

**Dissertation submitted for the RCVS
Diploma in Cattle Health and
Production**

Alastair Ian Macrae

2012

**Use of Acute Phase Proteins for
Monitoring Cow Health and
Productivity in Dairy Cattle**

Word Count (excluding references) = 9,483 words

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INTRODUCTION

Assessment of cow health and productivity in dairy herds

Whilst individual animal medicine will always be important for the diagnosis, treatment and prognosis of specific conditions, the modern dairy practitioner is also required to assess cow health and productivity on a herd basis. There are a number of tools available for this including milk production records, milk composition (butterfat and protein levels), body condition score (BCS) and associated changes, milk quality (for example Somatic Cell Count [SCC] on a bulk tank and/or individual cow level), rumen pH monitoring, bulk tank monitoring for infectious disease status (for example Bovine Virus Diarrhoea (BVD), Infectious Bovine Rhinotracheitis (IBR) and leptospirosis), mobility scoring, disease and culling records, analysis of fertility data and cow signals to name but a few (Zaaijer and Noordhuizen, 2003; Statham 2011).

The aim with all of these assessment tools is to ask: 1) What is the current status of cow health and productivity, compared to expectations? 2) Is there a current problem(s)? 3) If so, what is it? and 4) What is the best solution(s) in terms of cow health, welfare, productivity and cost:benefit? The available tools all vary considerably in their cost, practicality, speed, reliability and accuracy in pinpointing issues on the farm. For example body condition scoring is inexpensive and easy to perform, but will only give a clue as to nutritional issues over a period of weeks or even months, and so is relatively long-term.

Use of metabolic profiles for monitoring cow health and productivity

Metabolic profiles were first developed in the 1970s as a method of assessing nutritional status in dairy herds (Payne et al., 1970), and have been utilised with modifications since (Blowey 1975, Ward et al., 1995, Whitaker 2004). Despite slight differences in the parameters measured, all metabolic profile systems involve taking blood samples from cows at key stages of the production cycle (typically late pregnancy and early lactation) and analysing for biochemical parameters that give information on cow health and potential limitations to productivity. Due to economic considerations, the aim is to provide the most amount of information from the minimum amount of parameters (and thus least cost). The advantage is that metabolic profiles enable quick, accurate information on metabolic status at key stages, pinpointing constraints and enabling rapid corrective action.

Initial work using metabolic profiles established optimum values based on population distributions (Payne et al., 1970). However subsequent work derived optimum values and thresholds on the basis of research work that has shown effects on cow health, fertility and productivity (Whitaker et al., 1993; Miettinen and Setälä 1993; Pushpakumara et al., 2003). Recent work has looked at the thresholds with reference to clinical disease and production, especially in association with assessment of energy balance using parameters such as β -hydroxybutyrate (BOHB) and non-esterified fatty acids (NEFA). Cows with elevated NEFA levels in the last 10 days of pregnancy are at an increased risk of developing left displaced abomasum (LDA), retained foetal membranes (RFM), culling before 60 days in milk and reduced milk production in early lactation (LeBlanc et al., 2005, Ospina et al., 2010a, Ospina et al., 2010b, Chapinal et al., 2011). Elevated NEFA

and BOHB levels in the first two weeks of lactation have been associated with an increased risk of LDAs, clinical ketosis, endometritis and lowered milk production in early lactation (Ospina et al., 2010a, Ospina et al., 2010b, Chapinal et al., 2011, reviewed by LeBlanc 2010). In relation to thresholds for minerals and trace elements, there is significant experimental work available to show responses when levels fall outside critical thresholds (for example Kelly 1998, Underwood and Suttle 1999).

Given the work referenced previously, most metabolic profile systems usually assess energy balance (using parameters such BOHB and NEFA), protein status (via urea and albumin), disease status (albumin and globulin), major minerals (magnesium and phosphate) and trace elements (including copper and GSHPx for selenium status). However other parameters such as liver enzymes, cholesterol and bile acids are sometimes analysed (Whitaker 2004). Any biochemical parameter that would provide useful additional information on current cow health and productivity, as well as predict clinical outcomes (such as fertility and milk production) more promptly than current parameters would be worth further investigation.

Acute Phase Proteins (APP)

Acute phase proteins are regarded as “biomarkers of inflammation, infection and trauma” (Eckersall and Bell 2010), and are a group of proteins produced by the liver in the initial stages of the inflammatory response. They have a number of functions as part of the immune response including opsonisation of microbes (to enhance destruction by phagocytosis), recruitment of inflammatory cells to sites of inflammation and regulation of the inflammatory response (Petersen et al., 2004). Although most work has

concentrated on the positive APP which increase during inflammation, there are another subset of APP that decrease during the inflammatory response (albumin is one example of a negative APP). Examples of the positive APP routinely measured in human and veterinary medicine include haptoglobin, serum (or milk) amyloid A, ceruloplasmin, C-reactive protein and fibrinogen.

By definition, the concentration of APP in serum increases or decreases by greater than 25% in response to conditions such as inflammation, infection and trauma (Eckersall, 2007), but the degree of response varies between species and APP. Indeed there is a large degree of species difference in the production of APP, and what might be a positive APP in one species may be non-existent in another species. APPs can be divided into major and minor. A major APP has a low concentration in the serum of healthy animals, but the concentration increases up to 1000 fold on stimulation, reaching a peak 24-48 hours after the insult and falling rapidly during recovery. In cattle, serum amyloid A (SAA) and haptoglobin are regarded as major APP, whilst fibrinogen and ceruloplasmin are regarded as minor APP that show a more gradual increase of 50-100% of normal resting levels (Petersen et al., 2004).

In human medicine, APP measurements are used as a diagnostic tool to detect the presence or absence of inflammation, to monitor the inflammatory response and are also used as a prognostic indicator (Gabay and Kushner 2001). Erythrocyte sedimentation rate is used as an indirect measure of the acute phase response as it is determined by fibrinogen and other APP in the blood, but most human studies have concentrated on C-reactive protein (CRP). CRP is a major APP in humans that is used to monitor progress of bacterial infections such as meningitis (Gabay and Kushner 2001), and has also been

shown to have predictive value in conditions such as coronary heart disease (Buckley et al., 2009), and even more generally mortality in critically ill patients (Zhang and Ni 2011).

APP therefore have the potential to be used in the diagnosis of disease in individual animals. However although the major APP are very sensitive, they are relatively non-specific being elevated in a wide range of inflammatory conditions including diseases such as mastitis and pneumonia, surgery and prolonged transport (Petersen et al., 2004). What might be of more value would be their use in the prognosis of disease (for example recumbency in cattle), early detection of disease (such as mastitis) and general health screening for welfare assessment and future productivity.

Haptoglobin in cattle

In healthy cattle, serum haptoglobin levels have been reported as being less than 0.02 g/l but increase rapidly to over 2 g/l within two days of inflammation occurring (Eckersall and Bell 2010). The main function of haptoglobin is to form stable complexes with free haemoglobin in the blood that prevents the loss of iron, which is thought to have a bacteriostatic effect by restricting iron availability for bacterial growth (Petersen et al., 2004).

Haptoglobin has been shown to become elevated in a wide range of inflammatory conditions including pneumonia, viral infections such as Respiratory Syncytial Virus (RSV) and Foot and Mouth Disease (FMD), metritis, trauma and transportation (reviewed by Petersen et al., 2004; Murata et al., 2004; Eckersall 2007; Eckersall and Bell, 2010). Haptoglobin has also been shown to increase in fatty liver (Nakagawa et al.,

1997), which could potentially explain why some studies have shown a rise in haptoglobin in the immediate periparturient period in dairy cattle (Bionaz et al., 2007).

Although higher haptoglobin concentrations have been found in animals with disease problems compared to healthy animals, the predictive value has been poor due to the non-specific nature of the APP response. So although “bobby” veal calves and cows with disease problems had higher haptoglobin levels prior to slaughter, this did not correlate with either the specific lesions discovered post-mortem or their severity (Gray et al., 1996; Hirvonen et al., 1997, Tourlomooussis et al., 2004). However levels were higher in cattle classified as having acute conditions compared to non-acute pathological conditions, and so it is proposed that haptoglobin could be used to assess animal health and welfare ante-mortem as an aide to meat inspection (Tourlomooussis et al., 2004).

Serum Amyloid A (SAA) in cattle

In healthy cattle, SAA levels have been reported as being less than 24 mg/l (Eckersall, personal communication) although a study of 22 healthy dairy cattle ante-mortem which had no visible pathological lesions post-mortem showed a mean \pm SD SAA concentration of 51 mg/l \pm 38 (Tourlomooussis et al., 2004). SAA is proposed to have an inhibitory effect on fever and the immune response, as well as a chemotaxic effect on white blood cells (Petersen et al., 2004).

Like haptoglobin, SAA has been shown to become elevated in a wide range of chronic inflammatory conditions such as mastitis, experimental infection with BVD and IBR and inflammation (reviewed by Petersen et al., 2004; Eckersall 2007; Eckersall and Bell, 2010). In a study comparing SAA levels and clinical examination for the diagnosis of

inflammatory conditions in dairy cows, low SAA and negative clinical findings (ie. no evidence of inflammation) were in agreement in 95% of cases. However only 26% of animals with a high SAA level had clinical signs of inflammation (Karreman et al., 2000), and the authors concluded that the high SAA levels were due to subclinical disease. A similar study concluded that both haptoglobin and SAA had low sensitivity but higher specificity in determining disease status compared with clinical examination (Humblet et al., 2006), and that both were significantly elevated in the week after calving. In dairy cattle, the discovery of a mammary isoform of SAA (termed M-SAA3) has led to much interest in the use of SAA/M-SAA3 in the early detection of mastitis. A number of studies have shown that cows with either naturally-occurring or experimentally- induced mastitis have increased levels of both SAA and haptoglobin in milk (Eckersall et al., 2001; Eckersall et al., 2006; Pyörälä et al., 2011), and this occurred within 12 hours of experimental inoculation (Eckersall et al., 2006; Suojala et al., 2008). SAA in milk appears to rise more quickly than haptoglobin, therefore making it a potential candidate for the early detection of subclinical and clinical mastitis using “in-line” sampling systems during milking.

Use of Acute Phase Proteins for the early diagnosis and prognosis of disease in cattle

There are a number of recent studies which have looked at the use of APP for the diagnosis and prognosis of diseases in cattle.

Most work has been performed looking at metritis in freshly calved dairy cows. As might be expected, a number of research studies have shown that cows with metritis have elevated APP (Sheldon et al., 2001; Humblet et al., 2006). Recent work has shown that

cows with mild and severe metritis had higher haptoglobin concentrations from the day of calving until day 12 post-partum compared to healthy controls, although there was no significant difference at any of the timepoints prepartum (Huzzey et al., 2009). Using a threshold of 1 g/l, the authors concluded that cows with elevated haptoglobin levels on day 3 post-partum (on average two days before clinical signs were noticed) were 6.7 times more likely to develop severe or mild metritis. This predictive threshold has a sensitivity of 50% and specificity of 87% (Huzzey et al., 2009). Another study by Chan et al. (2010) showed that cows with acute metritis had higher haptoglobin and SAA concentrations than healthy controls and heifers at all timepoints of the study, including one week prepartum. Although another study showed that haptoglobin concentrations tended to be higher prepartum in cows that developed more than one health disorder (defined as RFM, LDA or subclinical ketosis) or died by 30 days in milk, this was not significant once calving assistance was included in the analysis (Huzzey et al., 2011). The authors concluded that measuring haptoglobin did not provide any additional information compared to NEFA measurement prepartum.

