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Health and growth of Finnish beef calves and the relation to acute phase response

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ABSTRACT
Healthy, thriving calves are essential for beef calf production. We studied the health status and factors associated with the growth of beef calves in six cow-calf herds during the first month of the calves’ lives and at weaning age (200 days).

The six herds were visited three times, when calves were approximately 3 days, 16 days and 30 days of age. On each visit calves (n=37) were clinically examined, weighed or measured, blood samples were collected, faecal samples obtained and deep nasopharyngeal swabs were taken. Each blood sample was analysed for acute phase proteins (haptoglobin, serum amyloid-A, fibrinogen), total proteins and albumin, the faecal sample for intestinal tract pathogens (rotavirus, bovine coronavirus, enterotoxigenic Escherichia coli and Salmonella, oocysts of Eimeria coccidia and Cryptosporidium, and nematode eggs), and the nasopharyngeal swab for respiratory tract pathogens (bovine coronavirus (BCV), respiratory syncytial virus (RSV), bacteria and mycoplasma).

Clinical diagnosis of respiratory tract disease, diarrhoea or umbilical disease was set at 15.0% for all the three consecutive examinations combined (n=107), but only few pathogens were detected from the samples. The increased levels of acute phase proteins were neither associated with any of the diseases nor with the pathogens. Random intercept linear models were used to explore factors affecting early (3–30 days) and long-term (3–200 days) growth, showing that calves with elevated serum amyloid-A concentrations at the age of 16 days had lower long-term growth. Increased albumin concentration at 30 days of age and higher parity of the dam increased early-term growth. The lack of association between a disease and the acute phase protein may stem from the low disease prevalence in the beef calves examined. The measurement of acute phase proteins of a young calf can help identify animals with possible future growth deficiencies, although the mechanisms through which the association between acute phase proteins and growth has yet to be explained.

1. Introduction
A good growth rate of beef calves is essential for an efficient beef production. Various factors contributing for the differences of growth among individuals have been revealed over the years, including gender (Krupa et al., 2005; Toušová et al., 2014) and breed (Lundborg et al., 2003; Krupa et al., 2005) to diseases (Windeyer et al., 2014). Also various management practices, e.g. feeding method of colostrum and group size of the calves in group pens in dairy calves are associated with the growth of the calves (Brickell et al., 2009).

The dams of the beef calves have an important role on the health and subsequently growth of the calves, as the dams are the main source of nutrition and care for the beef calves. However, at least the age and breed (Krupa et al., 2005) or parity of the dam (Lundborg et al., 2003; Murray et al., 2014) have shown associations with the average daily gain of beef or dairy calves. More direct association was recorded for getting adequate amount of colostrum, lower diseases incidence and daily weight gain (Dewell et al., 2006; Furman-Fratczak et al., 2011). Diseases reduce growth rate (Thompson et al., 2006; Windeyer et al., 2014), mostly by reducing feed intake and increasing muscle catabolism (Gabay and Kushner, 1999). In addition, cytokines released in the inflammatory process directly reduce appetite (Borghetti et al., 2009).
Calves are thought to compensate the cessations of growth, but compensatory growth has been mostly observed after experimental feed restriction (Hornick et al., 2000); the ability of the calves to compensate after recovering from a disease is less studied.

Acute phase proteins, synthesized by the liver at the onset of the inflammation are valid markers of inflammatory diseases (Petersen et al., 2004). The major positive acute phase proteins in cattle are haptoglobin (Hp), serum amyloid-A (SAA) and fibrinogen (Fb) (Humblet et al., 2004; Nikunen et al., 2007). Albumin is a negative acute phase protein, whose concentration decreases during inflammation (Jacobson et al., 2004; Petersen et al., 2004). Decreased albumin concentrations have been detected at least with uterine infections (Schneider et al., 2013) and experimentally induced acute phase response (Jacobson et al., 2004). Acute phase proteins have also been used as prognostic markers or assessing severity of diseases (Horadagoda et al., 1999; Humblet et al., 2004; Schneider et al., 2013).

