 1974). A low to moderate heritability for DA and ketosis has been found in some studies (Heringstad *et al.*, 2005), but not in a study

of ancestry (Martin *et al.*, 1978b). Swedish Holsteins have a higher risk of DA (Stengärde & Pehrson, 2002) while SR cows have a higher risk of hyperketonemia (Andersson & Emanuelson, 1985). Detrimental housing factors include both large (Coppock, 1974) and small herds, cubicle housing (Simensen *et al.*, 2010), and suboptimal feed bunk management, including limited bunk space (Cameron *et al.*, 1998). Feeding factors associated with DA and ketosis include component and total mixed rations (TMR), and feed components (e.g., Stengärde & Pehrson, 2002; Østergaard & Grohn, 2000; Shaver, 1997; Dawson *et al.*, 1992; Coppock, 1974). Overfeeding and over-conditioning during the dry period increase the risk of DA or ketosis (Rukkwamsuk *et al.*, 1999a). Seasonal factors have been associated with both diseases; for example, risk is higher in winter than in summer (Constable *et al.*, 1992; Andersson & Emanuelson, 1985) and weather change has been associated with a higher risk of DA (Cannas da Silva *et al.*, 2004).

1.6 Biological markers of metabolic status

Metabolic changes in cattle can be estimated by alterations in blood parameters and BCS (Ingvartsen & Friggens, 2005). Blood parameters, BCS, and change in BCS can thus be used to monitor nutrient supply, although BCS is subjective and retrospective, and thus not as useful in the early detection of health problems (Ingvartsen *et al.*, 2003). Certain blood parameters have been used to predict an increased risk of metabolic disease in individual cows and to identify the acute phases of disease (Ospina *et al.*, 2010; LeBlanc *et al.*, 2005; Itoh *et al.*, 1998).

Metabolic profiles of cattle first came into use in the seventies, when the Compton metabolic profile test was developed to monitor production diseases in dairy herds (Payne *et al.*, 1970). This blood profile test included measures of blood cells, glucose, protein parameters, and minerals known to change when metabolic disorders were present. Cows in several lactational stages were tested. In Sweden, early work on blood profiles in dairy cows was carried out by Hewett (1974). To improve the usefulness of the blood tests, the blood parameters included in the test, sampling periods, and number of cows to test have been modified over time. In addition, indexes that combine blood parameters have been tested (Trevisi *et al.*, 2010; Kaneene *et al.*, 1997).

Blood concentrations of NEFA, beta-hydroxybutyrate (BHB), glucose, insulin, and cholesterol have been associated with energy metabolism in dairy cows and may be used as indicators of NEB (Macrae *et al.*, 2006;

Oetzel, 2004; Agenäs *et al.*, 2003; Ingvartsen *et al.*, 2003; Kim & Suh, 2003; Kronfeld, 1972). NEFA and BHB have also been used to assess energy metabolism at the herd level (Herdt, 2000b; Kaneene *et al.*, 1997). However, Herdt et al. (Herdt *et al.*, 1981a) suggested that neither BHB concentration nor glucose concentration was a valid indicator of energy balance, but that BHB could be used to identify herds with a higher prevalence of ketosis (Herdt *et al.*, 1981b). Liver-related biomarkers include cholesterol, aspartate aminotransferase (AST), and glutamate dehydrogenase (GD). Haptoglobin is an inflammatory marker.

1.6.1 Body condition score

From the dry period to early lactation, a physiological decline in body condition can be anticipated as a response to the use of body reserves. However, extensive decline in condition is negative for the individual and has been associated with increased incidence of DA and ketosis (Kim & Suh, 2003). Overconditioned dry cows risk a deeper NEB around the time of calving, as feed depression before calving will be more pronounced than in cows with lower BCS (Hayirli *et al.*, 2002; Rukkwamsuk *et al.*, 1999b), In addition, overconditioned cows will mobilize more adipose tissue before and after calving than will cows with a lower BCS (Kokkonen *et al.*, 2005).

Body condition scoring is widely used in cattle to evaluate nutritional status and several scoring charts exist (e.g., Gillund *et al.*, 1999; Edmonson *et al.*, 1989; Wildman *et al.*, 1982). Both actual BCS (e.g., at dry off, at calving, and in early lactation) and BCS change over time may be used (Roche *et al.*, 2009; Mulligan *et al.*, 2006). Body condition score targets of 3–3.5 at calving and a maximum loss of 0.5 points over the entire lactation period have been suggested (Mulligan *et al.*, 2006).

1.6.2 Glucose

The monosaccharide glucose is used as an energy source in body tissue, and also during lactation as a basis for producing lactose (i.e., milk sugar). The blood concentration of glucose is strictly regulated through homeostasis (Bauman & Currie, 1980). Glucose concentrations decrease at parturition and are lower in the first weeks of lactation than before calving or later in lactation (Ingvartsen *et al.*, 2003). In addition, dairy cows have lower plasma glucose concentrations than do young ruminants and non-ruminants (Kaneko *et al.*, 2008; Coppo, 2001). Glucose concentration in blood can undergo short-term fluctuations in response to stress hormones as well as display diurnal variation.

The ruminal microflora degrades dietary carbohydrates to volatile fatty acids, and only a small amount of glucose bypasses the forestomachs and is absorbed from the intestines. Of these volatile fatty acids, which are transported to the liver, propionate is the substrate for gluconeogenesis. Cows rely on gluconeogenesis, as most of the glucose demand in dairy cows is met by glucose synthesis in the liver (Young, 1977). During periods of high glucose demand, especially in early lactation, amino acids from the breakdown of muscle tissue are utilized as glucose substrate as well.

Glucose uptake in cells is mediated by glucose transporters. Glucose uptake in skeletal muscle, adipose tissue, and cardiac muscle is dependent on insulin, but in the udder, glucose uptake occurs independently of insulin (Zhao & Keating, 2007a; Zhao & Keating, 2007b; Joost & Thorens, 2001).

1.6.3 Fructosamine

Fructosamines are glycated proteins formed by an irreversible non-enzymatic reaction between glucose and blood proteins (in cattle, most commonly with albumin; Armbruster, 1987). The fructosamine concentration in blood depends on both glucose and total protein concentrations. The half-life of albumin varies between species, and in cattle, fructosamine is said to mirror serum concentrations of glucose over the preceding 1–3 weeks (Jensen *et al.*, 1993; Ropstad, 1987). Fructosamine is used in retrospective monitoring to detect elevated glucose concentrations in companion animals and in diabetic humans. A small study of ovine pregnancy toxaemia and a case report of a dog with insulinoma found lower concentrations of fructosamine, indicating that fructosamine can be used to detect periods of low glucose concentrations as well (Mellanby & Herrtage, 2002; Cantley *et al.*, 1991). Fructosamine concentration in dairy cows has been demonstrated not to change due to short-term fluctuations in glucose concentration and to display minor diurnal variations (Jensen *et al.*, 1993). Fructosamine is thus potentially suitable as a marker for monitoring long-term glucose concentrations in dairy cows.

A positive correlation between fructosamine and glucose has been established in calves (Coppo, 2001) and in dairy cows pp (Ropstad, 1987). In lactating dairy cows, a weak positive correlation between blood glucose and fructosamine was found when glucose was measured 12–30 days prior to fructosamine (Tråvén *et al.*, 2007). However, a study of dairy cows before and after calving, found no correlation between fructosamine and retrospective concentrations of total protein or glucose (Sorondo & Cirio, 2009).

1.6.4 Insulin

Insulin is an anabolic peptide hormone, synthesized and stored in beta cells located in the islets of Langerhans in the pancreas. Insulin is one of the hormones that regulate glucose concentration and is released from the beta cells to the bloodstream in response to increase in levels of nutrients (e.g., glucose and propionate) in the serum, gastrointestinal hormones, and parasympathetic stimuli (Hayirli, 2006). Insulin facilitates the cell uptake of glucose and downregulates adipose tissue mobilization. Insulin concentration is high before parturition and decreases to low levels after parturition (van Knegsel *et al.*, 2007; Kokkonen *et al.*, 2005). The low concentration of insulin pp stimulates adipose tissue breakdown to counteract the NEB in early lactation. The amino-acid sequence of insulin is species-specific, but varies only slightly between mammalian species. Some assays to determine insulin concentrations in serum manufactured for one species may be used to determine insulin concentrations in other species.

1.6.5 Non-esterified fatty acids

Non-esterified fatty acid concentrations rise before calving and peak 1–3 weeks pp (Cavestany *et al.*, 2005; Ingvartsen *et al.*, 2003). The increase in NEFA is part of the physiological response necessary to meet higher energy demands at the onset of lactation, but excess NEFA concentrations are detrimental; for example, high NEFA concentration is toxic to peripheral tissue and negative affects fertility (Adewuyi *et al.*, 2005; Leroy *et al.*, 2005).

The main source of NEFA is triglycerides stored in adipose tissue, where they serve as an energy supply. Non-esterified fatty acids are transported to the liver and taken up by the hepatocytes to be completely oxidized, incompletely oxidized, or esterified. Complete oxidation in the citric acid cycle produces carbon dioxide and energy, whereas incomplete oxidation produces ketone bodies. The major esterification product is TAG, which is either redistributed into the bloodstream as part of very-low-density lipoproteins (VLDL) or stored in the hepatocyte. VLDL formation depends on apolipoproteins and cholesterol. Because apolipoprotein supply is limited in cattle, the ruminant liver has a low capacity to secrete VLDL (Grummer, 1993), and overproduction of TAG will accumulate in the hepatocyte cytosol, causing hepatic lipidosis.

1.6.6 Beta-hydroxybutyrate

The ketone bodies i.e., BHB, acetoacetate, and acetate, are formed by incomplete oxidation of NEFA in the liver. Another source of BHB in blood is ruminal butyrate that is oxidized to BHB in the rumen wall. Ketogenesis is

part of the normal energy metabolism in ruminants. If glucose concentrations are low, more ketone bodies are produced in the liver to meet the energy needs of body tissues. Concentrations of BHB increase pp and peak 2–4 weeks pp (Cavestany *et al.*, 2005; Ingvartsen *et al.*, 2003); excess is detrimental, and may cause ketosis. In dairy cows, there is an individual variation in tolerance of elevated ketone bodies (Kessel *et al.*, 2008). Ketone bodies are excreted through the kidneys and, as well as in blood and urine, are also found in milk; cow-side tests available for detecting ketone bodies.

1.6.7 The *revised* quantitative insulin sensitivity check index

The *revised* quantitative insulin sensitivity check index (RQUICKI) is a metabolic index used in humans to assess insulin sensitivity (Rabasa-Lhoret *et al.*, 2003; Perseghin *et al.*, 2001). RQUICKI is based on fasting plasma glucose, insulin, and NEFA, and a low value is indicative of decreased insulin sensitivity. The metabolic index is calculated a follows (Rabasa-Lhoret *et al.*, 2003; Perseghin *et al.*, 2001):

$$\text{RQUICKI} = 1/[\log_{10}(\text{glucose in mg/dL}) + \log_{10}(\text{insulin in } \mu\text{U/mL}) + \log_{10}(\text{NEFA in mmol/L})]$$

RQUICKI has been evaluated for lactating dairy cows, in which a negative linear relationship between body condition and RQUICKI was found (Holtenius & Holtenius, 2007). However, it is not known how well RQUICKI reflects reduced insulin sensitivity in lactating dairy cows and there is no gold standard for assessing insulin sensitivity in them. In calves, RQUICKI values have been demonstrated to be correlated with the response to an intravenous glucose tolerance test (Bossaert *et al.*, 2009); however Kerestes et al. (Kerestes *et al.*, 2009) did not find RQUICKI values to be correlated with insulin resistance in cows with ketosis and signs of puerperal metritis. The usefulness of RQUICKI in cows with disease or reduced feed intake merits further evaluation.

1.6.8 Cholesterol

Cholesterol is produced and degraded in the liver and is a precursor of steroid hormones, vitamin D, and bile acids. Cell membranes and bile micelles consist partly of cholesterol. Cholesterol is secreted into the blood stream as VLDL, and low concentrations of cholesterol may impair the transport of TAG from the liver (Holtenius, 1989). Cholesterol concentrations are low at calving and increase slowly over the first weeks

after calving (van Knegsel *et al.*, 2007; Cavestany *et al.*, 2005). Low cholesterol concentrations have been associated with metabolic disorders (Itoh *et al.*, 1998), hepatic lipidosis (Bobe *et al.*, 2004; Grum *et al.*, 1996), and feed intake (Janovick Guretzky et al., 2006).

Excessive BCS decline in early lactation is associated with deep NEB. This is not reflected in glucose concentrations, but cholesterol has been proposed as a better predictor of energy balance in early lactation (Kim & Suh, 2003).

1.6.9 Aspartate aminotransferase

Aspartate aminotransferase is an enzyme present in the cytoplasm and in mitochondria of several types of cells (e.g., hepatocytes and skeletal muscle cells). The enzyme facilitates the conversion of aspartate and 2-oxogltatarate to oxaloacetate and glutamate (Kaneko *et al.*, 2008). Only low concentrations of the enzyme are present in the blood of healthy animals, but concentrations of AST rise in cows around calving (Cavestany *et al.*, 2005; Geishauser *et al.*, 1999). When there is cell damage or necrosis of fat-infiltrated hepatocytes, the enzyme can leak into the bloodstream and increased AST serum activity may be detected. Aspartate aminotransferase, although not liver specific, is used to detect liver cell damage in cattle and has been associated with liver cell damage and hepatic lipidosis (Bobe *et al.*, 2004; Komatsu *et al.*, 2002; Rehage *et al.*, 1996).