Diagnosis and prognosis of respiratory disease in fattening animals is another focus of APP research, primarily due to the reduced growth rates and poorer feed conversion efficiency of affected cattle especially under feedlot conditions. Again it would be expected that animals with respiratory disease would have higher APP (Petersen et al., 2004; Eckersall 2007; Eckersall and Bell, 2010), although their association with fatal disease is unproven (Aich et al., 2009). Animals with raised haptoglobin concentrations on arrival at a feedlot had lower Dry Matter intakes and liveweight gains in the initial

month upon arrival, but long-term there was no difference in morbidity or carcass characteristics (Holland et al., 2011).

As previously mentioned there is also much interest in the early and rapid diagnosis of mastitis in dairy cattle. There are a number of biomarkers for mastitis including APP, as well as enzymes such as N-acetyl- β -D-glucosaminidase (NAGase) and Lactate Dehydrogenase (LDH) (Pyörälä 2003; Akerstedt et al., 2011). LDH is now used commercially as an “in-line” marker for the early detection of mastitis, primarily in robotic milking systems (Herd Navigator 2012). Presumably if the technology was developed to measure SAA/M-SAA3 in milk as a “cow-side” test or “in-line”, then this could also be used as an early diagnostic test for subclinical and clinical mastitis.

Aims of this study

Most of the previous research work on APP has focused on the diagnosis of acute disease processes, and used serial sampling to try and detect subclinical disease problems such as metritis and mastitis. However there are few (if any) studies looking at the use of APP for the assessment of health and future productivity in commercial dairy herds. Therefore this study aims to answer the following questions:

1. What is the distribution of APP (haptoglobin and SAA) in clinically normal dairy cows at different stages of the production cycle?
2. What is the relationship of APP (haptoglobin and SAA) and existing biochemical parameters used in metabolic profiles?
3. What is the association of APP (haptoglobin and SAA) with clinical disease and production parameters in dairy cows?
4. Are APP (haptoglobin and SAA) useful for the monitoring of dairy cow health and productivity?

5. MATERIALS AND METHODS

Blood samples were obtained from 12 commercial dairy herds in Central and South East Scotland as part of a routine nutritional monitoring programme using metabolic profiles as described by Whitaker (2004). To summarise, farmers were requested to present cows for blood sampling in late pregnancy (DRY: ideally within 10 days of their predicted calving date), early lactation (EARLY: 10 – 20 days calved) and mid lactation (MID: 90 – 150 days calved). The farmers were instructed that any cows with evidence of clinical disease problems were not to be presented for blood sampling, as this would not give accurate information on the nutritional status of the animal. At the same time as blood sampling, liveweights were estimated using a weighband to measure heart girth (Heinrichs et al., 1992), and cows were body condition scored on a 1 to 5 scale (DEFRA 2001). Details were also collected of calving dates, lactation numbers and daily milk yields for each cow sampled.

Blood samples were taken from the coccygeal vein into oxalate (for glucose analysis) and lithium heparin vacutainers (all other metabolites), or with no anti-coagulant (for APP analysis). Samples were analysed for biochemical metabolites within 24 hours of collection using an Instrumentation Laboratory IL600 wet chemistry system using reagents supplied by Randox (BOHB and NEFA) and Instrumentation Laboratory (all other metabolites). Samples were analysed for: β -OH butyrate (BOHB), glucose, NEFA, urea-N, albumin, globulin, magnesium and phosphate. A smaller subset of these samples were also analysed for plasma copper levels, and GSHPx for the estimation of long-term selenium status. Results were collated in an Access database.

Aliquots of serum were frozen, stored at -20°C and subsequently analysed for the APP haptoglobin and Serum Amyloid A (SAA) by Reactivlab Ltd., University of Glasgow. The concentration of haptoglobin in bovine serum was measured using a modification of the procedure described in Eckersall et al., (1999) which is a haemoglobin binding automated biochemical assay based on the peroxidase activity of haptoglobin-haemoglobin complex at low pH. The concentration of serum amyloid A in bovine serum was measured by ELISA (Tridelta Development plc, Ireland) according to the manufacturer's instructions, with standards and samples being assayed in duplicate.

Table 1 provides some background information on the farms that were included in the study. For those nine farms that milk recorded, the two milk recordings that were closest to the actual date of blood sampling were selected. For the DRY and EARLY cows, this was usually the first two milk recordings of the lactation. For the MID group, this was the milk recording before and the one after the date of blood sampling. The date of the milk recording (and thus time in relation to blood sampling) and the Individual Cow Somatic Cell Count (ICSCC) was recorded for each cow.

For the nine milk recorded herds, milk recording data was downloaded from either the CIS or NMR website as a CDL file, and imported into Interherd (NMR and PAN Livestock Services, UK). For the lactation in which the blood samples were taken (or subsequent lactation for DRY cows), the total lactation milk yield, 305 day lactation milk yield and milk per day of lactation were recorded for each cow.

Appropriate disease records were obtained for each farm, and any disease occurrences within 3 months of the blood sampling date were recorded along with the date they occurred. It was assumed that if the farm had appropriate records and no disease was

recorded, that it meant that there was no clinical disease observed by the farm staff in that animal.

Table 1. Summary of twelve farms in the study

	Number of cows	Breed	Average lactation yield (kg)	System	Milk recording	Disease records	Fertility records
Farm A	120	Hol/Fr	10,056 kg	Standard	CIS	Farmdata	Farmdata
Farm AM	240	Jersey	7,161 kg	Standard	CIS	Paper	CIS
Farm B	150	Hol/Fr	7,459 kg	Organic	NMR	Interherd	Interherd
Farm BH	300	Hol/Fr	8,000 kg	Standard	No	Uniform-Agri	Uniform-Agri
Farm C	140	Hol/Fr	6,773kg	Standard	CIS	None	CIS
Farm CT	230	Ayrshire	7906 kg	Standard	CIS	Paper	CIS
Farm G	170	Hol/Fr	7,247 kg	Standard	CIS	Paper	CIS
Farm H	100	Hol/Fr	7,000 kg	Organic	No	SAC	SAC
Farm HC	170	Hol/Fr	10,820 kg	Standard	CIS	Uniform-Agri	Uniform-Agri
Farm L	240	Hol/Fr	9,302 kg	Standard	CIS	Uniform-Agri	Interherd
Farm NP	90	Hol/Fr	8,692 kg	Organic	CIS	Paper	CIS
Farm YM	300	Hol/Fr	8,000 kg	Standard	No	None	DairyComp 305

Relevant fertility data recorded included date of 1st service, number of services, date of successful service and whether the cow was back in calf by the end of that lactation. This enabled the calculation of calving to 1st service interval, calving to conception interval and whether the cow was back in calf by 100 days of lactation. For all DRY cows, the actual calving date was also recorded.

All data was collated in Microsoft Excel 2010, and statistical analyses performed using Minitab 15 and R Version 2.14.1 (R Foundation for Statistical Computing). Data for both haptoglobin and SAA was highly skewed, and so either non-parametric tests were used where appropriate or the data was log₁₀ transformed (SAA) prior to analysis to normalise the residuals.

For analyses of the \log_{10} transformed SAA values, linear mixed effect (LME) models were used with farm identity entered as a random effect to account for intra-farm correlation, and system (standard or organic), lactation number (primiparous or multiparous) and group (EARLY, MID or DRY) added as fixed effects. Over 50% of the haptoglobin values were 0 (ie. below the detection limit of the assay used), which made analysis of haptoglobin values not possible.

Therefore in order to examine the relationships with haptoglobulin logistic analysis using generalised linear mixed effect models with binomial errors (GLMEb) were used for the analysis of threshold data, when data was represented as “high” or “low” referenced to a threshold of 0.02 g/l for haptoglobin. Again farm identity was entered as a random effect to account for intra-farm correlation, and system (standard or organic), lactation number (primiparous or multiparous) and group (EARLY, MID or DRY) added as fixed effects. An equivalent GLMEb threshold analysis was also carried out for SAA at a threshold of 24 mg/l.

Generalised linear mixed effect models with Poisson errors (GLMEp) were used for the analysis of body condition score, where system (standard or organic), lactation number (primiparous or multiparous) and \log_{10} SAA values were added as fixed effects

For binomial data (for example SAA and haptoglobin thresholds), Relative Risk was calculated with 95% confidence intervals (CI). If the 95% CI included 1.000, then it was considered that the Relative Risk was not-significant.

RESULTS

Descriptive statistics, and distribution of APP measurements in dairy cows

A total of 388 blood samples were received from individual cows from the twelve farms. Although not the focus of this study, the results of the metabolic profile analyses were consistent with previous results reported by Macrae et al., (2006), in that the majority of issues were identified with negative energy balance in cows during late pregnancy and early lactation, as evidenced by elevated levels of BOHB (38% of samples in the EARLY group had BOHB values ≥ 1.0 mmol/l) and/or NEFA (33% of samples in the EARLY group had NEFA values ≥ 0.7 mmol/l).

A summary of the APP results is given in Figures 1 and 2 below, as well as Table 2. Using a threshold of 0.02 g/l as proposed by Eckersall and Ball (2010), 155 cows (40% of the cows sampled) had elevated haptoglobin results. Using a threshold of 24 mg/l, 165 cows (42.5 % of the cows sampled) had elevated SAA results.

Figure 1. Distribution of haptoglobin results in the 386 samples analysed. The majority of cows had haptoglobin results below the detectable limit of the assay, and 155 cows had haptoglobin results greater than 0.02 g/l.