The aim of this study was to explore the health of beef calves, follow growth and examine factors associated with diseases, growth and acute phase response before weaning.

2. Material and methods

Calves from six herds were included in the study. Convenience sampling was done from farms located in southern and south-western Finland and those participating in the Beef Cattle Development Project of the HKScan slaughterhouse. Inclusion criteria were having a compact calving season in the spring and providing means and facilities for weighing the calves. All herds were free from bovine viral diarrhea virus (BVDV) and were not subject to any vaccination protocol. Farms had typical Finnish beef calf herds where during the time of serving. The mean cow number on the farms was 58, ranging from 39 to 78 dams.

On each farm, 5–8 calves born within 2–5 days were included, resulting in 37 calves from 6 farms. The calves were either Herefords (farms A, B and C; n=19) or Charolais (farms D, E and F; n=18) breed. 57% of the calves were heifers, 30% Herefords and 27% Charolais heifers.

Calves were born in March and April 2009 and farm visits were scheduled to begin when the farm had as many as possible calves born within 3–4 days. The farms were visited at approximately two week intervals by the one or two veterinarians working in the research project, resulting in visits when the calves were 3.3 ± 1.1 days, 16.4 ± 1.4 days and 30.2 ± 1.2 days old. At each visit calves were weighed (or measured with weight tape on one farm), clinically examined, and blood samples were obtained. Cows had ad libitum feeding on silage. Dams and calves grazed on grass, oaks and straw bedding. Cows had free lying space but on one farm lying stalls were used. Cows had ad libitum feeding on silage. Dams and calves grazed starting from mid-May and the bull was kept within the herd to breed. Dams usually calve inside the barn, but some also on the pasture depending on the time of serving. The mean cow number on the farms was 58, ranging from 39 to 78 dams.

Blood samples were collected by venapuncture from the jugular vein into EDTA and plain serum tubes (BD Vacutainer, New Jersey, USA). The samples were analysed for complete blood count and total protein, albumin, Fb, Hp and SAA concentration. Globulin concentration was calculated as globulins = total protein - albumin. The complete blood count analysis was performed using an automatic system (Coulter-Counter Model T850, Coulter Electronics, Luton UK) as well as the total protein and albumin measurements (KONE Pro, version 5.4, Konelab, Finland). Fibrinogen concentration was determined with the heat precipitation method (Millar et al., 1971). For the haptoglobin measurements, a modified haptoglobin-haemoglobin binding method was used (Makimura and Suzuki, 1982), where o-dianisidine was substituted by tetrathionate benzidine as a chromogen (Alsemgeest et al., 1994). SAA measurements were performed by sandwich ELISA according to manufacturer's instructions (Phase SAA assay, Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland).

Deep nasopharyngeal swabs were collected using a 27 cm long sterile, guarded swab Dryswab™ (Medical Wire Equipment Ltd., Corsham, England) (DeRosa et al., 2000). From the deep nasopharyngeal swabs, anaerobic and aerobic bacterial culturing was performed and Ureaplasma diversum, Mycoplasma bovis, respiratory syncytial virus (RSV) and bovine coronavirus (BCV) were examined as described by Autio et al. (2007).

Faecal samples were collected from the rectum into plastic bags and analysed for salmonella, Escherichia coli (ETEC), rotavirus, coronavirus, Cryptosporidium spp., paraite egg counts and Eimeria spp. oocyst count. Salmonella culture was made according to ISO 6579:2002 and virulence factors for E. coli/ETEC (F5, F41, ST1) were detected with PCR described by Wieler and Bauerfeind (2003). Presence of rotavirus and bovine coronavirus was detected using a commercial ELISA kit (Duo Digestive Kit, Bio-X, Jemelle, Belgium). Parasite egg count and Eimeria spp. oocyst count were performed using the conventional McMaster's method. Cryptosporidium spp. was detected using Ziehl Neelsen staining and Cryptosporidium spp. positive samples were examined with a restriction length polymorphism PCR for species identification (Feng et al., 2007).