1.6.10 Glutamate dehydrogenase

Glutamate dehydrogenase, an enzyme present in hepatocyte mitochondria, is necessary for urea synthesis in the conversion of glutamate to alpha-ketoglutarate. In cattle, GD is liver specific and used to detect liver cell damage (Kaneko *et al.*, 2008). When there is cell damage or necrosis of fat-infiltrated hepatocytes, the enzyme can leak into the blood stream and increased serum activity GD of may be detected (Bobe *et al.*, 2004). Increased GD concentration has been associated with hepatic lipidosis (Rehage *et al.*, 1996).

1.6.11 Haptoglobin

Haptoglobin is a species-specific acute-phase protein the levels of which rise in response to trauma, inflammation, and infection (Earley & Crowe, 2002; Hirvonen *et al.*, 1999a; Hirvonen *et al.*, 1999b; Skinner *et al.*, 1991). In healthy cattle, haptoglobin concentration is low and may be undetectable. Haptoglobin concentrations rise at calving and stay elevated for a period after calving (Hiss *et al.*, 2009). Haptoglobin is synthesized by hepatocytes in response to inflammatory cytokines. The major role of haptoglobin is to

bind haemoglobin, thus preventing iron loss, formation of free iron radicals, and renal damage. The haptoglobin level rises in response to assault and reaches maximum concentrations within 24–48 hours, thereafter staying elevated for up to two weeks (Petersen *et al.*, 2004). In chronic disease, haptoglobin may be elevated for longer periods of time but at lower concentrations than in acute disease. In addition to being an inflammatory marker, haptoglobin has been proposed as an indicator of animal welfare in pigs, a non-specific indicator of health in cattle, and may also be useful in monitoring herds for subclinical disease (Hiss *et al.*, 2009; Piñeiro *et al.*, 2007; Petersen *et al.*, 2004). Haptoglobin has also been proposed to affect lipid metabolism or to react to hepatic lipidosis (Katoh & Nakagawa, 1999; Uchida *et al.*, 1993).

2 Aims

Overall, this thesis aims to gain insight into the potential use of blood parameters and BCS for cows to identify herds at risk of DA or ketosis and to investigate herd-level risk factors for these disorders. The first hypothesis is that there are metabolic differences between cows in herds with higher and lower incidences of energy-related metabolic disorders. The second hypothesis is that these metabolic differences should be possible to find by studying herd means of BCS and blood parameters reflecting energy metabolism, liver cell damage, and inflammatory processes in cows around the time of calving and in early lactation. To accomplish this, the specific aims were:

- to compare blood profiles that reflect energy metabolism, liver cell damage, and inflammatory processes in dairy cows with and without DA (Paper I);

- to study how blood profiles that reflect energy metabolism, liver cell damage, and inflammatory processes vary over time in relation to calving in dairy cows with DA (Paper I);

- to assess variation in blood profiles that reflect energy metabolism, liver cell damage, and inflammatory processes and BCS between dairy herds with a long-term high incidence of DA or clinical ketosis (Paper II);

- to assess variation in blood parameters that reflect energy metabolism, liver cell damage, and inflammatory processes and BCS over time in relation to calving in dairy herds with a long-term high incidence of DA or clinical ketosis (Paper II);

- to assess the association of long-term high incidence of DA or clinical ketosis in dairy herds with blood profiles that reflect energy metabolism, liver cell damage, and inflammatory processes and BCS in the periparturient period and early lactation (Papers II and III); and

- to assess housing, management, and feeding risk factors related to a high long-term herd incidence of DA or clinical ketosis (Paper IV).

3 Materials and methods

This is an overview of the materials and methods used in the four field studies included in this thesis, which were conducted between January 2005 and July 2007. Full descriptions of each study are found in papers I–IV. Study I was an observational study at the individual cow level, studies II and III were observational studies at the herd level, and study IV was a case-control study at the herd level; the same herds were used for studies II (five herds), III, and IV.

Table 1. *Overview of the studies included in this thesis. In studies II–IV, displaced abomasum (DA) and clinical ketosis were used as herd-level indicators of the occurrence of metabolic disorders in the transition period*

		Level		Herd incidence of DA or ketosis	
Study	Objective	Cow	Herd	High	Low
I	Blood profiles and BCS[a] for cows with or without DA 2–56 days pp[b]	x			
II	Blood profiles and BCS over time in healthy cows 4 weeks ap[c] to 9 weeks pp		x	x	
III	Blood profiles and BCS in cows 4 weeks ap to 7 weeks pp		x	x	x
IV	Risk factors for DA or ketosis		x	x	x

[a]body condition score

[b]postpartum

[c]antepartum

3.1 Definitions and classification of herds

The following definitions of clinical ketosis, DA, and herd-level incidence of DA or clinical ketosis were used:

- **Clinical ketosis** was defined as ketosis diagnosed by a veterinarian. The diagnosis was most commonly based on case history, clinical examination, and cow-side testing to verify presence of ketone bodies in milk.
- **Displaced abomasum** was defined as a displacement of the abomasum to the left or right (with or without suspected torsion in the case of right-displaced abomasum), diagnosed by a veterinarian. The diagnosis was based on clinical examination, including auscultation to locate the characteristic metallic ping sounds found in cows with DA.
- A **high herd-level incidence of DA or clinical ketosis** was defined as an incidence rate of DA or clinical ketosis (number of cows with either of the two diagnoses divided by the average number of cows over the year) above the 3rd quartile in the Swedish National Animal Disease Recording System (NADRS; Mörk *et al.*, 2010; Emanuelson, 1988) during the year before sampling and in at least one of the preceding two years. The 3rd quartiles were 0.04, 0.04 and 0.03 cases of DA or clinical ketosis per cow per year for each of the three periods. Likewise, a **low herd-level incidence of DA and clinical ketosis** was defined as an incidence rate of DA and clinical ketosis below the 1st quartile in the NADRS during the year before sampling and in at least one of the preceding two years. The 1st quartile was 0 cases of DA and clinical ketosis per year for each of the three periods.

The final classification of herds with a high or low incidence differed between studies III and IV. In study III, an additional evaluation of disease frequency occurred during the farm visit, and the incidence rate of DA and clinical ketosis in the 12-month period when the farm visit took place completed the classification. To be classified as a high-incidence herd, a herd had to have 0.04 or more cases per cow per year of DA or clinical ketosis in the 12-month period on which the herd was visited, whereas the low-incidence herds had at most 0.01 cases per cow per year over that period. Herds that did not meet the disease incidence criteria during the 12-month period when they were visited, although they had high or low incidences when selected for inclusion in the study, were classified as inconsistent herds. In study III, the classification of herds was thus based on the historical and present incidence of DA and clinical ketosis. In study IV,

only the historical incidence over the three year-period for the inclusion criteria was used to classify the herds as having either a high or a low herd-level incidence of DA or clinical ketosis.

3.2 Source population

Dairy herds enrolled in SOMRS were eligible for inclusion in all four studies. In addition, the herds included in study II had to have more than 100 cows and produce >9500 kg of energy-corrected milk (ECM) per cow annually. Furthermore, the herds had to be located in either of two Swedish provinces (Västergötland and Uppland) and to have at least 6 cases of DA, clinical ketosis, or both per 100 lactating cows within the year before the study to be eligible for inclusion. In studies III and IV, herds had to have more than 50 cows, be located in any of four provinces (Skåne, Halland, Västergötland, and Uppland), and have either a high or low herd-level incidence of DA or clinical ketosis, respectively.

In study I, the cows with DA and the controls were chosen by the practicing veterinarians who did the field work. Fourteen veterinary ambulatory practices across Sweden participated. In study II, practicing veterinarians in the two provinces were asked to suggest eligible herds; the first five eligible herds whose owners were asked to participate and agreed to do so were enrolled. In studies III and IV, owners of all eligible herds were asked to enroll their herds in the studies; all herds whose owners were willing to participate were included.

3.3 Data collection and sampling

In all studies information about herd management, housing, and feeding together with specific information about the sampled cows was gathered by the visiting veterinarian using standardized questions. Herd-level data on milk yield, herd size, and disease incidence as well as individual data such as breed, calving date, parity, and diseases over the study period, were retrieved from SOMRS and NADRS.

In studies I–III, the cows were clinically examined and blood samples were drawn. Blood samples were collected from each cow in evacuated tubes, both without additives and containing FluoridHeparin (BD Vacutainer Systems, Plymouth, UK), and serum and plasma samples were frozen within 8 hours and kept frozen until analysis. Both primiparous and multiparous cows were included in the studies and samples from 1 day ap to 1 day pp were excluded.

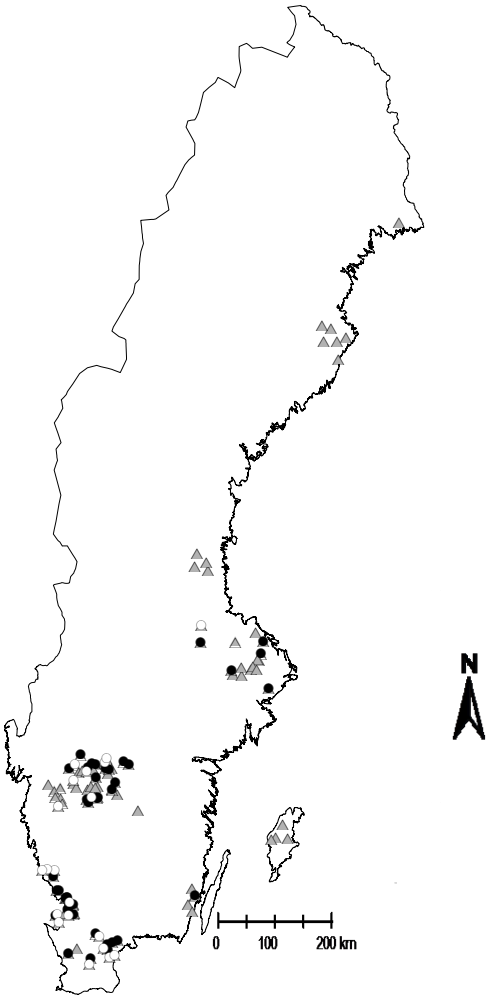

Figure 1. Location of the herds examined in studies I–IV. Triangles: herds in study I, White circles: low-incidence herds in study IV, Black circles: high-incidence herds in study IV

In study I, dairy cows 2–56 days pp diagnosed with DA (n = 69) and up to two healthy control cows (n = 104) in the same herds (n = 60) were included. The body condition of the sampled cows was assessed as thin, average, or over-conditioned.

Study II included 94 cows in five herds with a high incidence of DA and clinical ketosis. The cows were blood sampled repeatedly at three-week intervals from 4 weeks ap to 9 weeks pp. Each cow was sampled on at least

two occasions (n = 326), A minimum of 15 cows were examined in each herd. The BCS was assessed on a five-point scale with half-point increments (Edmonson *et al.*, 1989). The ap BCS was defined as BCS four weeks to one day ap and BCS loss was defined as the difference between BCS ap and BCS 4–6 weeks pp.

In study III, 943 cows (6–26 cows per herd) in 19 herds with high incidence of DA and clinical ketosis, 15 herds with low incidence, and 26 herds with inconsistent incidence were included. The cows were blood sampled once and their BCS was assessed on a five-point scale (Edmonson *et al.*, 1989). The samples included 10% of the cows in each herd up to a maximum of 25 cows. In most herds, all eligible cows were sampled. In the 10 herds that contained more eligible cows than required, cows were convenience sampled as they were found in the housing systems.

In study IV, the same herds as in study III were included, classified according to historical incidence as 40 high- and 20 low-incidence herds. The study herds were visited during the stable season and the farmers were interviewed using standardized questionnaires. The questions focused on long-term housing, feeding, and management practices for the following groups of animals: heifers in late gestation (i.e., the last 8 weeks before expected first parturition), dry cows, and cows in the first 8 weeks of lactation. The questions were similar for all cow groups.

3.4 Statistical analyses

Studies I–III used linear mixed models for the data analyses and statistical significance was assessed at $p < 0.05$. Aspartate aminotransferase, BHB, cholesterol, GD, glucose, haptoglobin, insulin, and NEFA were used as outcome variables in studies I–III. In addition, fructosamine was included as an outcome variable in studies I and II, BCS was included in studies II and III, and RQUICKI was included in studies I and III. The predictor variables used in the models in each study were chosen based on biological plausibility. Study I assessed the association between the blood parameters and cows with or without DA. In DA cows, additional analyses were performed to evaluate the effects of symptom duration and medical treatment. Study II assessed the association of the blood parameters and BCS with time in relation to calving in five herds with a high incidence of DA or clinical ketosis. Study III assessed the association of the blood parameters and BCS with a high or low herd-level incidence of DA or clinical ketosis. The inconsistent herds were retained in the statistical models to increase the analytical power. Herd identity was included as a random effect to account for the clustering of

within-herd observations in studies I and III. In study II, only cows represented by more than one observation were included and the repeated with-in cow and with-in herd sampling was accounted for. In addition, the proportion of cows included in study I (all cows) and study III (cows sampled within certain timeframes) outside suggested reference ranges for some of the blood parameters were calculated. In study III, receiver operator characteristics (ROC) curves were constructed and area under the curve (AUC) was determined to evaluate the association of glucose, insulin, NEFA, cholesterol, and RQUICKI with being a high-incidence herd (Dohoo *et al.*, 2009). Herd size and herd mean milk yield were tested non-parametrically by means of a Kruskal-Wallis test.