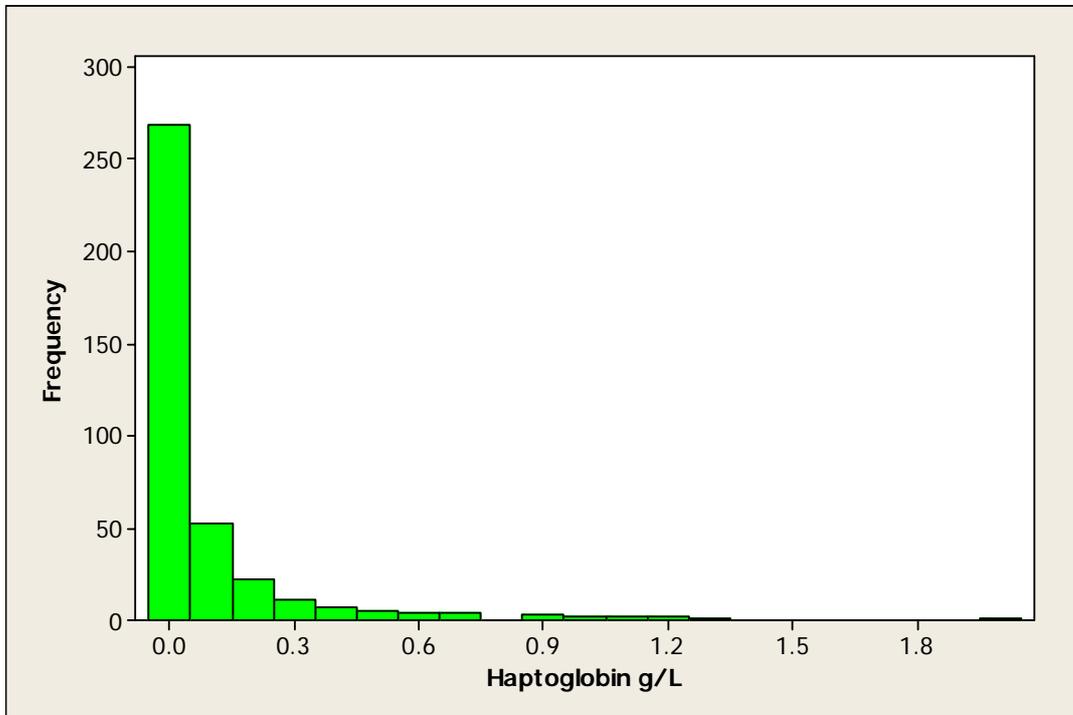


Figure 2. Distribution of SAA results in the 388 cows sampled. 165 cows had SAA results greater than 24 mg/l.

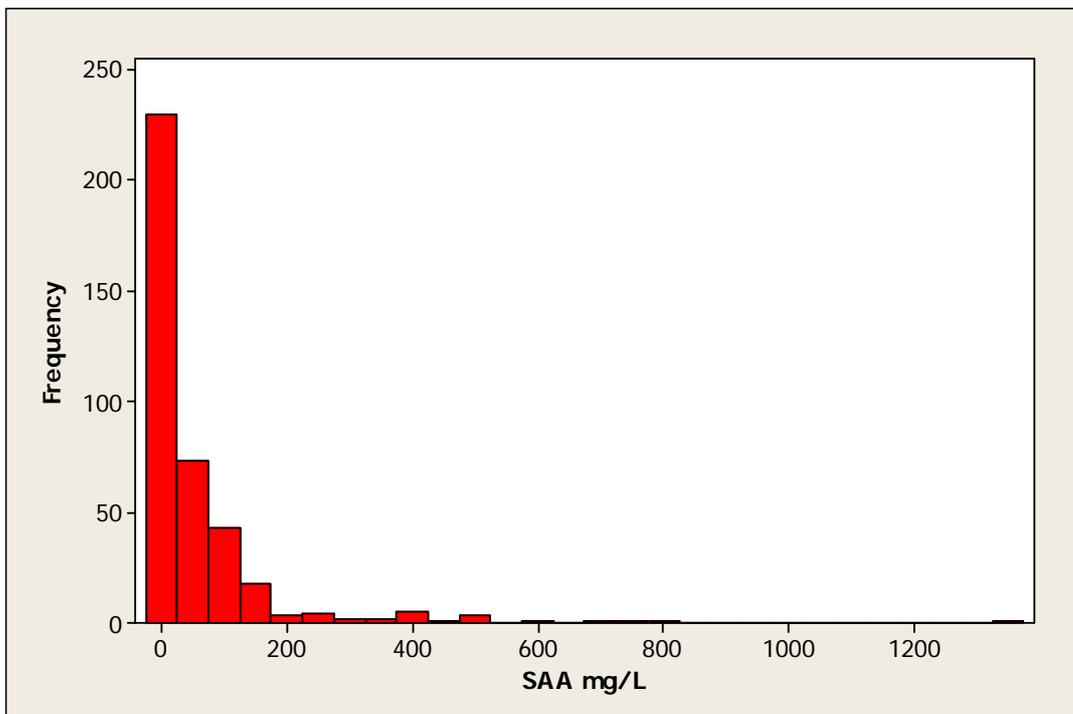


Table 2. Summary of haptoglobin and SAA results in the three groups of cows sampled.

Group	Number of samples analysed for haptoglobin	Mean Haptoglobin (g/l) \pm SD	Median Haptoglobin (range) (g/l)	Number of samples analysed for SAA	Mean SAA (mg/l) \pm SD	Median SAA (range) (mg/l)
EARLY	156	0.135 \pm 0.29	0 (0 – 2.025)	157	78.4 \pm 161.4	24 (1 – 1340)
MID	120	0.071 \pm 0.16	0 (0 – 1.27)	121	59.8 \pm 93.3	18 (1 – 520)
DRY	110	0.064 \pm 0.17	0 (0 – 1.16)	110	36.9 \pm 88.4	8 (2 – 815)

There was no significant difference in haptoglobin between the three groups (Figure 3), although the EARLY and MID lactation groups had significantly higher SAA results compared to the DRY group (Kruskal-Wallis test; $P < 0.001$)(Figure 4).

Figure 3. Variation in haptoglobin results by stage of lactation and parity. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.

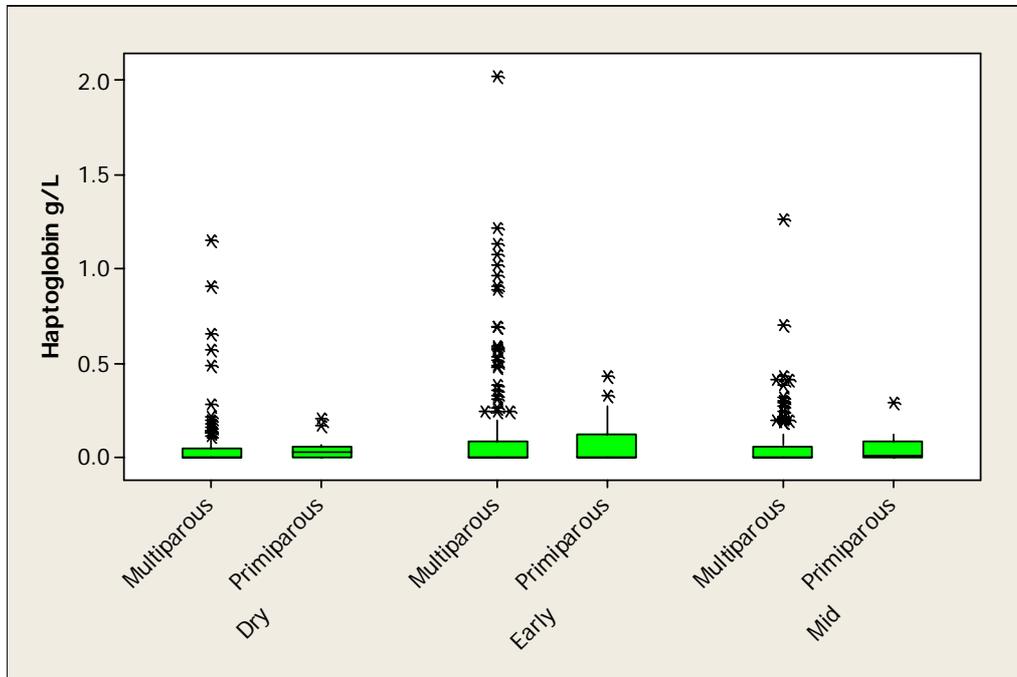
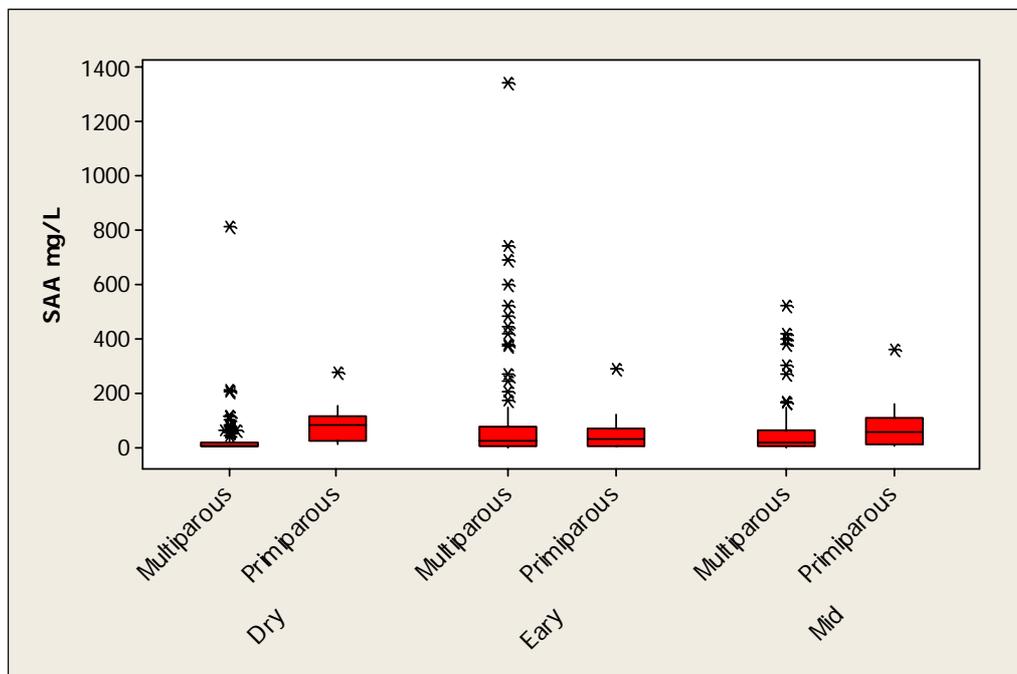


Figure 4. Variation in SAA results by stage of lactation and parity. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks. There is a significant interaction between SAA levels, parity (primiparous or multiparous) and group ($P=0.007$).



More detailed analysis of SAA results using LME models showed that this interaction depended on a combination of parity (primiparous or multiparous) and group (Figure 4, $P=0.007$). SAA levels in the DRY group differed in primiparous and multiparous, but no difference was observed for the EARLY and MID groups for SAA. Using GLMEb and a threshold of 24 mg/l for SAA, there was a significant interaction between parity, group and SAA ($P=0.007$). 75% of primiparous cows in late pregnancy (DRY) had an elevated SAA result, compared to 52% of the EARLY and 57% of the MID heifers. However, only 22% of the multiparous cows in late pregnancy had a high SAA result, compared to 51% of the EARLY and 43% of the MID groups. There was no similar effect for haptoglobin ($P>0.2$).

Using GLMEb, primiparous cows had a significantly higher SAA result than multiparous cows when looking at either the SAA value ($P=0.004$) or the number of cows over the 24 mg/l threshold ($P=0.008$). The Relative Risk of having a high SAA result in heifers compared to multiparous cows was 1.476 (1.135 – 1.920). However there was no significant difference in the proportion of cows with elevated haptoglobin results between heifers (48%) and multiparous cows (39%) ($P=0.079$).