2.2. Statistical methods

Factors related to the serum proteins (albumin and globulins) and APP (Hp, SAA and Fb) concentrations during the neonatal period of beef calves were studied using random intercepts linear models. In those models protein concentrations were included as outcome variables and farm and calves as random factors. First order autoregressive covariance structure was included in the model to account for repeated
sampling of calf (at average age 3, 16 and 30 days). Sampling times (at average age 3, 16 and 30 days of age) were included as a categorical fixed factor and calf age at first sampling time as a covariate fixed variable in all models. The general equation of the linear mixed models for evaluating factors related to the protein concentrations was as follows:

\[ Y_{ijk} = \mu + \beta X_{ijk} + X_{ij} + \text{farm}_i + \text{calf}_j + e_{ijk} \]

In which \( Y_{ijk} \) is the outcome variable, \( \mu \) is the intercept, \( \beta \) are the sizes of the effect of independent covariate variable \( X_{ijk} \) (calf age at the first sampling time) and \( X_{ij} \) is a three level grouping variable (sampling time: at average age of 3, 16 and 30 days). \( \text{farm}_i \) is the random effect of farm, \( \text{calf}_j \) is the random effect of calf with the first order autoregressive covariance structure by sampling time for repeated measures, and \( e_{ijk} \) is an error term.

Firstly all calf (breed, gender) and dam (dam parity as 3-level factor: 1, 2–4, ≥5 and dam BCS as a 2-level factor: < 3 and ≥3) related factors and their interactions with sampling times were included in the models. A backward elimination procedure was used to establish the optimal models. Subsequently clinical disease related variables (respiratory disease: yes/no, diarrhoea: yes/no and umbilical disease: yes/no) and respiratory or intestinal tract pathogen variables (Pasteurella multocida: yes/no, Trueperella pyogenes: yes/no, Fusobacterium spp. yes/no, U. diversum: yes/no and rotavirus: yes/no) and their interaction with sampling times were included and a backward elimination procedure was performed. The model assumptions were verified with scatter and normality plots of the standardized residuals. A logarithmic transformation of Fb and square root transformation for SAA were used in the models. Bonferroni coefficients for multiple comparisons were used to correct P values.

For studying factors related to the daily weight gains, random intercept linear models were used. In first model early growth (from first sampling at approximately 3 days to last sampling at approximately 30 days of age) was used as an outcome variable and farm as a random factor. Protein concentrations from the first and second sampling times (at 3 and 16 days of age), breed and gender, dam parity (3-level variable), and BCS scores (2-level variable) were included initially as fixed factors and a backward elimination procedure was used for producing the final model.

In a second similar model the long term growth (from first sampling at approximately 3 days to weighing at weaning at an average 200 days of age) was used as an outcome variable. The protein concentrations from all sampling times (at 3, 16 and 30 days of age) were used with all the same variables as in the early growth model and a backward elimination procedure was used for the final model. Biologically important interactions were tested in both models and the model assumptions were checked by assessing visually the scatter and normality plots of standardized residuals. Bonferroni coefficients for multiple comparisons were used to correct P values.

Described models were fitted using the mixed procedure with SAS/STAT 9.2 (SAS Institute Inc., Cary, NC, USA) software.

2.3. Missing data

Of the 37 calves 3 died during the sampling period (2 calves) or before weaning (1 calf). The weaning weight was not obtained for four calves. Therefore, the data for the early growth model contain findings from 35 calves and data for the long term growth model from 30 calves.

3. Results

3.1. Clinical diseases and pathogens of intestinal and respiratory tracts

In general, calves were healthy, with very few clinical diseases. Clinical diagnosis (Table 2) was set at 15.0% of the consecutive examinations of 37 calves, the most common diagnosis being umbilical disease (prevalence of 10.8%, 5.7% and 5.7% at 3, 16 and 30 days of age, respectively). Respiratory tract disease and diarrhoea were rare during sampling and none of the calves had arthritis.