Study IV used univariable and multivariable logistic regression models to assess associations between herd-level risk factors and being a herd with a high or low incidence of DA or clinical ketosis. In the multivariable model, statistical significance was assessed using Wald's test at $p < 0.05$. All one-way interactions among variables in the final model were tested, but none was found to be significant. One confounding variable was found and added to the final model.

All statistical analyses were performed using the SAS statistical software package (SAS Institute, Cary, NC, USA).

4 Results

This section presents the main results of studies I–IV are; full presentations of the study results are found in papers I–IV.

4.1 Herd characteristics

The mean herd size in study I was 84 cows and the milk production was 9501 kg ECM per cow per year. In study II, the herds had 150–300 cows and produced 9500–11,500 kg ECM per cow per year. Recorded cases of DA and clinical ketosis in the herds varied between 3.0% and 9.4% in the 12-month period preceding the last herd visit. Two herds had fewer than 6 cases of the two diseases per 100 cows.

In study III, high-incidence herds were significantly larger (mean 123 cows) than the low-incidence herds (mean 74 cows). Herd mean milk production was significantly higher in high-incidence herds than in low-incidence herds (10,471 and 9077 kg ECM per cow per year, respectively).

In study IV, high-incidence herds were larger (mean 125 cows) and had a higher milk production (9818 kg ECM per cow per year) than did the low-incidence herds (mean 72 cows and 9308 kg ECM per cow per year, respectively). Most herds were kept in loose housing systems. In the herds with component ration feeding, concentrates were fed separately 3–12 times daily. Most herds were SH (32 herds), or consisted of mixed breeds (20 herds), while SR herds (8 herds) were less common (defined as an SH or SR herd if >80% of the cows were of one breed). Most herds used prophylactic measures to avoid hypocalcaemia and to increase blood glucose in the transition period.

4.2 Clinical signs and treatments in cows with DA

In most of the 69 DA cows examined in study I, general condition was reported as mildly (n=31) or moderately (n=29) disturbed. A reduced feed intake was reported in all cows with DA. Thirty-seven per cent of the cows had symptoms of DA for 1–3 days, 34% had symptoms for 4–7 days, and 28% had symptoms for 8–26 days. Seventy-two per cent of the DA cows had at least one other disease in the period from 1 week ap to 1 week after sampling. Seventy-two per cent of the DA cows were treated in the last week before sampling, i.e., before diagnosis of DA, with one or more of the following: calcium orally or intravenously, oral infusions to increase blood glucose, corticosteroids systemically, and antibiotics.

4.3 Body condition score

In study I, body condition was reported as thin (42% DA cows vs. 5% controls), average (51% DA cows vs. 81% controls), and overconditioned (6% DA cows vs. 8% controls). In study II, all herds had a mean BCS above 3.5 ap and only 11% of the cows had a BCS of 3 or lower ap. All the cows with a BCS loss of 1.0 or greater (n = 21) had a BCS of 4 or higher ap except for two cows. In study III, no differences in mean BCS values between high-incidence and low-incidence herds were found ap (mean 3.8 for high-incidence herds and 3.7 for low-incidence herds) or pp (mean 3.1 and 3.0). The proportion of cows with a BCS of 4.5–5 ap differed ($p = 0.04$) between high-incidence herds (20%) and low-incidence herds (8%). The range (10^{th} to 90^{th} percentiles) of BCS 1-2 weeks ap was 3–4.5 for high-incidence herds and 3–4 for low-incidence herds. Proportion of cows included in study III with a BCS above 3.5 ap is shown in Table 2.

4.4 Blood parameters

In study I, DA cows had significantly higher concentrations of NEFA, AST, GD, and haptoglobin and lower concentrations of insulin and cholesterol than did control cows. A tendency towards lower RQUICKI ($p = 0.056$) values was found, but glucose and fructosamine were not significantly different between DA cows and controls. For insulin, NEFA, BHB, cholesterol, AST, GD, and haptoglobin, statistically significant differences in serum concentrations between DA cows and controls were found in one or more sampling period. Symptom duration and medical treatment were not significantly associated with any of the blood parameters.

In study II, statistically significant differences between the herds were found in concentrations of NEFA ap and pp, insulin ap, cholesterol pp, and haptoglobin pp. In study III, glucose concentration ap and cholesterol concentration pp were significantly higher, and RQUICKI pp was significantly lower in high- than in low-incidence herds. Tendencies for concentrations of glucose, NEFA, and insulin to be higher pp in high- than low-incidence herds were evident. The range (10^{th} to 90^{th} percentiles) of NEFA 1–3 weeks pp was 0.15 to 1.23 mmol/L for high-incidence and 0.19 to 0.82 mmol/L for low-incidence herds. The proportions of cows in studies I and III with blood values outside suggested reference ranges are shown in Tables 2 and 3.

Table 2. *Proportions of cows with blood values outside suggested reference ranges, among sampled cows in herds with high or low incidence of displaced abomasum or clinical ketosis (study III)*

		Cows in high-incidence herds		Cows in low-incidence herds	
Parameter[a]	Reference range and time span[b]	n	%	n	%
Glucose	<3.0 mmol/L day 2–21 pp[c]	60/108	56	45/80	56
NEFA	≥0.4 mmol/L day 14–2 ap[d]	21/69	30	5/34	15
NEFA	≥0.7 mmol/L day 10–20 pp	14/58	24	8/40	20
BHB	≥1.4 mmol/L day 5–50 pp	41/212	19	33/146	23
BCS	>3.5 in cows ap	56/98	57	23/52	44
Cholesterol	<2.0 mmol/L day 2–21 pp	10/108	9	12/80	15
AST	>2.2 μkat/L day 2–21 pp	3/108	3	4/80	5
GD	>250 μkat/L day 2–21 pp	9/108	8	7/80	9
Haptoglobin	>0.5 g/L day 2–21 pp	34/108	31	30/80	38

[a]NEFA, non-esterified fatty acids; BHB, betahydroxybutyrate; BCS, body condition score; AST, aspartate amino transferase; GD, glutamate dehydrogenase

[b]Glucose, NEFA pp (Whitaker, 2004)

NEFA ap reference range according to Whitaker (Whitaker, 2004) and time span according to Oetzel (Oetzel, 2004)

BCS (Edmonson *et al.*, 1989)

BHB (Oetzel, 2004)

Cholesterol (Van den Top *et al.*, 2005; Holtenius *et al.*, 1986)

AST (Kaneko *et al.*, 2008)

GD Reference according to Clinical Pathology Laboratory, University Animal Hospital, SLU, Uppsala (Personal communication B. Jones)

Haptoglobin reference range according to study II

[c]pp postpartum

[d]ap antepartum

Table 3. *Proportions of cows with displaced abomasum (DA) and healthy controls with blood parameter values outside suggested reference ranges. All cows in study I sampled 2–56 days postpartum have been included*

		DA cows n=69		Control cows n=104	
Parameter[a]	Reference range[b]	n	%	n	%
Glucose	<3.0 mmol/L	41	59	64	63
Glucose	>3.5 mmol/L	16	23	3	3
NEFA	≥0.7 mmol/L	69	100	26	25
BHB	≥1.4 mmol/L	44	64	31	30
Cholesterol	<2.0 mmol/L	24	35	5	5
AST	>2.2 μkat/L	31	45	2	2
GD	>250 μkat/L	26	38	24	23
Haptoglobin	>0.5 g/L	44	64	21	20

[a]NEFA, non-esterified fatty acids; BHB, betahydroxybutyrate; AST, aspartate amino transferase; GD, glutamate dehydrogenase

[b]Glucose, NEFA (Whitaker, 2004)

BHB (Oetzel, 2004)

Cholesterol (Van den Top *et al.*, 2005; Holtenius *et al.*, 1986)

AST (Kaneko *et al.*, 2008)

GD Reference according to Clinical Pathology Laboratory, University Animal Hospital, SLU, Uppsala (Personal communication B. Jones)

Haptoglobin- reference range according to study II

4.5 Test characteristics for herd-level biomarkers

To evaluate the association of RQUICKI, glucose, insulin, NEFA, and cholesterol with being a herd having a high incidence of DA or ketosis (study III), AUC for ROC curves was determined. Before calving, glucose had an AUC of 0.67. In the first 3 weeks pp, the AUC for RQUICKI was 0.70, while the AUC for glucose (0.57), insulin (0.57), and NEFA (0.61) were lower than for RQUICKI. In weeks 4–7 pp, the AUC for RQUICKI and cholesterol were 0.61 and 0.63, respectively.

4.6 Risk factors for displaced abomasum and ketosis

Herd size was confounded by stall system (classified as tie-stalls, cubicles with automated milking-systems, cubicles with milk parlour), so stall system was added to the final logistic regression model. Tendencies for a higher

maximum daily milk yield in multiparous cows (defined as the highest amount of milk in kg ECM per day the most high-yielding multiparous cows produced according to the farmer) and larger herd size (defined as number of cows in the herd) were associated with being a high-incidence herd, where the association with maximum daily milk yield was non-linear. Compared with the overall median herd size in the study (77 cows), the odds of being a high-incidence herd were 0.4 times lower (95% confidence interval (CI) 0.2–1.1) at the 25th percentile (58 cows) and 9.8 times higher (CI 0.9–112) at the 75th percentile (130 cows). At the 25th percentile (50 kg ECM per cow-day) of maximum daily milk yield among multiparous cows, the odds of being a high-incidence herd were 0.9 times lower (CI 0.6–1.5) than at the overall median in the study (53.8 kg), while at the 75th percentile (60 kg) they were 1.9 times higher (CI 0.1–3.8). Not cleaning the feeding platform for the heifers daily (classified as daily cleaning or less frequently than daily cleaning) and keeping all cows in one group in the dry period (classified as one group or more than one group) were also associated with being a high-incidence herd. The odds ratio (OR) for being a high-incidence herd when all cows were kept in one group in the dry period was 8; if the heifer feeding platform was not cleaned daily the OR for being a high-incidence herd was 12.

5 Discussion

5.1 Cows with DA

In study I, cows with DA were studied to establish their blood profiles. Cows with DA and healthy controls were sampled in the same herds, at the same time, and at the same stage of lactation. In other studies, DA cows have been sampled after transport to a clinic (Komatsu *et al.*, 2002; Itoh *et al.*, 1998; Rehage *et al.*, 1996) while control cows have been sampled in the original herds or even in other herds (Zadnik, 2003; Itoh *et al.*, 1998; Muylle *et al.*, 1990). Therefore, comparisons of blood parameters in cows with and without DA may not be valid. Some studies have used reference values for comparisons instead of control cows (Stengärde & Pehrson, 2002; Hirvonen & Pyörälä, 1998; Rehage *et al.*, 1996). Transport, change of management, and change in feed can affect several blood parameters, and DA and control cows should therefore be sampled under similar conditions. Because the cows in study I were sampled in their home herds, effects of differences in feeding and stress were kept to a minimum. Thus, the differences noticed in blood parameters between cows with and without DA will reflect changes due to the disease, and not other stress related factors.

5.1.1 Blood parameters reflecting energy metabolism in DA cows

In study I, a larger variety of blood parameters were also studied compared with earlier studies, including RQUICKI, insulin and fructosamine. A tendency for lower RQUICKI was found in cows with DA. The lower values may indicate decreased insulin sensitivity. However, the cows with DA had lower concentrations of insulin than control cows, and there was no difference in glucose and fructosamine concentrations between DA and control cows. The fructosamine concentrations indicated that glucose concentrations had been similar for cows with DA and controls prior to the

diagnosis of DA. Even though there were no differences in mean glucose concentrations, there was a larger span, and also a larger proportion of cows with DA with glucose concentrations >3.5 mmol/L, compared with glucose concentrations for control cows. Presumably, DA may not cause any consistent changes in plasma glucose concentrations. A reduced feed intake leads to a rapid decrease in glucose and insulin concentrations (Agenäs *et al.*, 2003), whereas stress, for example related to pain, may result in high glucose concentrations. Insulin sensitivity is difficult to assess in cows that are off feed, and results in insulin, glucose, and fructosamine concentrations do not contradict that DA cows may have reduced insulin sensitivity.

5.1.2 Effect of time on blood parameters in cows with DA

The effect of time in relation to calving on blood profiles for cows with DA was also studied (study I). For most blood parameters related to energy balance, differences between DA and control cows were similar over time. In early lactation, major alterations in energy related biomarkers for metabolism may be expected because the drive to sustain lactation is strong (Herdt, 2000a). Later in lactation, cows instead respond with down-regulation of milk synthesis in response to disease or deficient nutrient intake, and this may result in a less pronounced response in metabolic biomarkers. On the other hand, cows diagnosed later in relation to calving might have had a longer period of disease before diagnosis and thus a substantially disturbed blood profile would be expected.