Given the difference in the DRY, EARLY and MID groups between SAA values, it was decided to look at this more closely by seeing if there was an effect on APP levels by days in relation to calving when the blood samples were taken (Figures 5 and 6), specifically days -50 to calving (precalving) and days from calving to 50 days in milk (postcalving). Using LME to analyse actual SAA values and GLMEb for the analysis of the proportion of cows with haptoglobin and SAA values over the threshold levels, there

was no statistically significant effect of days in relation to calving on haptoglobin levels either pre- or post-calving ($P>0.777$)(Figure 5).

Figure 5. Variation in haptoglobin results by time relative to calving.

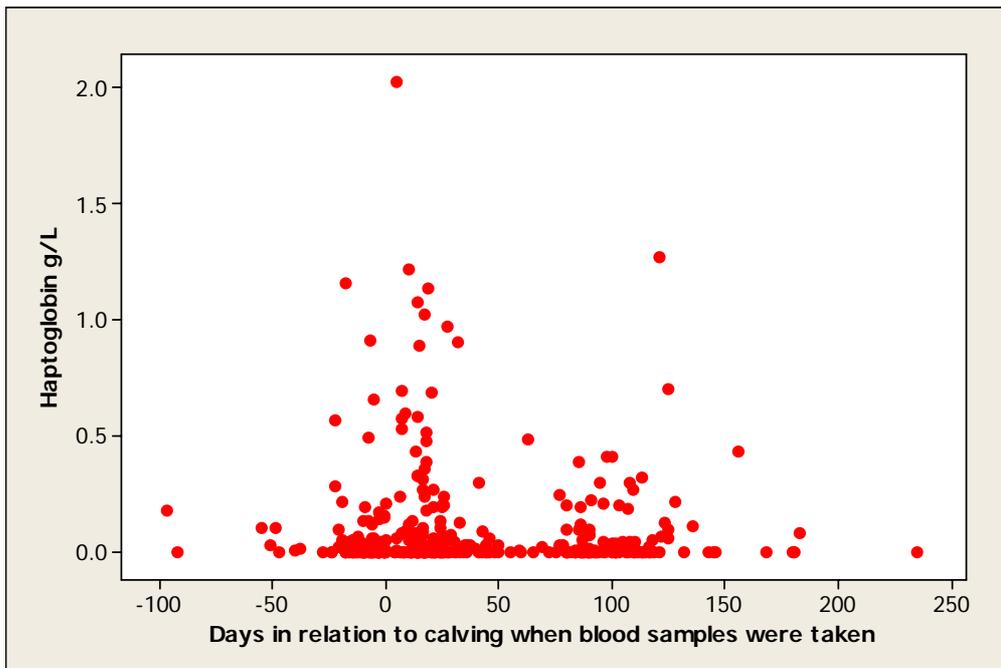
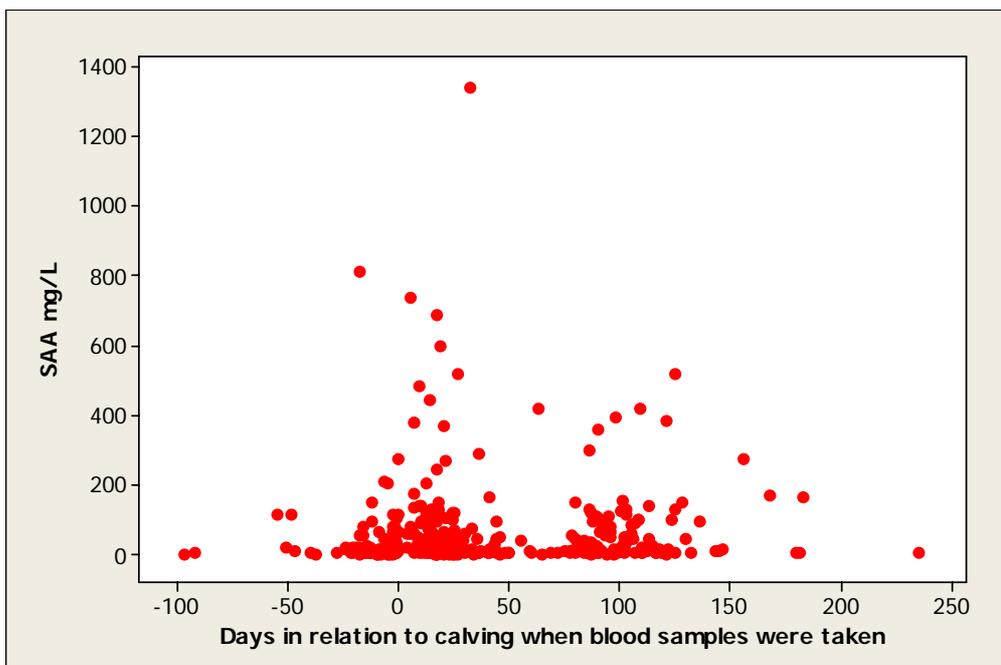


Figure 6. Variation in SAA results by time relative to calving.



Although there was no significant association between SAA and time in relation to calving in the 50 days before calving (precalving), there was a significant negative association between SAA and days in milk postcalving for both actual SAA value ($P=0.009$) and proportion of cows with an elevated SAA over the threshold value ($P=0.014$).

Figures 7 and 8 show the variation in haptoglobin results between individual farms, which was accounted for in the statistical analysis by including farm identity as a random effect to account for intra-farm correlation.

Figure 7. Variation in haptoglobin results between individual farms. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.

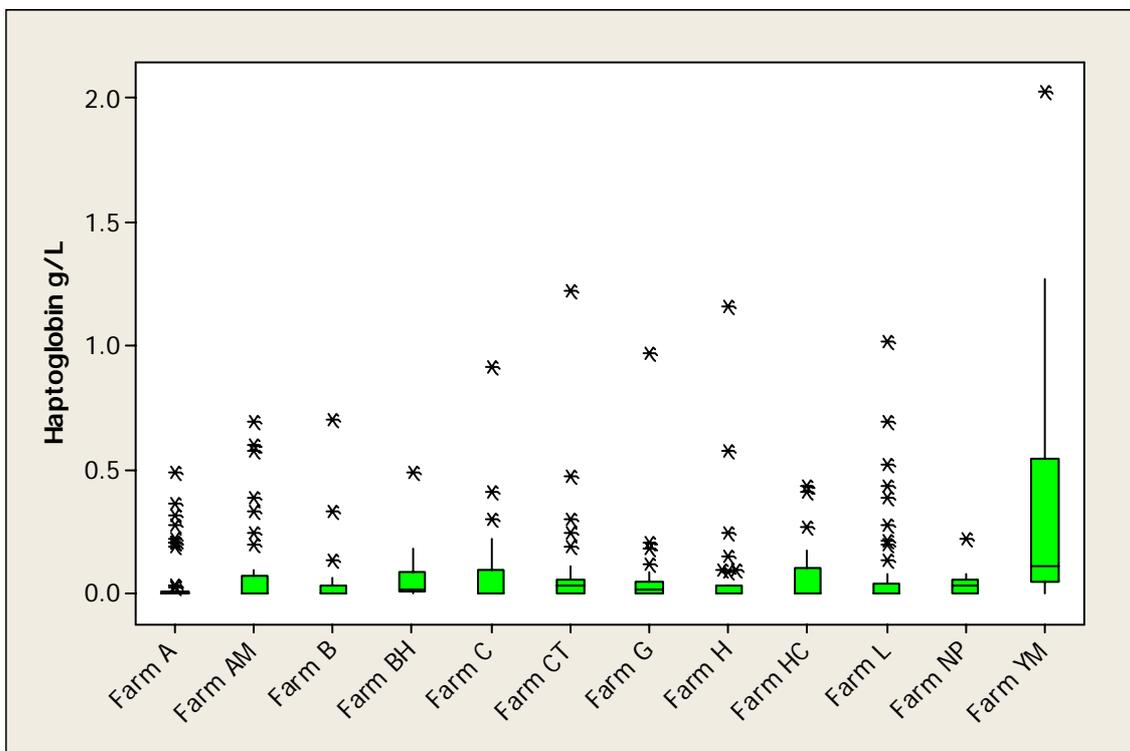
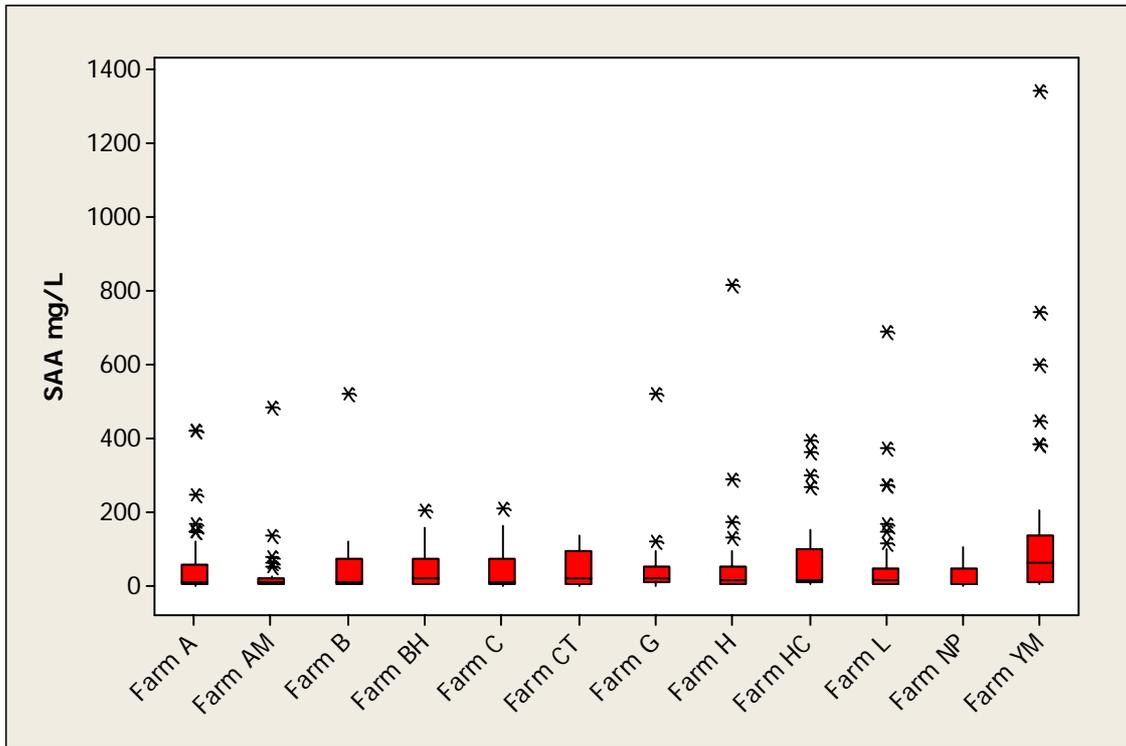


Figure 8. Variation in SAA results between individual farms. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.