Findings in faecal samples were scarce, except for rotavirus and Cryptosporidium spp. Rotavirus was detected on four farms (farm B 4.2%, farm D 6.7%, farm E 31.3% and farm F 11.1% of the samples taken). Farms B and E reported a rotavirus outbreak between first and second visits. On farm B three calves from the study and three calves from outside the study fell ill for rotavirus confirmed by faecal sample examination. Farm E reported of two calves with clinical signs of rotavirus, and one of those died at 6 days of age.

Cryptosporidium spp. positive samples were recorded on second or third sampling times on all farms but farm C. The proportion of the samples positive for Cryptosporidium ranged from 4.2–27.8% on positive farms. No C. parvum was detected, neither Salmonella spp., Escherichia coli (ETEC) nor BCV. Eimeria spp. were detected from the samples taken at 30 days of age from one calf on farm A (6900 oocysts per gram faeces) and from four out of six calves on farm F (median 20250 oocysts per gram faeces). Nematode eggs were similarly detected only in samples from 30 d old calves, on farms A, B and C with mean egg count in positive samples of 450, 375, and 1200 eggs per gram faeces, respectively.

One quarter (27.1%) of the respiratory tract samples were positive for one or more bacteria or ureaplasm. P. multocida was the most commonly found (detailed results of each farm in Table 3). No M. bovis, bovine corona virus or bovine respiratory syncytial virus were detected.

![Table 2](https://example.com/table2.jpg)

**Table 2**

Clinical findings of Finnish beef calves on three consecutive visits.

| Diagnosis        | 3 days | 16 days | 30 days | Overall |
|------------------|--------|---------|---------|---------|
| Umbilical disease| 4/37   | 10.8    | 2/35    | 5.7     | 7.5     | 8/107   |
| Respiratory tract disease| 4/37 | 10.8 | 1/35 | 2.9 | 5.9 | 7/106 | 6.6 |
| Diarrhoea        | 2/37   | 5.4     | 0/35    | 0.0     | 1/35    | 2.9     | 3/107   | 2.8    |
| Total, calves    | 9/37   | 24.3    | 3/35    | 8.6     | 4/35    | 11.4    | 16/107  | 15.0   |

\(^a\) one calf had both umbilical disease and respiratory tract disease.

\(^b\) the two calves having both umbilical disease and respiratory tract disease are only counted once in the total.

![Table 3](https://example.com/table3.jpg)

**Table 3**

Percentage values of positive bacterial findings of deep nasopharyngeal swab samples of calves in six Finnish beef herds, results of three consecutive samplings at ages 3, 16 and 30 days combined.

| Farm | N | Pos\(^a\)% | PM | TP | F | U | M | RSV | BCV |
|------|---|------------|----|----|---|---|---|----|-----|
| A    | 6 | 11.1       | 5.6| 0.0| 5.6| 0.0| 0.0| 0.0| 0.0  |
| B    | 8 | 25.0       | 20.8| 0.0| 4.2| 0.0| 0.0| 0.0| 0.0  |
| C    | 5 | 6.7        | 6.7 | 0.0| 0.0| 0.0| 0.0| 0.0| 0.0  |
| D    | 5 | 13.3       | 6.7 | 13.3| 0.0| 0.0| 0.0| 0.0| 0.0  |
| E    | 7 | 35.3       | 17.6| 11.8| 0.0| 5.9| 0.0| 0.0| 0.0  |
| F    | 6 | 66.7       | 66.7| 5.6| 0.0| 0.0| 0.0| 0.0| 0.0  |
| Total| 107 | 27.1 | 19.6| 4.7| 1.9| 2.8| 0.0| 0.0| 0.0  |

N=number of calves per sampling, Pos=proportion of positive samples of all the samples from the farm. PM=Pasteurella multocida, TP= Trueperella pyogenes, F=Fusobacterium spp., U=Ureaplasma diversum, M=Mycoplasma bovis, RSV=respiratory syncytial virus, BCV-bovine corona virus.