5.1.3 Symptom duration and other diseases in cows with DA

In agreement with another study (Stengärde & Pehrson, 2002), a large proportion of the cows with DA in study I had symptoms for more than 4 days before day of diagnosis, and 26% of the cows had symptoms already at calving. In addition, 42% of the cows with DA had a low BCS, also indicating a long duration of symptoms because extensive weight loss has been associated with DA (Østergaard & Gröhn, 1999). Symptom duration was not significantly associated with any of the blood parameters, possibly because all cows with DA had a reduced feed intake

Seventy per cent of the cows with DA in study I had concurrent or previous diseases, which is in agreement with other studies (Stengärde & Pehrson, 2002; Rohrbach *et al.*, 1999). Haptoglobin concentrations were increased in DA cows compared with control cows and may be useful in determining if there are other disorders that need treatment. It would be advantageous to diagnose cows with DA earlier in relation to onset of symptoms to improve animal welfare and reduce economic losses due to

BCS loss and milk reduction in cows with DA. Blood analysis of haptoglobin may be one way to find cows in need of extra attention earlier than with only animal inspections. This is in accordance with notions to use acute phase proteins to monitor for subclinical disease (Petersen *et al.*, 2004).

5.2 Markers of metabolism in herds with DA or clinical ketosis

5.2.1 Blood parameters reflecting energy metabolism

In study III, differences in metabolism were found between cows in herds with high incidence of DA or clinical ketosis and cows in herds with low incidence. These differences were most pronounced in RQUICKI, but were also found for glucose, NEFA, insulin, and cholesterol. The mean value of RQUICKI pp was lower for high-incidence herds than for low-incidence herds. The RQUICKI is used in humans to assess insulin sensitivity and a lower RQUICKI indicates decreased insulin sensitivity (Rabasa-Lhoret *et al.*, 2003; Perseghin *et al.*, 2001). A similar relationship was recently shown in calves (Bossaert et al., 2009). Few studies in lactating cows have been carried out. In one study, a negative linear relationship was demonstrated between RQUICKI and body condition in lactating dairy cows (Holtenius & Holtenius, 2007) and overconditioned cows have been shown to have a decreased insulin sensitivity or a reduced insulin response (Holtenius *et al.*, 2003; Rukkwamsuk *et al.*, 1999c; Rukkwamsuk *et al.*, 1998). In another study, the RQUICKI values were not correlated with insulin sensitivity in cows with ketosis and signs of puerperal metritis (Kerestes et al., 2009). Results from studies I and III suggest that RQUICKI is most useful in apparently healthy cows, and could be used both at the individual or at the herd level to find cows or herds in need of interventions. Further research in lactating dairy cows to determine the usefulness, and if RQUICKI measures insulin sensitivity in lactating dairy cows is advocated.

5.2.2 Effect of milk yield and herd size

The herds in study III with a high incidence of DA or clinical ketosis were larger and had a higher average milk production than the herds with a low incidence. The metabolic differences may be partially explained by differences in milk yield, feeding or management factors. In the risk factor study (IV), herd size, high individual daily milk yield, but not average herd yield, were found to be risk factors for DA and clinical ketosis. The average milk yield in high-incidence herds in study III was higher than the average milk yield in high-incidence herds in study IV. The average milk yield in low-incidence herds in study III was lower than the average milk yield in

low-incidence herds in study IV. The herds included in study III had a one year longer history of being high- or low-incidence herds than the herds in study IV, and the apparent difference in association with milk yield in studies III and IV implies that high average herd milk yield may be a risk factor in herds with long-term high-incidences of DA or clinical ketosis. On the other hand, the results in study IV implies that the mean level of herd milk yield may be of less importance than the maximum milk yield of individual cows, and a high maximum daily milk yield is most influential in the development of DA and ketosis. A high maximum daily milk yield will probably result in a deeper or more long-lasting negative energy balance around calving and could therefore be more stressful for the cow.

Several studies have found a high milk yield to increase the risk of DA or ketosis at an individual level (Fleischer *et al.*, 2001; Constable *et al.*, 1992; Gröhn *et al.*, 1989) and herd level (Constable *et al.*, 1992; Gröhn *et al.*, 1989; Coppock, 1974), but others have found no such association (Rohrbach *et al.*, 1999; Rajala & Gröhn, 1998; Gröhn *et al.*, 1995; Martin *et al.*, 1978a). Conflicting results may be explained by differences in feeding and management, as well as an effect of the disease on current lactation, confounding the effect of milk yield.

In study III, cholesterol concentrations were higher in high- than in low-incidence herds. This, along with a higher average milk yield in the high-incidence herds, indicated a higher feed intensity in the high-incidence herds than in the low-incidence herds. In addition, lower cholesterol concentrations were found in cows with DA compared with control cows in study I and variability in cholesterol concentrations was found among high-incidence herds pp in study II. Cholesterol concentrations have been associated with feed-intake (Janovick Guretzky et al., 2006). Cholesterol could potentially be useful as an indicator of feed intake the first weeks of lactation in herds where it is difficult to determine feed intake, for example in systems with ad lib feeding strategies and/or TMR, but further research is needed before specific recommendations can be given.

5.2.3 Proportions of cows outside reference ranges

In other countries, proportions of cows with NEFA and BHB outside reference ranges are used to evaluate energy balance or to find subclinical ketosis at the herd level (Oetzel, 2004). In study III, the proportion of cows outside given reference ranges were different between the herd categories. Oetzel (2004) have suggested alarm levels at 10% of the sampled cows above the reference ranges for NEFA ap (0.4 mmol/L) and BHB pp (>1.4 mmol/L), respectively. A higher overall proportion of cows in high-incidence herds

were above the suggested reference range for NEFA ap, compared with low-incidence herds. However, both herd categories had more than 10% of the sampled cows above the alarm level for prepartum negative energy balance. Neither mean values nor proportion of cows above reference range differed for BHB, between high- and low-incidence herds, but both herd categories had more than 10% of cows with BHB concentrations above the alarm level for subclinical ketosis. Determination of BHB is recommended to find herds with subclinical ketosis (Oetzel, 2004), but seems less useful under Swedish conditions. Differences in feed composition between Sweden and other countries may be an explanation to why differences in BHB concentrations were not detected between high- and low-incidence herds in study III. Reference ranges for NEFA and BHB may need to be established for Swedish conditions before proportions of cows are applicable in herd health work.

5.2.4 Blood parameters associated with liver cell damage

Mean concentrations of AST and GD were similar in high- and low-incidence herds (study III) and among high-incidence herds (study II). The blood parameters were thus not useful at the herd level to find herds with liver cell damage and possible occurrence of hepatic lipidosis. In study I, the concentrations of AST and GD were elevated in cows with DA, and these enzymes can be used to find individual cows with liver cell damage, possibly indicating hepatic lipidosis. In addition, the concentrations of NEFA and BHB were increased in the cows with DA and increased concentrations of BHB and NEFA in the blood, have been associated with hepatic lipidosis (Bobe *et al.*, 2004; Grum *et al.*, 1996).

5.3 Blood parameters and BCS as tools on cow and herd level

Blood parameters and BCS can be used to find herds at risk for subclinical or clinical manifestations of metabolic disorders, or to monitor energy metabolism (Quiroz-Rocha *et al.*, 2009; Macrae *et al.*, 2006; Mulligan *et al.*, 2006; LeBlanc *et al.*, 2005; Eckersall & Conner, 1988). In most countries national disease data bases are not available and it is uncommon that disease records are kept in all dairy herds. As a consequence, disease incidence is unknown. When incidence data are not available or under circumstances where the validity of disease records is uncertain, blood parameters may be an alternative way of monitoring herd health. In addition, records of disease incidence may more be a reflection of the past health status, while diagnostics based on blood metabolites reflect the current status.

The usefulness of blood profiles on herd level is, however, influenced by factors such as disease incidence, management and feeding routines, and level of milk production.

There is a large between country variability in these aspects which needs to be considered. Swedish dairy herds have a fairly low disease incidence, especially of DA and clinical ketosis, a high average milk yield, and cows are kept indoors during the winter season. The usefulness of blood sampling to find herds at risk for metabolic disorders may be questionable in a situation with low disease incidences. In addition, blood sampling must be carried out by veterinarians under Swedish legislation, and some of the parameters in a blood profile, for example insulin, may be expensive to analyze because they are not part of a routine analysis in many laboratories.

5.3.1 Usefulness of blood parameters

Study II showed that there were differences in blood parameters and BCS among herds with a high incidence of DA or clinical ketosis. Insulin ap, NEFA ap and pp, cholesterol pp, BCS in dry cows, and body condition loss in early lactation were found useful to identify problem areas in herds with metabolic disorders. Elevated NEFA concentrations can be used to pinpoint problem areas in the herd, and to give advice on in what animal groups to focus on to improve herd performance. The results in study III indicated that mean blood values of RQUICKI, glucose, NEFA, insulin, and cholesterol identified differences in metabolism at the herd level under Swedish conditions. Mean concentrations of haptoglobin were not different between the herds in study II or between herd categories in study III. However, cows with DA had increased haptoglobin concentrations in study I. The studies in this thesis suggest that haptoglobin can be useful in individual cows to find cows with an inflammatory response, but less so at the herd level to find problem herds.

The variability in blood profiles among high-incidence herds indicated that it may be difficult to assess differences in blood profiles between high- and low-incidence herds with singles blood parameters. Blood profiles should be used as a complement to examinations and evaluations of feeding and management in herd health work.

5.3.2 Usefulness of RQUICKI

In the ROC analysis in study III, the predictive value for RQUICKI for 1–3 weeks pp was higher than for each of the parameters included in the index (NEFA, glucose, insulin; paper III). This implied that RQUICKI may be a more sensitive marker of metabolic imbalances than separate evaluations of

glucose, insulin, and NEFA. The advantage, however, is only slight, as exemplified by the positive predictive value (i.e. the proportion of test-positive cows correctly identified as being in a high-incidence herd) of 0.15 for RQUICKI (assuming a prevalence of 10%) versus 0.12 for NEFA (the second best indicator); the corresponding negative predictive values (i.e. the proportion of test-negative cows correctly identified as being in a low-incidence herd) were 0.95 for RQUICKI and 0.94 for NEFA, respectively. Before the RQUICKI can be used in preventive herd health work, further research into how to improve the test characteristics, for example by selecting sub-populations of herds or animals for testing or by targeting specific periods after calving, is necessary.

By serial interpretation of test results from several cows, sensitivity for RQUICKI can be improved, although the risk for false positive test results will increase. Likewise, specificity can be improved, but with an increased risk of false negative test results, with a parallel interpretation of test results from several cows. By erroneous classification of cows in a high-incidence herd, clinical disease may become manifest with consequences for animal welfare as well as economic losses. The costs due to left-sided DA and ketosis have been estimated in USA, in 1997, to 340 USD and 145 USD, respectively (Kelton *et al.*, 1998).

5.4 Risk factors for DA or ketosis

The risk factors for being a herd with a high incidence of DA or ketosis identified in study IV were all biologically relevant. An increasing milk yield is something most farmers strive for and herd size is increasing for economical reasons. These two risk factors are thus not easily influenced although there are discussions about the most profitable level of milk yield not necessarily being the highest possible. Larger herd size was not found to be a risk factor for either DA or ketosis in an earlier study (Erb & Gröhn, 1988).

Not cleaning the feeding platform daily increased the odds of being a high-incidence herd twelvefold. Suboptimal feed bunk management and feed freshness prepartum have previously been shown to be a risk factor for DA (Cameron *et al.*, 1998).

The advice to clean the feeding platform on a daily basis is highly relevant, easy to explain and possible to implement in all herds. However, cleaning the feeding platform may also be a proxy for good overall management. In herds where priority is given to cleaning the feeding platform, the herdsmen may also strive for good feed hygiene and feed

availability. In addition, close and frequent observation of cows during the work day probably facilitates early detection of sick cows and may have a positive effect on herd health.

Keeping all dry cows in one group increased the odds of being a high-incidence herd eightfold. It is of utmost importance to ensure proper feed rations and accessibility to feed for all dry cows. Several factors associated with overfeeding dry cows have been shown to predispose for DA and ketosis (Rukkwamsuk *et al.*, 1999a; Correa *et al.*, 1990). When keeping dry cows in one group, there is an obvious risk that far-off dry cows are overfed and close-up dry cows are underfed because of the higher energy demand closer to parturition and the common practice to lead-feed close-up dry cows, thereby giving all cows in the group access to more energy-dense feed. In addition, feed access may be limited in larger cow groups. Depending on the housing system, it can be difficult to change number of groups with dry cows.

Statistical power is a point of concern in studies with a limited number of study objects. In study IV, relatively large differences were needed for statistically significant differences to be found and thus, more subtle differences in potential risk factors may not have been detected. The lack of significant associations should therefore be viewed with caution.