Body condition score (BCS) was determined for each cow at the time of blood sampling using a standard 1 – 5 scale in ¼ points (DEFRA 2001). One observer was present at all sampling times to ensure consistency. Preliminary analysis showed that there was a negative relationship between SAA and body condition score using GLMEp ($P=0.038$), once the non-significant effects of system and parity were taken into account (Figure 9).

It was therefore decided to group cows into three groups based on body condition score: thin cows (BCS less than or equal to 2), fat cows (BCS greater than or equal to 4), and the remainder (BCS values between 2¼ and 3¾), which were deemed to be within acceptable limits given the quoted variation in BCS targets. Using GLMEb, there was a

significant association between thin cows and elevated SAA values ($P=0.016$), and thin cows and elevated haptoglobin levels ($P=0.049$)(Figures 9 and 10). The Relative Risk of being a thin cow given a high SAA value was 2.573 (95% CI: 1.138 – 5.817), although the Relative Risk of being a thin cow given a high haptoglobin value was 1.288 (95% CI: 0.899 – 1.846) indicating that it was not significant.

There was no significant association between fat cows (BCS 4 and over) and APP result, using either the actual SAA values ($P=0.155$), or SAA threshold values ($P=0.067$) or haptoglobin threshold values ($P=0.717$).

Figure 9. Variation in haptoglobin results by cow body condition score. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.

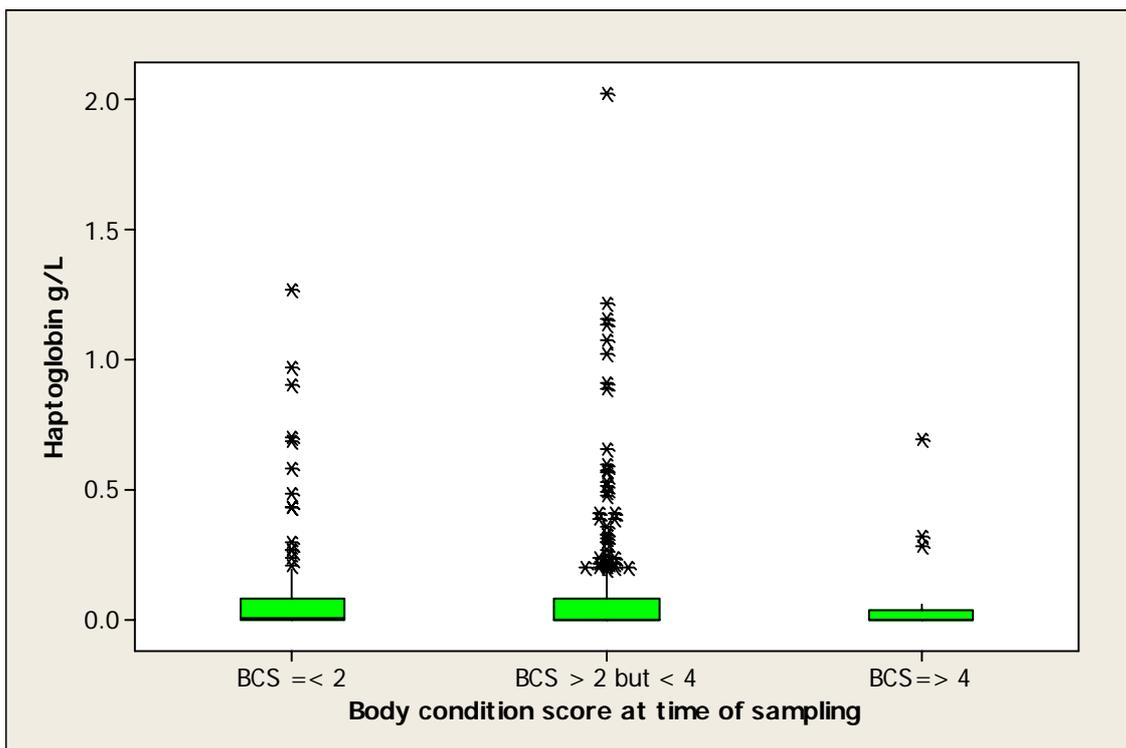
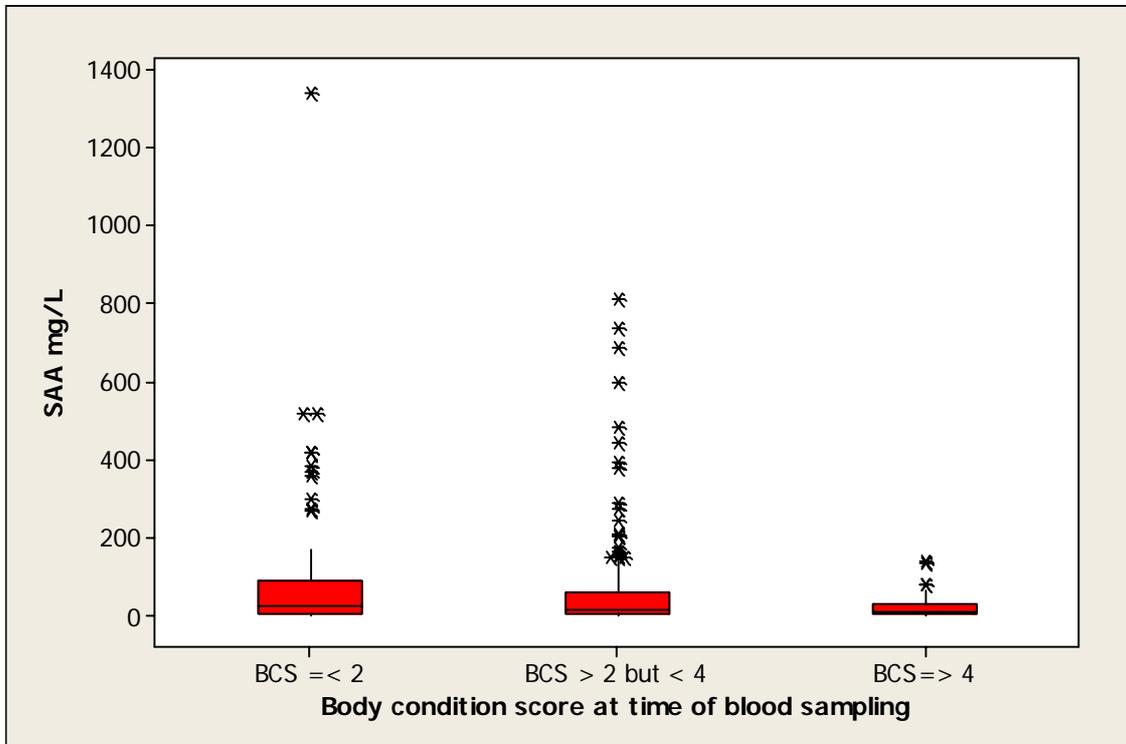


Figure 10. Variation in SAA results by cow body condition score. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.



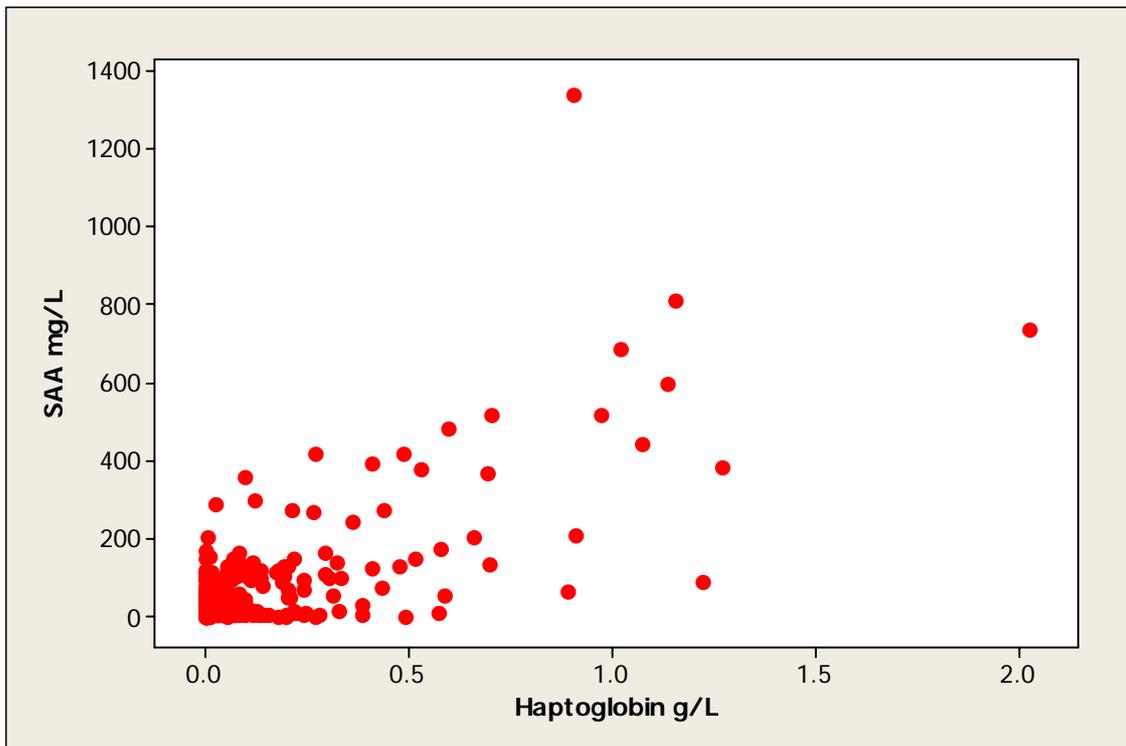
Relationship between APP and existing parameters used in metabolic profiles

Although both haptoglobin and SAA are acute phase proteins, due to the variation in APP and non-specific nature of the acute phase response, they are not necessarily measuring the same pathways (ie. there may be some inflammatory conditions where haptoglobin is increased but SAA remains low). It was decided to see if there was a correlation between haptoglobin and SAA, and other existing measures of inflammation used in metabolic profiles (albumin, globulin, copper/ceruoplasmin).

Haptoglobin and SAA were significantly positively correlated using Spearman Rank correlation ($r_s=0.377$, $P<0.001$)(Figure 11). However the correlation indicated that there

was not a perfect association, with low haptoglobin results associated with SAA levels greater than 300 mg/l and *vice versa*.

Figure 11. Correlation between SAA and Haptoglobin (P<0.001).



However there were interesting differences between haptoglobin and SAA when looking at the individual samples using standard thresholds. Although 64.5% of the samples were in agreement (ie. SAA and haptoglobin were either both high or both low), the remaining samples had only one APP value above the threshold value (see Table 3).

Table 3. Results of APP analysis using standard thresholds.

	SAA < 24 mg/l	SAA > 24 mg/l
Haptoglobin < 0.02 g/l	158 (40.9%)	73 (18.9%)
Haptoglobin > 0.02 g/l	64 (16.6%)	91 (23.6%)

Looking at correlations between SAA and haptoglobin on individual farms, nine of the twelve farms showed significant positive correlations between SAA and haptoglobin ($P < 0.05$). However three farms had no significant correlation between these two APP (Farm BH, $r_s = -0.451$, $P = 0.07$; Farm H, $r_s = 0.243$, $P = 0.174$ and Farm AM, $r_s = 0.115$, $P = 0.517$). There were no obvious reasons from the farm descriptions in Table 1 as to why this might be the case.

The milking cow groups (EARLY and MID) had significant positive correlations between SAA and haptoglobin ($P < 0.001$), although the DRY group was not significant ($P = 0.233$). Both primiparous and multiparous cows showed significant positive correlations ($P < 0.001$).

Albumin is considered a negative APP (Petersen et al., 2004), and so levels might be expected to correlate with SAA and/or haptoglobin levels. However there were only ten cows in this study which had albumin levels of 30 g/l or less, which is considered the threshold for use in dairy cows (Whitaker 2004). Using GLMEp, there was a negative relationship between albumin and SAA value ($P = 0.028$). This was also significant for SAA levels over the 24 mg/l threshold, once the non-significant effects of system and parity were excluded ($P = 0.043$). However there was no significant association between haptoglobin and albumin ($P = 0.214$).

Another parameter measured in metabolic profiles is globulin levels. Values over 50 g/l are considered to show evidence of chronic inflammation (Whitaker 2004). In this study, heifers had more elevated globulin results compared to older cows, and organic systems had more elevated globulin results compared to conventional systems ($P < 0.05$). Taking these into account using LME for actual values and GLMEb for binary data, there was a significant positive association between globulin levels and SAA ($P < 0.001$) and haptoglobin ($P < 0.001$). Excluding either system or parity had no impact in terms of significance. The Relative Risk of a cow having a raised globulin result (over 50 g/l) if she had a high SAA result (over 24 mg/l) was 3.1 (95% CI: 1.668 – 5.763), and 5.297 (95% CI: 2.613 – 10.737) if she had a haptoglobin result over 0.02 g/l.

Cows with evidence of negative energy balance were determined from BOHB values (greater than 1.0 mmol/l in a milking cow and 0.6 mmol/l in a dry cow) and NEFA results (greater than 0.7 mmol/l in a milking cow and 0.5 mmol/l in a dry cow). 1.39% of primiparous cows had evidence of negative energy balance, whereas 53% of multiparous cows had evidence of negative energy balance ($P < 0.05$). However there was no significant association between APP value and cows with evidence of negative energy balance ($P > 0.1$).

Samples from 113 cows in this study from 6 farms (Farms B, C, G, H, HC and NP) were analysed for plasma copper. This is of interest in that the majority of copper in the bloodstream is bound to ceruloplasmin (Underwood and Suttle 1999), and so plasma copper is an indirect measure of ceruloplasmin levels (a positive APP). There was no significant association between plasma copper levels and system (organic or conventional) or parity (multiparous or primiparous). Using LME, there was a positive

association between plasma copper levels and SAA ($P=0.003$) and haptoglobin ($P<0.001$). Cows with SAA values over 24 mg/l had mean plasma copper levels 1.89 ± 0.58 SEM $\mu\text{mol/l}$ higher than cows with low SAA results (Figure 13), and cows with haptoglobin values over 0.02 g/l had mean plasma copper levels 2.38 ± 0.58 SEM $\mu\text{mol/l}$ higher than cows with low haptoglobin results (Figure 12).

Figure 12. Variation in plasma copper results by haptoglobin result, using 0.02 g/l threshold for haptoglobin (Hp). The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.

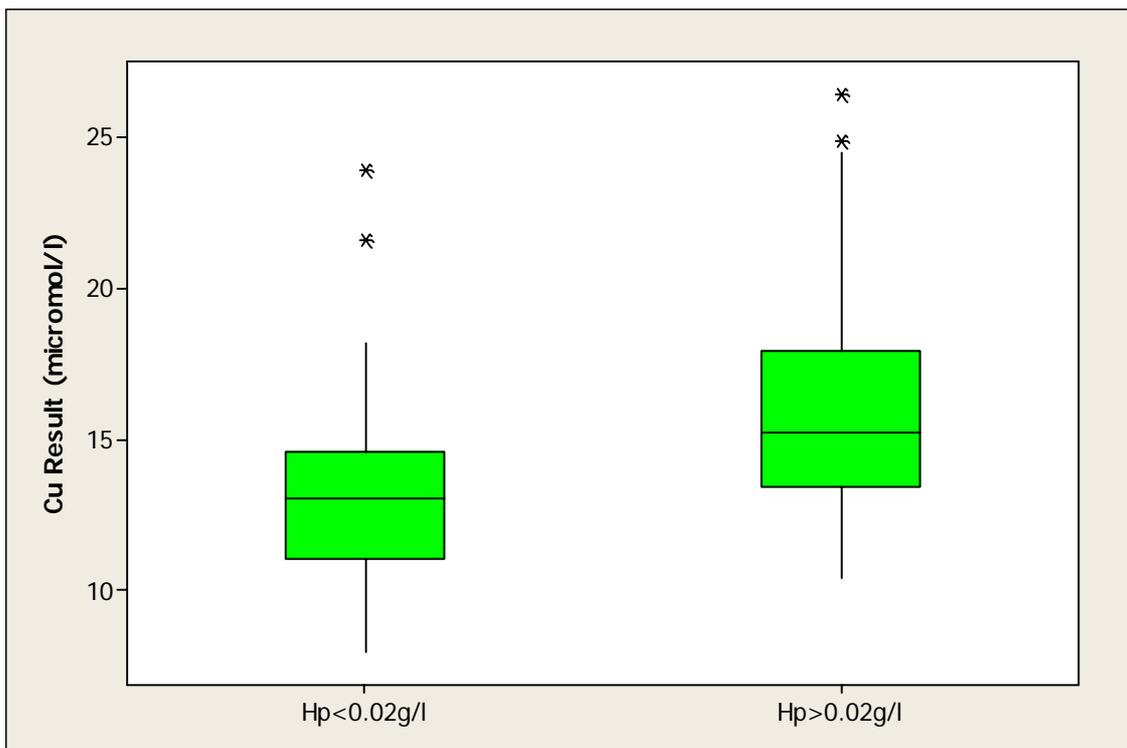
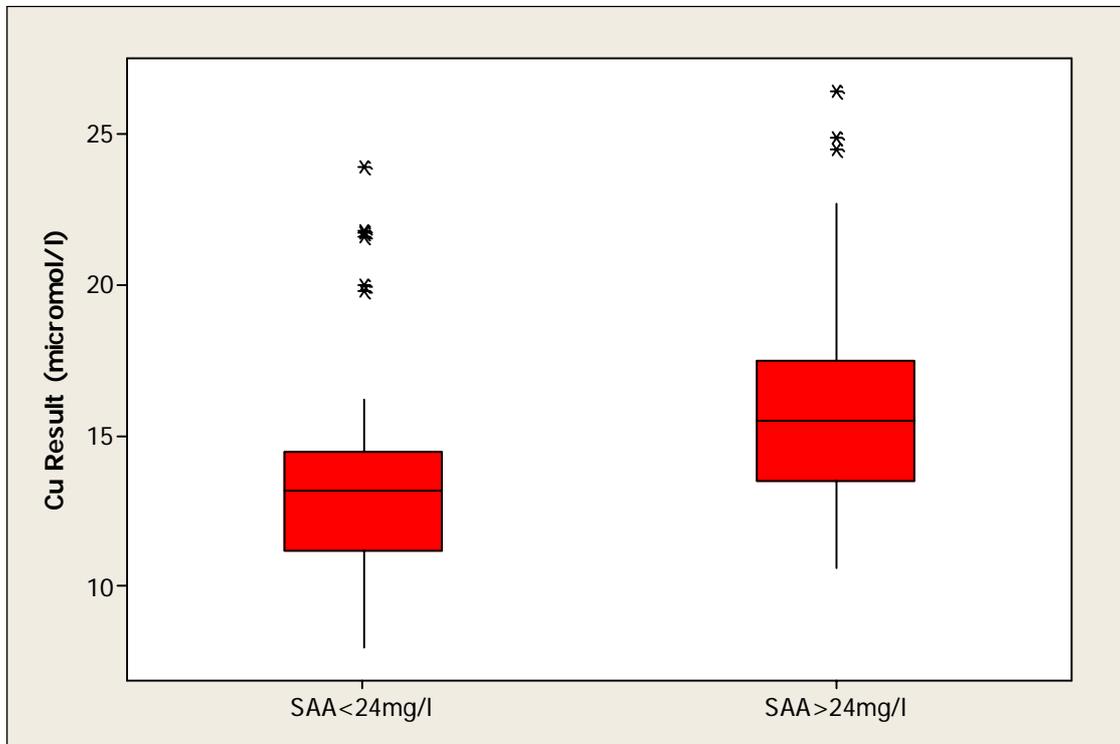


Figure 13. Variation in plasma copper results by SAA result, using 24mg/l threshold for SAA. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks.



Association between APP and clinical disease and production parameters in dairy cows

A) Association with clinical disease problems

All diseases were included if they occurred within 100 days of the date of blood sampling. Three farms (Farm YM, C and NP) had poor or non-existent recording systems, and so were excluded from the subsequent analyses. There were 75 disease events recorded, with 39 separate cases of mastitis, 15 cases of metritis or endometritis and 10 cases of lameness. Abortions and LDA cases occurred at very low levels (there was only one LDA recorded in the cows sampled), and so they were excluded as specific disease conditions. On the nine remaining farms, data from animals in which no disease events were recorded was assumed to be accurate (ie. it was assumed that all clinical disease events had been recorded).

Looking at all disease conditions grouped together (ie. was any disease condition present within 100 days of the date of blood sampling), there was no significant association between APP and disease occurrence.

There were no significant positive associations between mastitis or metritis/endometritis and APP result, although there was a slight negative statistical association between cows with SAA results over 24 mg/l and mastitis occurrence (45% of cows with no reported mastitis had a high SAA result, whereas 28% of cows with mastitis had a high SAA result)($P=0.036$).

However there were significant associations between lameness and APP results. Using LME to analyse \log_{10} SAA values, there was a positive association between SAA high values and lameness occurrence ($P=0.008$). This was also significant when using a threshold of 24 mg/l to interpret SAA values: 42% of non-lame cows had a high SAA

value, compared to 80% of lame cows ($P=0.022$). The Relative Risk of a cow having a lameness event if she had a high SAA result (over 24 mg/l) was 6.203 (95% CI: 1.363 – 28.24). There was no association between lameness and haptoglobin result ($P=0.823$).

B) Association with Somatic Cell Counts (SCC)

Nine of the twelve farms milk recorded, and so monthly individual cow somatic cell counts (ICSCC) were available. For DRY cows sampled, the first two ICSCC of the following lactation were recorded as these were the closest to the time of blood sampling. For EARLY cows, again the first two ICSCC of the lactation were recorded. For MID cows, the milk recording prior to blood sampling and the milk recording immediately after the blood sampling date were taken. Recording 1 was the milking recording date closest to the blood sampling, and occurred at a median of – 5 days in relation to the blood sampling date (IQ range: -14 to + 20 days). Recording 2 occurred at a median of + 42 days in relation to the blood sampling date (IQ range: 23 to 63 days).