5.5 Metabolic disorders

In studies II–IV, DA and clinical ketosis were considered marker diseases for the complex of energy-related metabolic disorders around calving and in early lactation. Displaced abomasum and clinical ketosis were chosen because they require veterinary treatment. Ketosis can be prevented by prophylactic treatment in dairy herds, but cases of clinical ketosis that need medical treatment are treated by veterinarians and should be reported to the NADRS. The incidence of veterinary treated ketosis, however, most likely underestimates the true incidence of clinical ketosis. Several risk factors are similar for DA and ketosis and the diseases have been shown to interrelate and to be associated with other disorders in the transition period (Erb & Gröhn, 1988). Ketosis can be both a risk factor for and a consequence of DA (Curtis *et al.*, 1985). Examples of other metabolic disorders are hepatic lipidosis and hypocalcemia (Bobe *et al.*, 2004; Goff & Horst, 1997), and disorders with a presumed metabolic component are retained placenta, udder edema, subclinical rumen acidosis and laminitis (Kleen *et al.*, 2003; Goff & Horst, 1997; Nocek, 1997; Gröhn *et al.*, 1989).

Results from study I confirmed that DA and subclinical ketosis were common findings in the same herds. The suggested cut-point for excessive concentrations of BHB in cows with subclinical ketosis varies between authors (Oetzel, 2004; Whitaker, 2004; Nielen *et al.*, 1994), but using a cut-point of 1.4 mmol/L (Duffield *et al.*, 2009), 64% of the DA cows and 30% of the healthy controls in study I had a subclinical ketosis. Subclinical ketosis was found to increase the risk for both DA and clinical ketosis (Duffield *et al.*, 2009; Duffield, 2000). The assumption to use these two diseases as a marker for metabolic disorders seems reasonable.

5.6 Change in blood profiles- cause or effect

A point of discussion is whether the disorders with a metabolic component are caused by a change in metabolism, or if the disease itself causes a change in metabolism. Studies that show differences in blood parameters before the disease is clinically manifest indicate that there are differences in markers of metabolism before the diseases are diagnosed (Quiroz-Rocha *et al.*, 2009). Increases in concentrations of NEFA, BHB and AST of the same magnitude as in DA cows in study I have been found in cows that later developed DA (LeBlanc *et al.*, 2005; Van Winden *et al.*, 2003; Geishauser *et al.*, 1997). This implies that some of the changes may last over some time, related to other diseases foregoing the DA or to early stages in the development of DA. Indeed many of the DA cows in study I had a long duration of symptoms before the DA was diagnosed. The changes prior to diagnosis in NEFA and BHB may be caused by excessive NEB (Van Winden *et al.*, 2003). In addition, metabolic changes were found at the herd level (papers II and III), indicating that cows in herds where metabolic disorders were more common indeed had a compromised metabolism.

5.7 Methodological considerations

5.7.1 Assessing blood parameters in dairy cows

When assessing blood parameters in dairy cows, there are several sources of variation, for example, between animal-handling regimes, between sample-handling regimes, and between laboratory methods. Thus, comparing blood parameters between cows and herds, and between studies, is challenging.

To minimize bias, blood sampling procedures and analysis methods were standardized in each of the studies in this thesis. In study I, DA and control cows were sampled at the same time, in their own herds. In studies II and III, the herds were sampled after feeding, as recommended in earlier studies,

to avoid diurnal variation (Whitaker, 2004; Eicher *et al.*, 1999). These recommendations apply to herds fed concentrates twice daily; since none of the herds in studies II and III was fed concentrates only twice daily, diurnal variation was probably less pronounced. Another recent study of cows fed TMR found diurnal variation in NEFA concentrations, but less variation in BHB (Quiroz-Rocha *et al.*, 2010). The authors concluded that consistency in sampling time relative to feeding time was important to be able to compare blood results. In addition, glucose, NEFA, and BHB concentrations have been found to rise in response to the mild stress associated with handling (Leroy *et al.*, 2009; Nielsen *et al.*, 2003). Cows in studies II and III were confined immediately before sampling and stress was kept to a minimum.

5.7.2 Selection bias

Misclassification can be present in the studies included in this thesis if there are discrepancies between farmer records of diseases and those registered at NADRS for the study herds. In a validation study of disease records for Swedish herds, overall completeness of the NADRS was estimated to be 71–75%, though the completeness for metabolic disorders was found to be higher, and DA incidence rates estimated from farmer records (0.9 cases per 100 cow-years) and from the NADRS (0.8) were not statistically significantly different (Mörk *et al.*, 2010; Mörk *et al.*, 2009). Corresponding incidence rates for the category clinical ketosis and inappetence were 1.5 cases per 100 cow-years in farmer recordings and 1.4 cases in the NADRS. At the herd visit, the disease incidences for DA and clinical ketosis were double-checked with the herdsman and low-incidence herds did not have cases of DA or clinical ketosis. Hence, for metabolic disorders and DA and clinical ketosis specifically, the incidences in NADRS can be considered reliable, so high- and low-incidence herds were most likely classified correctly.

The herds included in studies III and IV had to meet inclusion criteria based on incidence rates over three years. This limited the possible number of study herds and all eligible herds whose owners were willing to participate were included. In addition, in most herds, all eligible cows were blood sampled. A random selection of herds or cows was not possible in any of the studies, which may reduce the external validity of studies III and IV. However, on the basis of geographical location, herd size, and milk production, the herds examined in all four studies were representative for Swedish average- to high-producing dairy herds with more than 50 cows. Such herds will be the dominant future type in Sweden, so inferences to the target population, i.e., Swedish dairy herds enrolled in SOMRS with more than 50 cows, can be drawn, but with caution.

In studies III and IV, there was moderate interest among herd owners to participate. However, the willingness was similar for both high- and low-incidence herds, approximately 30%, and should thus not have introduced any bias.

5.7.3 Information bias

The same investigator visited all the herds in studies II–IV, and herd-level data were collected through interviews and observations to ensure that all herds were evaluated similarly and that misunderstanding regarding questions were avoided.

The high-incidence herds examined in studies II–IV could have a combination of cases of DA and clinical ketosis. It was thus possible for herds with only one of the two diseases to be included in studies III and IV. Furthermore, because both DA and ketosis are multi-factorial diseases, manifest problems in a herd may have various aetiologies. This could have led to a heterogeneous classification of high-incidence herds, which makes it less likely to find differences between high- and low-incidence herds. The diseases used to categorize herds and the variability in risk factors may contribute to a non-differential misclassification biasing the association measure towards the null hypothesis.

5.7.4 Statistical methods

Linear mixed models were used in studies I–III. The use of linear mixed models made it possible to control for differences in factors such as breed and parity that may affect the results of blood parameters. In addition, clustering within herd and within cows (i.e., between repeated sampling occasions, study II) could be taken into account in the modelling.

All blood parameters and BCS were modelled separately; however, the parameters likely co-vary because they are somewhat related to the same biological processes. It is therefore possible that modelling a combination of parameters is more useful than modelling each parameter separately. An approach to the statistical modelling where several blood parameters are included may thus be advantageous and should be addressed in future research.

6 Conclusions

- Overall, this thesis aims to gain insight into the potential use of blood parameters and BCS for cows to identify herds at risk of DA or ketosis and to investigate herd-level risk factors for these disorders.

- There were distinct differences in blood profiles between cows diagnosed with DA and healthy control cows, indicating a severe NEB, liver cell damage, and an inflammatory response.

- In DA cows, no significant differences were found in blood parameters at different time intervals from calving.

- Many cows with DA had a long duration of symptoms before the DA was diagnosed. They also had other diseases before or at the same time as the DA were diagnosed.

- Mean blood concentrations in cows from dairy herds with a long-term high-incidence of DA, clinical ketosis or both, differed in parameters estimating energy metabolism including RQUICKI, NEFA, insulin, glucose, and cholesterol, in the periparturient period and early lactation versus in cows in low-incidence herds.

- There were variations in BCS and blood profiles among herds with a high incidence of metabolic disorders, therefore it may be possible to further pinpoint distinct problem areas within the herd.

- The most important time periods to sample cow to assess metabolism were before calving and in early lactation, in accordance with earlier work.

- RQUICKI may be a tool with which to identify specific problem herds.

- High maximum daily milk yield in multiparous cows, large herd size, keeping all dry cows in one group, and not having daily routines to clean the feeding platform were found to be risk factors for a herd having a high incidence of DA, clinical ketosis or both.

7 Recommendations for farmers, advisors and veterinarians

- Both BCS and change in BCS from calving to 6 weeks pp can be used as monitoring tools the first weeks of lactation.
- Avoid overconditioned dry cows, i.e. BCS 4 or higher.
- Avoid excessive BCS loss from calving to 6 weeks pp.
- For the individual cow, blood parameters such as NEFA, BHB, cholesterol, AST, GD and haptoglobin can be useful to assess cow health.
- Haptoglobin is an inflammatory marker that may be useful to find cows that need extra attention in the transition period.
- At the herd level, NEFA concentrations can be used to screen dairy herds to find cows with a compromised energy metabolism. High concentrations of NEFA in blood indicate excessive adipose tissue mobilization.
- A large proportion of cows with DA had symptoms more than 4 days. Strive for an early diagnosis and treatment.
- Do not keep all cows in one group during the dry period.
- Clean the feeding table daily.

8 Future research

As dairy farming undergoes changes in housing, management, feed, and the genetic material of the dairy cows, higher milk production levels than today's can be anticipated. In the future, the transition period will become an even greater challenge for the dairy cows and farmers to get through without the cow succumbing to diseases related to NEB. Hence, further research into metabolism, metabolic disorders, and effective prophylactic measures is needed.

8.1 Studies of biological markers of metabolism

- Further studies to determine whether fructosamine (or other long-term measures of glucose concentration) is useful in dairy cows for determining long-term glucose concentrations would be of interest.

- Around the time of calving, decreased insulin sensitivity in peripheral tissue is part of the physiological adaptation to a glucose-sparing state in the cow (Bell, 1995). Further studies of detrimental effects of decreased insulin sensitivity and the usefulness of RQUICKI to detect and possibly quantify the degree of decreased insulin sensitivity in lactating dairy cows is advocated.

- By preselecting herds or individuals, the diagnostic value of RQUICKI may be further improved.

8.2 Studies of metabolic disorders

- The studies in this thesis, as well as earlier studies, indicate that early changes related to DA may be evident several days before the disease is

manifested. To learn more about the pathogenesis of DA and other metabolic disorders, maybe cows should be studied in the dry period or even in late lactation. It would be of particular interest to sample blood from cows that later develop a DA or other metabolic diseases. However, such studies are difficult to carry out in countries with low incidences of the diseases.

- Displaced abomasum is a multifactorial disease with an economic impact in herds in which the incidence is high. In the USA, the economic losses due to left-sided DA were estimated to 340 USD as of 1997 (Kelton *et al.*, 1998). It would be useful to study the productivity and survival of cows treated for DA and their daughters, to assess whether it is economic to treat and breed cows diagnosed with DA.

- Another area of key interest is depressed immune function in cows around the time of calving and its relationship to metabolism. Further studies to elucidate how metabolism and immunology are associated with reproduction could potentially improve animal welfare in the transition period.

8.3 Studies of risk factors for metabolic disorders

- Future herds will likely be larger than current herds and will be housed in cubicle systems. Larger herd size implies less time spent per cow and new solutions for management, feeding, and housing. Sweden has a high proportion of AMS systems, so it would be useful to identify risk factors for metabolic disorders in herds managed using AMS. By matching production level and herd size between high- and low-incidence herds, further knowledge may be attained.

- Feed factors such as suboptimal chemical composition, physical structure or hygienic quality may all contribute to an increased risk for metabolic disorders. Further studies to elucidate this area would be very useful.

9 Populärvetenskaplig sammanfattning

Löpmagsförskjutning och ketos hos svenska mjölkkor
Blodprofiler och riskfaktorer

9.1 Bakgrund

I samband med kalvningen förändras förutsättningarna för kon. Hon går från att vara sinlagd och dräktig till att producera mjölk. Efter kalvningen ökar energibehovet flerfaldigt vilket medför att kon hamnar i en negativ energibalans. Det beror på att kon inte kan äta tillräckligt för att täcka energibehovet för underhållet av kroppen och för mjölkproduktionen. För att täcka energibehovet används reservenergi från fettdepåer och muskulatur. En viss negativ energibalans är normalt men vid kraftig negativ energibalans ökar risken för att kon skall bli sjuk.

Till ämnesomsättningssjukdomarna räknas till exempel löpmags-förskjutning, ketos, leverförfettning och kalvningsförlamning. Det finns också sjukdomar som delvis beror på ämnesomsättning, till exempel kvarbliven efterbörd och livmoderinflammation. Löpmagsförskjutning och ketos utgör ungefär 2 % av behandlingsorsakerna hos nötkreatur.

Alla sjukdomar har stor betydelse för kon. En sjukdom ökar risken för att hon skall insjukna även i andra sjukdomar. Genom att förebygga sjukdomar i stället för att behöva akutbehandla sjuka kor ökar djurvälfärden och arbetsinsatserna kan läggas på andra arbetsuppgifter än särbehandling av sjuka kor.