ICSCC data was analysed either using the actual ICSCC value (which was \log_{10} transformed prior to analysis) or a 200,000 cells/ml threshold (ICSCC were classified as either being HIGH or LOW). Data was analysed using LME for \log_{10} SAA values, or GLMEb for binary data (ie. SAA or haptoglobin values over their respective thresholds). The time of milk recording in relation to blood testing was included in the model as an additional fixed effect for the ICSCC analyses. Using either of these methods for the analysis of actual ICSCC value or a 200,000 cells/ml threshold, there was no significant association between APP (either haptoglobin or SAA) and ICSCC.

C) Association with culling

There was no significant association between system (organic or conventional) and likelihood of culling by the end of lactation, although 29% of multiparous cows were culled compared to 6% of primiparous cows ($P < 0.05$). Although there was no significant association of SAA levels and likelihood of culling, 32% of cows with high haptoglobin levels were culled compared to 22% of cows with low haptoglobin levels ($P = 0.044$, once the effect of parity had been removed). The Relative Risk of a cow being culled if she had a high haptoglobin result (over 0.02 g/l) was 1.506 (95% CI: 1.086 – 2.088).

D) Association with milk production

Production data was obtained from milk recording information that had been input into Interherd. There were ten cows with lactation milk yields less than 4,000 litres due to terminated lactations, and so data on milk production from these ten cows was removed from the analyses as it was not thought to be representative. There was a significant relationship between parity and total lactation yield (multiparous cows gave 375 litres more milk on average compared to heifers), 305 day lactation yield (multiparous cows gave 832 litres more milk compared to heifers) and milk per day of lactation (multiparous cows gave 2.7 litres more milk per day compared to heifers).

The milk production data is summarised in table 4 below, and figures 14 and 15. Data was analysed using LME with system (organic or conventional), parity and group added as fixed effects and farm added as a random effect. There was no significant effect of elevated APP (either haptoglobin or SAA) on production, whether measured by total lactation yield, 305 day lactation yield or milk per day of lactation.

Table 4. Milk production data for cows with either elevated haptoglobin or SAA results. Figures are given as mean kg of milk \pm SEM.

	Haptoglobin < 0.02 g/l	Haptoglobin > 0.02 g/l	SAA < 24 mg/l	SAA > 24 mg/l
Total lactation yield (kg)	10,901 (\pm 246)	11,064 (\pm 316)	10,897 (\pm 248)	11,047 (\pm 313)
305 day lactation yield (kg)	9,495 (\pm 156)	9,451 (\pm 190)	9,469 (\pm 157)	9,496 (\pm 193)
Milk per day of lactation (kg)	30.1 (\pm 0.55)	29.8 (\pm 0.68)	29.9 (\pm 0.57)	30.1 (\pm 0.65)

Figure 14. Variation in 305 day milk yield by haptoglobin result, using 0.02g/l threshold. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks. There was a 52 litre difference in median 305 day adjusted milk yields, but this was not statistically significant.

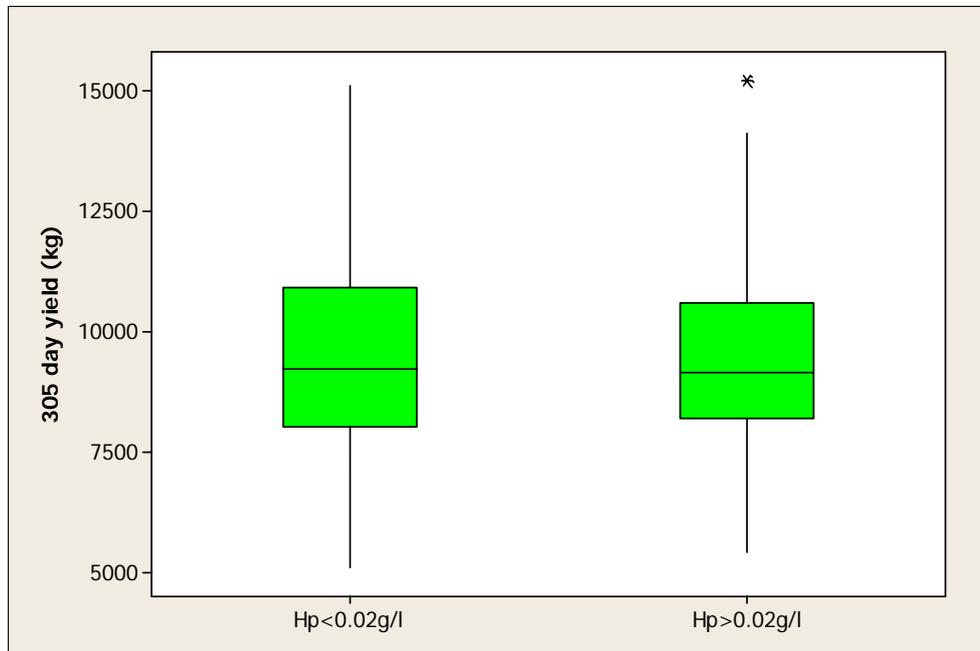
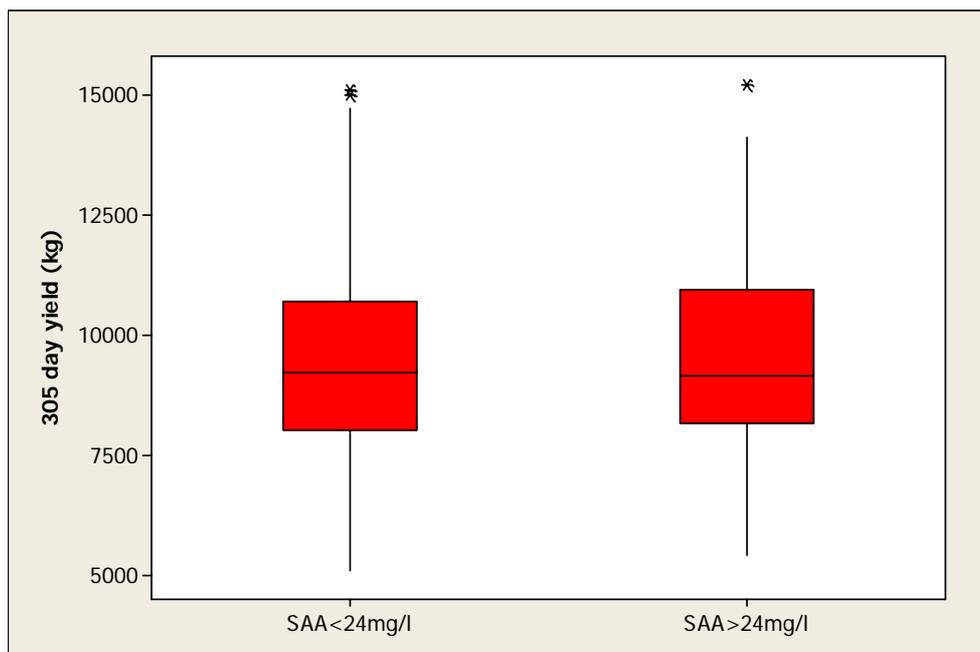


Figure 15. Variation in 305 day milk yield by SAA result, using 24mg/l threshold. The boxplot denotes the interquartile range, with the central bar denoting the median. Outlying values are indicated by asterisks. There was a 52 litre difference in median 305 day adjusted milk yields, but this was not statistically significant



E) Association with fertility parameters

Fertility data is summarised in table 5 below.

Table 5. Fertility parameters for cows with either elevated haptoglobin or SAA results. ^a indicates that the difference within the row was statistically significant.

	Haptoglobin < 0.02 g/l	Haptoglobin > 0.02 g/l	SAA < 24 mg/l	SAA > 24 mg/l
Calving to 1st service interval <i>Median days (IQ range)</i>	72 days (60 – 99)	74.5 days (57.3 – 96.8)	74 days (58.5 – 95.3)	72.5 days (60 – 95.3)
Number of services Median <i>(IQ range)</i>	2^a (1 – 3)	3^a (1 – 4)	2 (1 – 3)	2 (1 – 4)
Calving to conception interval <i>Median days (IQ range)</i>	115 days^a (80 – 164)	147 days^a (93.5 – 216.5)	123 days (84 – 190)	140 days (80 – 191.5)
% in calf by 100 days	33.2%^a	20.8%^a	28.5%	27.9%
% in calf by end of lactation	81.9%	75.9%	80.6%	78.0%

There was a significant difference between the individual farms in calving to 1st service interval (Kruskal-Wallis test; $P < 0.001$), in particular Farm C which had a median calving to 1st service interval of 164 days. However there was no significant association between calving to 1st service interval and haptoglobin level ($P=0.161$) and SAA either as a numerical value ($P=0.321$) or using the 24 mg/l threshold ($P=0.704$) using LME.

There was no significant association between number of services and SAA either as a numerical value ($P=0.541$) or using the 24 mg/l threshold ($P=0.571$) using GLMEp. However there was a positive association between cows with haptoglobin levels over 0.02 g/l and number of services ($P=0.038$).

As previously, there was a significant difference between the individual farms in calving to conception interval (Kruskal-Wallis test; $P < 0.001$), in particular Farm C which had a median calving to conception interval of 217 days, and Farm B and H which had median calving to conception intervals under 90 days. There was no significant association between calving to conception interval and SAA either as a numerical value ($P=0.152$) or using the 24 mg/l threshold ($P=0.570$) using LME. However cows with a haptoglobin level over 0.02 g/l took an average of 21.5 days (± 9.62 SEM) longer to get in calf compared to those cows with low haptoglobin levels ($P=0.025$).

There was no significant association between 100 day in-calf rate and SAA either as a numerical value ($P=0.541$) or using the 24 mg/l threshold ($P=0.848$) using GLMEb. However cows with a haptoglobin level over 0.02 g/l had a 100 day in-calf rate of 20.8% compared to those cows with low haptoglobin levels (33.2%)($P=0.007$). The Relative Risk of a cow being in calf at 100 days in milk if she had a high haptoglobin result (over 0.02 g/l) was 0.616 (95% CI: 0.432 – 0.878).

Primiparous cows were significantly more likely to be in calf by the end of their lactation (96%) compared to multiparous cows (77%). However there was no significant association between the proportion of cows in calf by the end of their lactation and haptoglobin level ($P=0.536$) and SAA either as a numerical value ($P=0.977$) or using the 24 mg/l threshold ($P=0.556$) using GLMEb.