För att kunna bedriva förebyggande arbete krävs kunskaper om vad som orsakar ämnesomsättningssjukdomar och en effektiv övervakning av hälsoläget i besättningarna. Redan idag används förekomst av sjukdomar och andra hjälpmedel för att underlätta övervakningen av hälsoläget i besättningen. Syftet med studierna som ingår i denna avhandling var att

undersöka om blodprofiler och hull går att använda för att utreda ämnesomsättningsstörningar i svenska besättningar. Riskfaktorer för löpmagsförskjutning och ketos på besättningsnivå undersöktes också.

9.2 Sammanfattning av studier och resultat

Studierna utfördes under 2005 och 2006 i svenska mjölkkobesättningar. I tre av studierna studerades hull och blodvärden som avspeglar energiomsättning, skador på leverceller, inflammationer och ett ämnesomsättningsindex (RQUICKI) som används på människa för att bedöma insulinkänslighet.

I studie I jämfördes blodvärden mellan 69 kor med löpmagsförskjutning och 104 friska kor i samma besättningar och i samma laktationsstadier. Besättningarna hade i medeltal 84 kor och producerade 9501 kg energikorrigerad mjölk (ECM). Resultaten visade att det var stor skillnad i markörer för ämnesomsättning hos kor med löpmagsförskjutning och friska kontroller. Blodanalyserna visade att kor med löpmagsförskjutning var i djup negativ energibalans och mobiliserade stora mängder kroppsfett. Vidare hade de en ökad andel skadade leverceller och en högre grad av inflammationer i kroppen. Cirka 70 % av korna i studien hade eller hade haft minst en annan sjukdom tillsammans med löpmagsförskjutningen.

Det är ibland svårt att upptäcka löpmagsförskjutning på grund av att symptomen är ospecifika. Många av korna hade haft symptom mer än tre dagar innan de fick sin diagnos. Flera kor var magra och har troligen minskat i vikt under sjukdomen. Det är viktigt med en tidig diagnos och behandling av korna för att minimera tiden med störd ämnesomsättning. Flera blodparametrar kan vara värdefulla att undersöka; inflammationsmarkören och markörer för energiomsättning och skadade leverceller kan användas för att bedöma kons allmänstatus.

I studie II studerades 5 besättningar med hög förekomst av löpmagsförskjutning och ketos. Nittiofyra friska kor provtogs var tredje vecka från 4 veckor innan kalvning till 9 veckor efter kalvning. Besättningarna hade från 3 till 9,4 % löpmagsförskjutningar och ketoser. Besättningarna hade mellan 150 och 300 kor och en mjölkavkastning på 9500–11500 kg ECM. Samtliga besättningar hade sinkor som var över medelhull (minst 4 på en femgradig skala). Dessa kor förlorade mer än en hullpoäng från sinperioden till 4–6 veckor efter kalvning. Det fanns skillnader i blodprofiler mellan besättningarna som visade att kor i vissa besättningar började bryta ner fettreserver redan innan kalvningen medans andra kor använde sina fettreserver först efter kalvningen. Besättningarna med kor som började bryta ner fett innan kalvningen underutfodrade

troligen sina kor under sinperioden. Resultaten visade att det går att använda blodparametrar både för att hitta och för att förebygga hälsostörningar i besättningar.

I studie III jämfördes medelvärden från 943 kor i besättningar med hög (19 stycken) eller låg (15 stycken) förekomst av löpmagsförskjutning eller ketos. Besättningar med hög förekomst hade minst 4 fall av löpmagsförskjutning eller ketos per 100 kor och besättningar med låg förekomst hade som mest 1 fall av sjukdomarna per 100 kor under två till tre år innan studien. Proverna togs under perioden 4 veckor innan kalvning till 9 veckor efter kalvning. Det fanns skillnader i blodprofiler som mäter ämnesomsättning hos kor i besättningar med hög förekomst av löpmagsförskjutning eller ketos jämfört med besättningar med låg förekomst av sjukdomarna. Bland annat var RQUICKI lägre de första veckorna efter kalvningen i besättningarna med hög förekomst av de två sjukdomarna. Besättningarna med hög förekomst av sjukdomarna hade 1400 kg ECM högre medelmjölkavkastning och fler kor (10471 kg ECM och 123 kor i medeltal) än besättningarna med låg förekomst (9077 kg ECM och 74 kor i medeltal). Resultaten visade att det fanns skillnader i ämnesomsättningen mellan besättningskategorierna.

I studie IV studerades riskfaktorer för hög förekomst av löpmags-förskjutning eller ketos. Fyrtio besättningar med hög förekomst och 20 besättningar med låg förekomst över en tidsperiod på tre år undersöktes. Personalen besvarade frågor om avkastning, inhysning, utfodring och skötsel. Djurgrupperna som frågorna gällde var förstakalvare de sista veckorna innan kalvningen, sinkor och kor i tidig laktation. Besättningarna med hög förekomst av löpmagsförskjutning och ketos hade en avkastning på 9818 kg ECM och i medeltal 125 kor. Besättningarna med låg förekomst hade en avkastning på 9308 kg ECM och i medeltal 72 kor. Fyra riskfaktorer hittades. Högre daglig toppavkastning hos äldre kor och större besättningsstorlek ökade risken för en besättning att ha hög förekomst av löpmagsförskjutning eller ketos. Om alla sinkor hölls i en grupp i stället för i flera grupper och om foderbordet inte sopades dagligen för högdräktiga kvigor ökade oddsen för besättningen att vara en högriskbesättning med 8 respektive 12 gånger. Resultaten visar att skötsel av sinkorna och foderhygien är viktiga områden i besättningsarbetet.

9.3 Slutsatser

Blodprofiler kan användas på individnivå för att bedöma graden av fettnedbrytning, levercellskador och inflammation hos kor. På så vis går det

att hitta individer som behöver extra tillsyn. På besättningsnivå kan blodprofiler ge information om och när eller i vilka grupper korna bryter ner överdrivna mängder av sina fettreserver. Hull kan vara användbart i besättningar för att hitta feta kor till exempel under sinperioden eller för att hitta kor som minskar snabbt i hull och som behöver extra omvårdnad. Riskfaktorerna för hög förekomst av löpmagsförskjutning eller ketos var kopplade till större besättningsstorlek, högre avkastning och skötselfaktorer som kan tänkas vara vanligare i stora besättningar där mindre tid finns per djur. Daglig rengöring av foderbordet minskade risken. Troligen på grund av förbättrad foderhygien. Daglig rengöring av foderbordet är ett enkelt råd som kan genomföras i alla Sveriges besättningar. Att hålla alla sinkor i en grupp ökade risken. Troligen på grund av bristande foderstyrning vilket leder till att sinkor blir feta. Eftersom mjölkavkastning och besättningsstorlek troligen kommer fortsätta att öka i Sverige är det viktigt att komma fram till förebyggande åtgärder som kan minska förekomsten av ämnesomsättningssjukdomar i perioden kring kalvningen.

References

Adewuyi, A.A., Gruys, E. & van Eerdenburg, F.J.C.M. (2005) Non esterified fatty acids (NEFA) in dairy cattle. A review. *Veterinary Quarterly,* 27(3), 117–126.

Agenäs, S., Dahlborn, K. & Holtenius, K. (2003) Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. *Livestock Production Science,* 83(2-3), 153–164.

Andersson, L. (1988) Subclinical ketosis in dairy cows. *Veterinary Clinics of North America: Food Animal Practice,* 4(2), 233–251.

Andersson, L. & Emanuelson, U. (1985) An epidemiologic study of hyperketonemia in Swedish dairy cows- Determinants and the relation to fertility. *Preventive Veterinary Medicine,* 3(5), 449–462.

Armbruster, D.A. (1987) Fructosamine: structure, analysis, and clinical usefulness. *Clinical Chemistry,* 33(12), 2153–2163.

Bauman, D.E. & Currie, W.B. (1980) Partitioning of nutrients during pregnancy and lactation - A review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science,* 63(9), 1514–1529.

Begg, H. (1950) Diseases of the stomach of the adult ruminant. *The Veterinary Record,* 62(51), 797–808.

Bell, A.W. (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* 73(9), 2804–2819.

Björnhag, G., Jonsson, E., Lindgren, E. & Malmfors, B. (1989) *Husdjur- ursprung, biologi, avel.* Stockholm, Sweden: LTs förlag. pp. 9-17.

Bobe, G., Young, J.W. & Beitz, D.C. (2004) Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *Journal of Dairy Science,* 87(10), 3105–3124.

Bossaert, P., Leroy, J.L.M.R., De Campeneere, S., De Vliegher, S. & Opsomer, G. (2009) Differences in the glucose-induced insulin response and the peripheral insulin responsiveness between neonatal calves of the Belgian Blue, Holstein-Friesian, and East Flemish breeds. *Journal of Dairy Science,* 92(9), 4404–4411.

Cameron, R.E.B., Dyk, P.B., Herdt, T.H., Kaneene, J.B., Miller, R., Bucholtz, H.F., Liesman, J.L., VandeHaar, M.J. & Emery, R.S. (1998) Dry cow diet, management, and energy balance as risk factors for displaced abomasum in highproducing dairy herds. *Journal of Dairy Science,* 81(1), 132–139.

Cannas da Silva, J., Schauberger, G., Oliveira, M.R., Segao, S., Kumper, H. & Baumgartner, W. (2004) Does the weather influence the occurrence of abomasal displacement in dairy cows? *Deutsche Tierarztliche Wochenschrift,* 111(2), 51–57.

Cantley, C.E.L., Ford, C.M. & Heath, M.F. (1991) Serum fructosamine in ovine pregnancy toxaemia: a possible prognostic index. *The Veterinary Record,* 128(22), 525–526.

Cavestany, D., Blanc, J.E., Kulcsar, M., Uriarte, G., Chilibroste, P., Meikle, A., Febel, H., Ferraris, A. & Krall, E. (2005) Studies of the Transition Cow Under a Pasture-based Milk Production System: Metabolic Profiles. *Journal of veterinary medicine A,* 52(1), 1–7.

Constable, P.D., Miller, G.Y., Hoffsis, G.F., Hull, B.L. & Rings, D.M. (1992) Risk-factors for abomasal volvolus and left abomasal displacement in cattle. *American Journal of Veterinary Research,* 53(7), 1184–1192.

Coppo, J.A. (2001) Evolution of fructosaminaemia and glucaemia during the growth of unweaned and early weaned half-bred Zebu calves. *Veterinary Research Communications,* 25(6), 449–459.

Coppock, C.E. (1974) Displaced abomasum in dairy cattle: Etiological factors. *Journal of Dairy Science* 57(8), 926–933.

Correa, M.T., Curtis, C.R., Erb, H.N., Scarlett, J.M. & Smith, R.D. (1990) An ecological analysis of risk factors for postpartum disorders of Holstein-Friesian cows from thirty-two New York farms. *Journal of Dairy Science,* 73(6), 1515–1524.

Curtis, C.R., Erb, H.N., Sniffen, C.J., Smith, R.D. & Kronfeld, D.S. (1985) Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein Cows. *Journal of Dairy Science,* 68(9), 2347–2360.

Dawson, L.J., Aalseth, E.P., Rice, L.E. & Adams, G.D. (1992) Influence of fiber form in a complete mixed ration on incidence of left displaced abomasum in postpartum dairy-cows. *Journal of American Veterinary Medical Association* 200(12), 1989–1992.

Detilleux, J.C., Grohn, Y.T., Eicker, S.W. & Quaas, R.L. (1997) Effects of left displaced abomasum on test day milk yields of Holstein cows. *Journal of Dairy Science,* 80(1), 121–126.

Dohoo, I.R., Martin, W. & Stryhn, H. (2009) *Veterinary epidemiologic research. Second edition.* Charlottetown, VER Inc. pp. 107-108.

Doll, K., Sickinger, M. & Seeger, T. (2009) New aspects in the pathogenesis of abomasal displacement. *Veterinary Journal,* 181(2), 90–96.

Drackley, J.K. (1999) Biology of dairy cows during the transition period: the final frontier? *Journal of Dairy Science,* 82(11), 2259–2273.

Duffield, T.F. (2000) Subclinical ketosis in lactating dairy cattle. *Veterinary Clinics of North America: Food Animal Practice,* 16(2), 231–253.

Duffield, T.F., Lissemore, K.D., McBride, B.W. & Leslie, K.E. (2009) Impact of hyperketonemia in early lactation dairy cows on health and production. *Journal of Dairy Science,* 92(2), 571–580.

Dyrendahl, I. (1996) Peter Hernquist från Härlunda. In Swedish *Veterinärhistoriska muséets meddelandeserie* 38.

Earley, B. & Crowe, M.A. (2002) Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *Journal of Animal Science,* 80(4), 1044–1052.

Eckersall, P.D. & Conner, J.G. (1988) Bovine and canine acute phase proteins. *Veterinary Research Communications,* 12(2-3), 169–178.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. & Webster, G. (1989). A Body Condition Scoring Chart for Holstein Dairy Cows. *Journal of Dairy Science,* 72(1), 68–78.

Eicher, R., Liesegang, A., Bouchard, E. & Tremblay, A. (1999) Effect of cow-specific factors and feeding frequency of concentrate on diurnal variation of blood metabolites in dairy cows. *American Journal of Veterinary Research,* 60(12), 1493–1499.

Emanuelson, U. (1988) The national Swedish animal disease recording system. *Acta Veterinaria Scandinavica Supplementum*, 84, 262–264.

Emery, R.S., Liesman, J.S. & Herdt, T.H. (1992) Metabolism of long chain fatty acids by ruminant liver. *Journal of Nutrition,* 122(3), 832–837.

Erb, H.N. & Gröhn, Y.T. (1988) Epidemiology of metabolic disorders in the periparturient dairy cow. *Journal of Dairy Science,* 71(9), 2557–2571.

Erb, H.N., Smith, R.D., Hillman, R.B., Powers, P.A., Smith, M.C., White, M.E. & Pearson, E.G. (1984) Rates of diagnosis of 6 diseases of Holstein cows during 15-day and 21-day intervals. *American Journal of Veterinary Research,* 45(2), 333–335.

Fleischer, P., Metzner, M., Beyerbach, M., Hoedemaker, M. & Klee, W. (2001) The relationship between milk yield and the incidence of some diseases in dairy cows. *Journal of Dairy Science,* 84(9), 2025–2035.

Geishauser, T. (1995) Abomasal displacement in the bovine- a review on character, occurrence, aetiology and pathogenesis. *Journal of veterinary medicine A,* 42(4), 229–251.

Geishauser, T., Leslie, K.E. & Duffield, T.F. (1999) Prevention and prediction of displaced abomasum in dairy cows. In: *Proceedings of American Associations of Bovine Practitioners* 1999. pp. 203–207.

Geishauser, T., Leslie, K.E., Duffield, T.F. & Edge, V. (1997) Evaluation of aspartate transaminase activity and beta-hydroxybutyrate concentration in blood as tests for prediction of left displaced abomasum in dairy cows. *American Journal of Veterinary Research,* 58(11), 1216–1220.

Gillund, P., Reksen, O., Karlberg, K., Randby, Å.T., Engeland, I. & Lutnaes, B. (1999) Utprövning av en holdvurderingsmetode på NRF-kyr. In Norweigan. *Norsk Veterinaertidsskrift,* 111(10), 623–632.

Goff, J.P. (2006a) Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. *Animal Feed Science and Technology,* 126(3-4), 237–257.

Goff, J.P. (2006b) Major advances in our understanding of nutritional influences on bovine health. *Journal of Dairy Science,* 89(4), 1292–1301.

Goff, J.P. & Horst, R.L. (1997) Physiological changes at parturition and their relationship to metabolic disorders [1,2]. *Journal of Dairy Science,* 80(7), 1260–1268.

Grum, D.E., Drackley, J.K., Younker, R.S., LaCount, D.W. & Veenhuizen, J.J. (1996) Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. *Journal of Dairy Science*, 79(10), 1850–1864.

Grummer, R.R. (1993) Etiology of lipid-related metabolic disorders in periparturient dairy cows. *Journal of Dairy Science,* 76(12), 3882–3896.

Grummer, R.R. (1995) Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science,* 73(9), 2820–2833.

Grummer, R.R., Mashek, D.G. & Hayirli, A. (2004) Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice,* 20(3), 447–470.

Gröhn, Y.T., Eicker, S.W., Ducrocq, V. & Hertl, J.A. (1998) Effect of diseases on the culling of Holstein dairy cows in New York state. *Journal of Dairy Science,* 81(4), 966–978.

Gröhn, Y.T., Eicker, S.W. & Hertl, J.A. (1995) The association between 305-day milk yield and disease in New York State dairy cows. *Journal of Dairy Science,* 78(), 1693–1702.

Gröhn, Y.T., Erb, H.N., McCulloch, C.E. & Saloniemi, H.S. (1989) Epidemiology of metabolic disorders in dairy cattle: Association among host characteristics, disease, and production. *Journal of Dairy Science,* 72(7), 1876–1885.

Hayirli, A. (2006) The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. *Veterinary Research Communications,* 30(7), 749–774.

Hayirli, A., Grummer, R.R., Nordheim, E.V. & Crump, P.M. (2002). Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. *Journal of Dairy Science,* 85(12), 3430–3443.

Herdt, T.H. (2000a) Ruminant adaptation to negative energy balance. *Veterinary Clinics of North America: Food Animal Practice,* 16(2), 215–230.

Herdt, T.H. (2000b) Variability characteristics and test selection in herd-level nutritional and metabolic profile testing. *Veterinary Clinics of North America: Food Animal Practice,* 16(2), 387–403.

Herdt, T.H., Stevens, J.B., Linn, J. & Larson, V. (1981a) Influence of ration composition and energy balance on blood betahydroxybutyrate (ketone) and plasma glucose concentrations of dairy cows in early lactation. *American Journal of Veterinary Research,* 42(7), 1177–1180.

Herdt, T.H., Stevens, J.B., Olson, W.G. & Larson, V. (1981b) Blood concentration of betahydroxybutyrate in clinically normal Holstein-Friesian herds and in those with a high prevalence of clinical ketosis. *American Journal of Veterinary Research,* 42(3), 503–506.

Heringstad, B., Chang, Y.M., Gianola, D. & Klemetsdal, G. (2005) Genetic analysis of clinical mastitis, milk fever, ketosis, and retained placenta in three lactations of Norwegian red cows. *Journal of Dairy Science,* 88(9), 3273–3281.

Hewett, C. (1974) On the causes and effects of variations in the blood profile of Swedish dairy cattle. *Acta Veterinaria Scandinavica Supplementum,* (50), 1–152.

Hirvonen, J., Eklund, K., Teppo, A.M., Huszenicza, G., Kulcsàr, M., Saloniemi, H. & Pyörälä, S. (1999a) Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. *Acta Veterinaria Scandinavica,* 40(1), 35–46.

Hirvonen, J., Huszenicza, G., Kulcsàr, M. & Pyörälä, S. (1999b) Acute-phase response in dairy cows with acute postpartum metritis. *Theriogenology,* 51(6), 1071–1083.

Hirvonen, J. & Pyörälä, S. (1998) Acute-phase response in dairy cows with surgically-treated abdominal disorders. *The Veterinary Journal,* 155(1), 53–61.

Hiss, S., Weinkauf, C., Hachenberg, S. & Sauerwein, H. (2009) Short communication: Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. *Journal of Dairy Science,* 92(9), 4439–4443.

Holtenius, K., Agenäs, S., Delavaud, C. & Chilliard, Y. (2003) Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *Journal of Dairy Science,* 86(3), 883–891.

Holtenius, K. & Holtenius, P. (1996a) Metabolic effects of glucocorticoids in sheep - Studies with a long-acting somatostatin analogue. *Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde,* 76(4-5), 224–230.

Holtenius, P. (1989) Plasma lipids in normal cows around partus and in cows with metabolic disorders with and without fatty liver. *Acta Veterinaria Scandinavica,* 30(4), 441–445.

Holtenius, P. & Holtenius, K. (1996b) New aspects of ketone bodies in energy metabolism of dairy cows: A Review. *Journal of veterinary medicine A,* 43(10), 579–587.

Holtenius, P. & Holtenius, K. (2007) A model to estimate insulin sensitivity in dairy cows. *Acta Veterinaria Scandinavica,* 49:29.

Holtenius, P., Niskanen, R. & Holtenius, K. (1986) Blood lipids, and lipoproteins in cows with abomasal displacement. In: *Proceedings of 14 World Buiatrics Congress*, Dublin, Ireland1986. pp. 47–51.

Ingvartsen, K.L. & Andersen, J.B. (2000) Integration of metabolism and intake regulation: A review focusing on periparturient animals. *Journal of Dairy Science,* 83(7), 1573–1597.

Ingvartsen, K.L., Dewhurst, R.J. & Friggens, N.C. (2003) On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. *Livestock Production Science,* 83(2-3), 277–308.

Ingvartsen, K.L. & Friggens, N.C. (2005) To what extent do variabilities in hormones, metabolites and energy intake explain variability in milk yield? *Domestic Animal Endocrinology,* 29(2), 294–304.

Itoh, N., Koiwa, M., Hatsugaya, A., Yokota, H., Taniyama, H., Okada, H. & Kudo, K. (1998) Comparative analysis of blood chemical values in primary ketosis and abomasal displacement in cows. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine,* 45(5), 293–298.

Janovick Guretzky, N.A., Carlson, D.B., Garrett, J.E. & Drackley, J.K. (2006) Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *Journal of Dairy Science,* 89(1), 188–200.

Jensen, A.L., Petersen, M.B. & Houe, H. (1993) Determination of the Frucotsamine Concentration in bovine Serum Samples. *Journal of veterinary medicine A,* 40(2), 111–117.

Joost, H.-G. & Thorens, B. (2001) The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). *Molecular membrane biology,* 18(4), 247–256.

Jorritsma, R., Jorritsma, H., Schukken, Y.H., Bartlett, P.C., Wensing, T. & Wentink, G.H. (2001) Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in The Netherlands. *Livestock Production Science,* 68(1), 53–60.

Kaneene, J.B., Miller, R., Herdt, T.H. & Gardiner, J.C. (1997) The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Preventive Veterinary Medicine,* 31(1-2), 59–72.

Kaneko, J.J., Harvey, J.W. & Bruss, M., L. (Eds.) (2008) *Clinical biochemistry of Domestic Animals*. London: Academic Press Limited. pp. 356–358, 883–895.

Karatzias, H. & Panousis, N. (2003) A study on the occurrence and aetiology of abomasal displacement in dairy cattle in Greece. *Cattle Practice,* 11 (3), 135–139.

Katoh, N. & Nakagawa, H. (1999) Detection of haptoglobin in the high-density lipoprotein and the very high-density lipoprotein fractions from sera of calves with experimental pneumonia and cows with naturally occurring fatty liver. *Journal of Veterinary Medical Science,* 61(2), 119–124.

Kelton, D.F., Lissemore, K.D. & Martin, R.E. (1998) Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. *Journal of Dairy Science,* 81(9), 2502–2509.

Kerestes, M., Faigl, V., Kulcsár, M., Balogh, O., Földi, J., Fébel, H., Chilliard, Y. & Huszenicza, G. (2009) Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. *Domestic Animal Endocrinology,* 37(4), 250–261.

Kessel, S., Stroehl, M., Meyer, H.H.D., Hiss, S., Sauerwein, H., Schwarz, F.J. & Bruckmaier, R.M. (2008) Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *Journal of Animal Science,* 86(11), 2903–2912.

Kim, I.H. & Suh, G.H. (2003) Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. *Theriogenology*, 60(8), 1445–1456.

Kleen, J.L., Hooijer, G.A., Rehage, J. & Noordhuizen, J.P.T.M. (2003) Subacute ruminal acidosis (SARA): A review. *Journal of veterinary medicine A,* 50(8), 406–414.

Kokkonen, T., Taponen, J., Anttila, T., Syrjälä-Qvist, L., Delavaud, C., Chilliard, Y., Tuori, M. & Tesfa, A.T. (2005) Effect of body fatness and glucogenic supplement on lipid and protein mobilization and plasma leptin in dairy cows. *Journal of Dairy Science,* 88(3), 1127–1141.

Komatsu, Y., Itoh, N., Taniyama, H., Kitazawa, T., Yokota, H., Koiwa, M., Ohtsuka, H., Terasaki, N., Maeno , K., Mizoguchi, M., Takeuchi, Y., Tanigawa, M., Nakamura, T., Watanabe, H., Matsuguchi, Y., Kukino, T. & Honma, A. (2002) Classification of abomasal displacement in cows according to histopathology of the liver and clinical chemistry. *Journal of Veterinary Medicine, Series A,* 49(9), 482–486.

Kronfeld, D.S. (1972) Diagnosis of metabolic disease of cattle. *Journal of the American Veterinary Medical Association,* 161(11), 1259–1264.

Kronfeld, D.S. (1982).Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. *Journal of Dairy Science,* 65, 2204–2212.

Lagerlöf, N. (1925a) Acetonämi eller småbrukaresjuka hos nötkreatur. In Swedish, *Svensk veterinär tidskrift,* 13, 141–155.

Lagerlöf, N. (1925b) Ett fall av kongenital (?) lägeförändring och kronisk löpmagsutvidgning hos nöt med ett 50-tal sår å löpmagens slemhinna. In Swedish, *Skandinavisk Veterinär-tidskrift för bakteriologi, patologi samt kött- och mjölkhygien,* 15(1), 71–98.

LeBlanc, S.J., Leslie, K.E. & Duffield, T.F. (2005) Metabolic predictors of displaced abomasum in dairy cattle. *Journal of Dairy Science,* 88(1), 159–170.

Leroy, J.L.M.R., Bossaert, P., Opsomer, G. & Bols, P.E.J. (2009) The effect of animal handling procedures on the blood non-esterified fatty acid and glucose concentrations of lactating dairy cows. *The Veterinary Journal,* In Press, Corrected Proof. E-published ahead of print.

Leroy, J.L.M.R., Vanholder, T., Mateusen, B., Christophe, A., Opsomer, G., de Kruif, A., Genicot, G. & Van Soom, A. (2005). Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction,* 130(4), 485–495.

Luthman, J. & Holtenius, P. (1972).Norepinephrine-induced fatty liver and hypocalcemia in sheep. *Acta Veterinaria Scandinavica,* 13(1), 31–41.