DISCUSSION

Distribution of APP in clinically normal dairy cows at different stages of the production cycle

This study of 388 cows from 12 different dairy farms showed that a significant proportion of cows had elevated haptoglobin and SAA levels (over 40%). Given that all of the farms were requested not to submit animals for blood sampling with evidence of clinical disease problems, this would *in theory* suggest that there are widespread issues with subclinical inflammatory conditions in these dairy cattle under farm conditions. However one of the issues is that although APP are very sensitive, they are non-specific and so there could be a wide range of clinical conditions (from teat injuries to severe lameness) that are resulting in the elevated APP values observed. However even accounting for the non-specific nature of the APP response, there were associations with disease and production parameters in dairy cows which suggest that APP measurements may be useful for monitoring dairy cow health.

There were significant differences in APP results between cows in late pregnancy, which had lower SAA results compared to the milking cow groups. This could be explained by the presence of clinical and subclinical disease issues in milking cows such as mastitis or lameness, which are generally more prevalent than in late pregnancy when the cows are dry. Heifers also had significantly higher SAA results than multiparous cows, which was possibly more unexpected. However heifers are more prone to dystocia and so inflammation and trauma of the reproductive tract following calving (which would potentially elevate SAA values in the EARLY group), and may also be more subject to social stresses following the transition at first calving and start of milk production. What

was of interest was that SAA appeared to be much more sensitive to these influences, whereas there were no such significant differences observed for haptoglobin levels.

Similar to previous studies (Bionaz et al., 2007), there was also a significant increase in APP in early lactation, although this was only significant for SAA in this study. However it is still not clear whether this is a normal physiological phenomenon, or a response to various inflammatory processes which are very common in cattle immediate after calving. Examples of such common processes include reproductive tract trauma at calving, bacterial contamination of the uterus post-partum and fatty infiltration of the liver. For example, Bionaz et al. (2007) found that over 52% of the cows in their study had at least one clinical disease problem in the immediate period after calving, and over 40% were classified as “serious inflammations” that decreased milk yield by at least 20%.

It was also found that thin cows (BCS less than or equal to 2) had elevated APP values. It would be expected that cows with moderate to severe inflammatory conditions would have lower body condition scores as these conditions would either be affecting energy metabolism, feed intakes and/or diverting energy resources into these inflammatory processes.

Relationship between APP and existing parameters used in metabolic profiles

Although there was a significant correlation between haptoglobin and SAA, it was unexpected to see that there were 35.5% of samples (Table 3) where the two APP differed. The probable explanation for this is that SAA and haptoglobin are produced via different pathways, have different functions in the body and produced in response to

different sources of inflammation (Petersen et al., 2004). This has implications for their use in monitoring animal health, and SAA for example appeared to be much more prone in this study to variation by parity and stage of production compared to haptoglobin. This disparity between SAA and haptoglobin appeared to be more apparent on three farms, although there was no apparent reason why this should be the case.

As expected, there was a significant association between APP and other measures of inflammation (albumin and globulin). Although both of these are considered very crude indicators on inflammation (globulin for example is considered an indicator of chronic inflammation), there was a strong association between APP and globulin levels. 40 of the cows sampled (10%) had globulin levels over 50 g/l (considered the threshold for use in dairy cows by Whitaker (2004)), whereas there were many more cows with elevated APP. This would suggest that APP are more sensitive for the detection of inflammatory problems in dairy cows, compared to albumin and globulin levels.

There was no association between negative energy balance and APP response in this study, in contrast to other studies which have shown an association as a result of the inflammation in the liver associated with hepatic lipidosis (Nakagawa et al., 1997; Petersen et al., 2004). Huzzey et al. (2011) found a positive correlation between prepartum NEFA and haptoglobin levels, although the correlation co-efficient was low and the authors concluded that it was of little biological significance. This study analysed samples from cows at a wide variety of stages of production, including the MID group who would be expected to have few issues with fatty liver. The inter-relationships between inflammation and energy balance are likely to be complex, and looking for associations between only two variables is likely to be too simplistic an approach.

The other association of interest was between both APPs measured and plasma copper levels, which has implications for the interpretation of plasma copper levels. It has been previously noted that plasma copper levels increase during inflammation due to increases in the circulating levels of ceruloplasmin (Underwood and Suttle 1999), as ceruloplasmin is a positive APP and the main protein binding copper in the circulation. Plasma copper is acknowledged as a very crude indicator of copper status, as it gives little assessment of liver copper reserves where most of the copper is stored in the body. However there are various laboratories that promote the use of copper:ceruloplasmin ratios for the assessment of copper deficiency and/or molybdenum toxicity in ruminants. This variation in plasma copper levels according to APP response illustrates the potential external influences on plasma copper levels, and urges caution in their interpretation.

Association between APP and clinical disease and production parameters in dairy cows

There was a slight negative association between clinical mastitis occurrence and SAA, but this was not considered to be of biological significance given the previous research work which has highlighted SAA (and M-SAA3) as an early indicator of mastitis. Although there were only 10 cases of lameness recorded in the 388 cows, there was a significant positive association between SAA and lameness occurrence. It is widely acknowledged that lameness is under-recorded on farms (Barker et al., 2010), and it is likely that the number of subclinically and clinically lame cows in this study was higher which may have affected the results. Farm YM had particular problems with lameness and digital dermatitis at the time when the blood samples were taken, but unfortunately kept no lameness records. Regular mobility scoring or more detailed analysis from foot

trimmer records might be alternative approaches to take to more accurately assess lameness prevalence in the herds.

For those nine herds that milk recorded, there was no association between ICSCC and APP results. Subclinical and clinical mastitis represents a common and significant source of inflammation in dairy cows, and there have been previous studies showing an association between SAA in particular and mastitis (Eckersall 2007). However it is likely that there were too many confounding factors in this study, as it relied on monthly milk recording for the assessment of udder health. The time between blood sampling and the next milking recording could have been considerable (over 30 days in the case of the DRY group sampled), and so any inflammation and APP response would be resolved by this stage. While an attempt to account for this was attempted in the analyses with the time between blood sampling and milk recording entered into the model as a confounder, this does not take into account the resolution of any inflammation. Most of the studies on APP and mastitis have focused on the early detection of mastitis in automated systems, and it is likely that the approach in this study for the detection of mastitis has no benefits above regular monthly milk recording.

There was a significant association between elevated haptoglobin levels and an increased risk of culling. Cows with inflammatory conditions such as mastitis, metritis/endometritis and lameness are at an increased risk of being culled for involuntary reasons, and so it does follow that cows with elevated haptoglobin levels (and so signs of inflammation) are at an increased risk of being culled. However APP are relatively non-specific and short-term, and so it was of interest that this association was significant. Such an association would concur with a recent study which showed that cows treated for mastitis with a non-

steroidal anti-inflammatory drug were less likely to be culled than those that were not treated (McDougall et al., 2009). Prompt treatment and ideally prevention of any inflammatory condition in dairy cows has beneficial long-term effects on reducing culling rates.

Cows with inflammation (and so elevated APP) would have been expected to have reduced milk yields, due to the subclinical effects on production. However there were no significant effects of APP levels on milk yields in the present study. The milk yield data in this study was taken from monthly milk recordings which were then used to calculate lactation yields, and thus was potentially not accurate enough to detect any significant effect. In addition the level of APP gives no indication as to the severity or extent of the inflammation, and so any possible effects on production.

There were significant associations between elevated haptoglobin levels and reduced fertility with an increased number of services per cow, increased calving to conception interval and reduced number of cows in calf at 100 days post-partum for those cows with elevated haptoglobin levels (Table 5). The relationship between metabolism and reproduction in dairy cows is highly complex (Chagas et al., 2007), and it is likely that inflammation can affect the pathways that control reproduction at multiple sites. For example elevated APP might be a reflection of direct effects such as metritis/endometritis resulting in inflammation of the uterus and so reduced implantation, or indirect effects such as inflammation reducing feed intake and thus affecting energy metabolism. There is also the potential for inflammation to affect the hypothalamus-pituitary axis that controls reproduction in the cow. All of these complex pathways might be potential sites for the effects of inflammation on reproductive performance in the cow.

What are the appropriate thresholds for the interpretation of APP in adult dairy cows?

The reference ranges used for the interpretation of haptoglobin and SAA levels have been initially derived from experimental trials, where healthy animals were sampled before and after experimental infections. The question is to whether these reference ranges are appropriate for use in the farm situation. By definition positive APP (such as haptoglobin and SAA) are present in very low levels in healthy animals (Eckersall and Bell 2010), and so one interpretation of the high proportion of cows with elevated APP is that there were a large number of cows with subclinical inflammatory conditions. The issue is whether there are other thresholds that should be used, above which the productivity and welfare of the cows is compromised.

Indeed a number of previous studies have used different thresholds for the interpretation of APP in dairy cows. Studies looking at haptoglobin have used 0.03 g/l (Humblet et al., 2006), 0.8 g/l (Dubuc et al., 2010) and 1.0 g/l (Huzzey et al., 2009; Huzzey et al., 2011). However the higher thresholds quoted were used in studies looking to predict the risk of cows developing metritis in the immediate period after calving, and thus were focused on a specific condition in a specific timeframe. If a threshold of 1.0 g/l was used in this study, then only seven of the haptoglobin results were above this threshold, which is a lot lower than the 35% of samples with haptoglobin levels over 1 g/l reported by Huzzey et al., (2011). Studies using SAA have used a threshold of 60 mg/l (Humblet et al., 2006) in the first week after calving, and 25 mg/l at all other stages of the production cycle, which is similar to the threshold values used in this study.

Selection of appropriate thresholds for both haptoglobin and SAA is complicated by the skewed nature of the data (see Figures 1 and 2), with the majority of the haptoglobin

results being below the limit of detection (as would be expected in clinically normal animals with no inflammatory conditions). There might also be one optimum threshold for risk of culling, and another for reduced fertility in cows with elevated haptoglobin levels. Comparing these thresholds to a “gold standard” definition of lameness for example is also difficult, given how notoriously under-reported cases of lameness are. There were only 10 lameness cases reported in 305 cows in this study in the first 100 days of lactation, which is likely to be an under-estimate of lameness events.

Are APP useful for the monitoring of dairy cow health and productivity?

This study has shown that there are potential benefits to using APP alongside other biochemical markers for the assessment of dairy cow health and productivity. Overall SAA appears to be more prone to external influence, being affected by stage of production and parity. This may be a reflection of increased sensitivity, although the only link with clinical disease in this study was with lameness. Haptoglobin appears to be less prone to external influence, and there were significant associations with an increased risk of culling and reduced fertility parameters. However the non-specific nature of the APP response is their main drawback: they tell us that inflammation is present, but not what is causing it. However if the source of inflammation can be accurately pinpointed and rapidly treated, then this would help reduce effects on cow health and productivity.

ACKNOWLEDGEMENTS

The co-operation of the farmers involved in this study is gratefully acknowledged, as well as Wilson Lee who helped extract the farm data from the Uniform-Agri system. The metabolic profile analysis was performed by the Dairy Herd Health and Productivity Service at the University of Edinburgh. The APP analysis was performed by Reactivlab Ltd., with assistance from Professor David Eckersall and E McCulloch. The analysis of the APP was funded by a KTN Biosciences SPARK award. Dr Darren Shaw at the University of Edinburgh assisted with the statistical analyses.

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