Macrae, A.I., Whitaker, D.A., Burrough, E., Dowell, A. & Kelly, J.M. (2006) Use of metabolic profiles for the assessment of dietary adequacy in UK dairy herds. *The Veterinary Record,* 159(20), 655–661.

Martin, S.W., Kirby, K.L. & Curtis, R.A. (1978a) Left abomasal displacement in dairy cows: Its relationship to production. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 19(9), 250–253.

Martin, S.W., Kirby, K.L. & Curtis, R.A. (1978b). Study of role of genetic factors in etiology of left abomasal displacement. *Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee,* 42(4), 511–518.

Mellanby, R.J. & Herrtage, M.E. (2002) Insulinoma in a normoglycaemic dog with low serum fructosamine. *Journal of Small Animal Practice,* 43(11), 506–508.

Milian-Suazo, F., Erb, H.N. & Smith, R.D. (1988) Descriptive epidemiology of culling in dairy cows from 34 herds in New York State. *Preventive Veterinary Medicine,* 6(4), 243–251.

Morrow, D.A. (1976) Fat cow syndrome. *Journal of Dairy Science,* 59, 1625–1629.

Mulligan, F.J., O´Grady, L., Rice, D.A. & Doherty, M.L. (2006) A herd health approach to dairy cow nutrition and production diseases of the transition cow. *Animal Reproduction Science* 96 (3-4), 331–353.

Muylle, E., Vandenhende, C., Sustronck, B. & Deprez, P. (1990) Biochemical profiles in cows with abomasal displacement estimated by blood and liver parameters. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine,* 37(4), 259–263.

Mörk, M., Lindberg, A., Alenius, S., Vågsholm, I. & Egenvall, A. (2009) Comparison between dairy cow disease incidence in data registered by farmers and in data from a disease-recording system based on veterinary reporting. *Preventive Veterinary Medicine,* 88(4), 298–307.

Mörk, M.J., Wolff, C., Lindberg, A., Vågsholm, I. & Egenvall, A. (2010) Validation of a national disease recording system for dairy cattle against veterinary practice records. *Preventive Veterinary Medicine,* 93(2-3), 183–192.

Nielsen, N.I., Ingvartsen, K.L. & Larsen, T. (2003) Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. *Journal of veterinary medicine A,* 50(2), 88–97.

Nocek, J.E. (1997) Bovine acidosis: Implications on laminitis. *Journal of Dairy Science,* 80(5), 1005–1028.

Oetzel, G.R. (2004) Monitoring and testing dairy herds for metabolic disease. *Veterinary Clinics of North America: Food Animal Practice,* 20(3), 651–674.

Olsson, S.-O., Baekbo, P., Hansson, S.Ö., Rautala, H. & Østerås, O. (2001) Disease Recording Systems and Herd Health Schemes for Production Diseases. *Acta veterinaria Scandinavica Supplementum,* 42(1), 51–60.

Ospina, P.A., Nydam, D.V., Stokol, T. & Overton, T.R. (2010) Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *Journal of Dairy Science*, 93(2), 546–554.

Payne, J.M., Dew, A.M., Manston, R. & Faulks, M. (1970) The use of a metabolic profile test in dairy herds. *The Veterinary Record,* 87(6), 150–158.

Perseghin, G., Caumo, A., Caloni, M., Testolin, G. & Luzi, L. (2001) Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *Journal of Clinical Endocrinology and Metabolism,* 86(10), 4776–4781.

Petersen, H.H., Nielsen, J.P. & Heegaard, P.M.H. (2004) Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research,* 35(2), 163–187.

Piñeiro, M., Piñeiro, C., Carpintero, R., Morales, J., Campbell, F.M., Eckersall, P.D., Toussaint, M.J.M. & Lampreave, F. (2007) Characterisation of the pig acute phase protein response to road transport. *The Veterinary Journal,* 173(3), 669–674.

Quiroz-Rocha, G.F., LeBlanc, S., Duffield, T., Wood, D., Leslie, K.E. & Jacobs, R.M. (2009) Evaluation of prepartum serum cholesterol and fatty acids concentrations as predictors of postpartum retention of the placenta in dairy cows. *Journal of the American Veterinary Medical Association,* 234(6), 790–793.

Quiroz-Rocha, G.F., LeBlanc, S.J., Duffield, T.F., Jefferson, B., Wood, D., Leslie, K.E. & Jacobs, R.M. (2010) Short communication: Effect of sampling time relative to the first daily feeding on interpretation of serum fatty acid and beta-hydroxybutyrate concentrations in dairy cattle. *Journal of Dairy Science,* 93(5), 2030–2033.

Rabasa-Lhoret, R., Bastard, J.-P., Jan, V., Ducluzeau, P.-H., Andreelli, F., Guebre, F., Bruzeau, J., Louche-Pellissier, C., Maîtrepierre, C., Peyrat, J., Chagne, J., Vidal, H. & Laville, M. (2003) Modified quantitative insulin sensitivity check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. *Journal of Clinical Endocrinology and Metabolism,* 88(10), 4917–4923.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D. (Eds.) (2007a) *Diseases of the alimentary tract - II, Left-side displacement of the abomasum and Right-side displacement o the abomasum and Abomasal volvulus*: Saunders Elsevier. Veterinary medicine, A textbook of the diseases of cattle, horses, sheep, pigs, and goats. pp. 354-367.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D. (Eds.) (2007b) *Metabolic diseases*: Saunders Elsevier. Veterinary medicine, A textbook of the diseases of cattle, horses, sheep, pigs, and goats. pp. 1661-1668.

Rajala, P.J. & Gröhn, Y.T. (1998) Disease occurence and risk factor analysis in Finnish Ayrshire cows. *Acta Veterinaria Scandinavica* 39(1), 1–13.

Rasmussen, L.K., Nielsen, B.L., Pryce, J.E., Mottram, T.T. & Veerkamp, R.F. (1999) Risk factors associated with the incidence of ketosis in dairy cows. *Animal Science,* 68(3), 379–386.

Rehage, J., Mertens, M., StockhofeZurwieden, N., Kaske, M. & Scholz, H. (1996) Post surgical convalescence of dairy cows with left abomasal displacement in relation to fatty liver. *Schweizer Archiv Fur Tierheilkunde,* 138(7), 361–368.

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J. & Berry, D.P. (2009) Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science,* 92(12), 5769–5801.

Rohrbach, B.W., Cannedy, A.L., Freeman, K. & Slenning, B.D. (1999) Risk factors for abomasal displacement in dairy cows. *Journal of the American Veterinary Medical Association,* 214(11), 1660–1663.

Ropstad, E. (1987) Serum fructosamine levels in dairy cows related to metabolic status in early lactation. *Acta Veterinaria Scandinavica,* 28(3-4), 291–298.

Rukkwamsuk, T., Kruip, T.A. & Wensing, T. (1999a) Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. *The Veterinary Quarterly,* 21(3), 71–77.

Rukkwamsuk, T., Kruip, T.A.M., Meijer, G.A.L. & Wensing, T. (1999b) Hepatic fatty acid composition in periparturient dairy cows with fatty liver induced by intake of a high energy diet in the dry period. *Journal of Dairy Science,* 82(2), 280–287.

Rukkwamsuk, T., Wensing, T. & Geelen, M.J.H. (1998) Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. *Journal of Dairy Science,* 81(11), 2904–2911.

Rukkwamsuk, T., Wensing, T. & Geelen, M.J.H. (1999c) Effect of overfeeding during the dry period on the rate of esterification in adipose tissue of dairy cows during the periparturient period. *Journal of Dairy Science*, 82(6), 1164–1169.

Shaver, R.D. (1997) Nutritional Risk Factors in the Etiology of Left Displaced Abomasum in Dairy Cows: A Review. *Journal of Dairy Science,* 80(10), 2449–2453.

Simensen, E., Osteras, O., Boe, K.E., Kielland, C., Ruud, L.E. & Naess, G. (2010) Housing system and herd size interactions in Norwegian dairy herds; associations with performance and disease incidence. *Acta Veterinaria Scandinavica*, 52:14.

Skinner, J.G., Brown, R.A.L. & Roberts, L. (1991) Bovine haptoglobin response in clinically defined field conditions. *The Veterinary Record* 128(7), 147–149.

Sorondo, M.L. & Cirio, A. (2009) Evaluation of the serum fructosamine test to monitor plasma glucose concentration in the transition dairy cow. *Journal of Dairy Research,* 76(2), 173–178.

Stengärde, L. & Pehrson, B.G. (2002) Effects of management, feeding, and treatment on clinical and biochemical variables in cattle with displaced abomasum. *American Journal of Veterinary Research,* 63(1), 137–142.

SVA *Surveillance of zoonotic and other animal disease agents in Sweden 2009*. [online] Available from: http://www.sva.se/upload/pdf/Tj%c3%a4nster%20och%20produkter/Trycksaker/Surveillance2009.pdf. [Accessed 20 September 2010].

SVA *Boskapspest*. In Swedish [online] Available from: http://www.sva.se/sv/navigera/Djurhalsa/Epizootisjukdomar/Boskapspest/ [Accessed 20 September 2010].

Swedish Board of Agriculture. (2010) *Yearbook of agricultural statistics 2010 including food statistics*. In Swedish [online] Available from: http://www.jordbruksverket.se/download/18.32b12c7f12940112a7c80002078/JS%C3%85+2010+Hela.pdf [Accessed 15 September 2010].

Swedish Dairy Association. (1994) *Cattle statistics 1994: Husdjursstatistik,* Eskilstuna.

Swedish Dairy Association. (2009) *Cattle statistics 2009: Husdjursstatistik,* Eskilstuna.

Swedish Dairy Association *Milk yield*. [online] Available from: http://www.svenskmjolk.se/ImageVault/Images/id_1156/scope_128/ImageVaultHandler.aspx. [Accessed 15 September 2010].

Trevisi, E., Zecconi, A., Bertoni, G. & Piccinini, R. (2010) Blood and milk immune and inflammatory profiles in periparturient dairy cows showing a different liver activity index. *Journal of Dairy Research,* 77(3), 310–317.

Tråvén, M., Stengärde, L., Emanuelson, U., Holtenius, K., Hultgren, J. & Niskanen, R. (2007) Evaluation of fructosamine as a tool to assess glucose levels in dairy cows. *13th International Conference on Production Diseases in Farm Animals; Leipzig*, 168.

Uchida, E., Katoh, N. & Takahashi, K. (1993) Induction of Serum Haptoglobin by Administration of Ethionine to Cows. *Journal of Veterinary Medical Science,* 55(3), 501–502.

Van den Top, A.M., Van Tol, A., Jansen, H., Geelen, M.J.H. & Beynen, A.C. (2005) Fatty liver in dairy cows *post partum* is associated with decreased concentration of plasma triacylglycerols and decreased activity of lipoprotein lipase in adipocytes. *Journal of Dairy Research,* 72(2), 129–137.

van Knegsel, A.T.M., van den Brand, H., Graat, E.A.M., Dijkstra, J., Jorritsma, R., Decuypere, E., Tamminga, S. & Kemp, B. (2007) Dietary energy source in dairy cows in early lactation: Metabolites and metabolic hormones. *Journal of Dairy Science,* 90(3), 1477–1485.

Van Winden, S.C.L., Jorritsma, R., Müller, K.E. & Noordhuizen, J.P.T.M. (2003) Feed intake, milk yield, and metabolic parameters prior to left displaced abomasum in dairy cows. *Journal of Dairy Science,* 86(1), 1465–1471.

Van Winden, S.C.L. & Kuiper, R. (2003) Left displacement of the abomasum in dairy cattle: recent developments in epidemiological and etiological aspects. *Veterinary Research,* 34, 47–56.

Whitaker, D.A. (Ed.) (2004) *Metabolic profiles*. Oxford: Blackwell Science. (Bovine Medicine: Diseases and Husbandry of Cattle.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F., Jr. & Lesch, T.N. (1982) A dairy cow body condition scoring system and its relationship to selected production characteristics. *Journal of Dairy Science,* 65(3), 495–501.

Young, J.W. (1977) Gluconeogenesis in cattle: Significance and methodology. *Journal of Dairy Science,* 60(1), 1–15.

Zadnik, T. (2003) A comparative study of the hemato-biochemical parameters between clinically healthy cows and cows with displacement of the abomasum. *Acta Veterinaria-Beograd,* 53(5-6), 297–309.

Zhao, F.-Q. & Keating, A.F. (2007a) Expression and regulation of glucose transporters in the bovine mammary gland. *Journal of Dairy Science Supplement,* 90(1), E76–E86.

Zhao, F.-Q. & Keating, A.F. (2007b) Functional properties and genomics of glucose transporters. *Current Genomics,* 8(8), 113–128.

Østergaard, S. & Gröhn, Y.T. (2000) Concentrate feeding, dry-matter intake, and metabolic disorders in Danish dairy cows. *Livestock Production Science,* 65(1-2), 107–118.

Østergaard, S. & Gröhn, Y.T. (1999) Effects of diseases on test day milk yield and body weight of dairy cows from Danish research herds. *Journal of Dairy Science,* 82(6), 1188–1201.

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