\(^a\) Two samples were positive for two bacteria species, P. multocida+T. pyogenes and Fusobacterium spp.+T. pyogenes.

\(^b\) Two calves of farm E died after the first sampling which has been accounted for when calculating the proportions.
3.2. Age related changes in proteins and association with diseases and pathogens

Albumin and globulin concentrations showed continual change during the first month of life (P < 0.001, Fig. 1), albumin concentration increasing and globulin concentration decreasing. Albumin concentration also displayed differences between breeds: Charolais calves had higher concentrations than Hereford calves at first sampling time (LS means ± SD: 28.8 ± 0.37 g/l vs. 25.1 ± 0.37 g/l, P < 0.001). The mean SAA concentrations showed a clear decline and Fb a moderate decline during the first month (Fig. 1). The concentrations of Hp showed no significant changes over time (Fig. 1), and 81% of all the samples had Hp concentration below the detection limit of 60 mg/l. There were no significant associations between APP and gender or breed at any sampling time.

No association between the diseases and any of the acute phase proteins was detected. However, a calf that died after drenching on farm E at 12 days of age, had elevated inflammatory markers at 3 days of age (Hp 498 mg/l, SAA 121 mg/l, Fb 9.5 g/l and WBC 16.3×10⁹/l).

3.3. Factors associated with calf growth

In general, calf growth was good. The mean growth rate for the first month was 1100 g/day (SD 330 g/day) and from 3 days to weaning, 1200 g/day (SD 170 g/day). In the model for early growth, albumin at 16 days of age and the dam’s parity increased with early growth, but calf gender and dam body condition score at first visit were not associated (Table 4). The globulin concentrations after birth (3 days) showed a positive trend of association with early growth (Table 4). The mean parity of all the dams was 3.5 (SD 3.0), but parity varied among farms from 1.8 (SD 1.0) to 7.0 (SD 4.7) parities. The BCS showed less variation, with a mean of 2.8 (SD 0.7); mean BCS was 1.75 (SD 0.2) on the farm with the thinnest dams and 3.3 (SD 0.8) on the farm with the fattest dams.

In the model for long-term growth, differences between breeds and gender were observed: Charolais calves grew better than Hereford calves. Exploration of the interaction term (breed x gender) showed that Charolais bulls exhibited better growth rates than Charolais heifers but no difference between genders was recorded for Hereford calves (the results not shown). Dam BCS ≥3 after parturition increased the long-term growth of the calves. Increased SAA concentration at 16 days of age decreased the long-term growth of the calves (Table 4).

4. Discussion

In this study we established that the beef calves were mainly very healthy, had low intestinal pathogen loads and there were no associations between acute phase response and diseases. We found associations between growth, acute phase proteins, some dam characteristics and farm management level.

4.1. Clinical diseases and pathogens of intestinal and respiratory tract

Disease rates of the beef calves in this study reflect those of dairy calves in Nordic countries (Lundborg et al., 2005; Gulliksen et al., 2009; Seppä-Lassila et al., 2015), except that the beef calves had less diarrhoea. The health of beef calves in Central Europe was evaluated by farmers, resulting in higher (Busato et al., 1997) or slightly lower (Slavík et al., 2009) disease prevalence than in this study. Although clinical examinations were thorough and repeated biweekly, sample sizes on each farm and overall were small, so comprehensive conclusions on health status of Finnish beef calves cannot be drawn from this study.
Presumably beef calves react to diseases similarly as dairy calves where associations between acute phase response and respiratory tract infections and umbilical infections have been reported (Nikunen et al., 2007; Tóthová et al., 2012; Seppälä-Lassila et al., 2013). The low number of clinical diseases and low concentrations of especially Hp in this study restricted us from drawing any conclusions regarding associations between diseases and APPs. However, lower acute phase protein concentrations were reported for healthy beef cattle than for healthy dairy cows (Tourlomousis et al., 2004) which may indicate some differences between diseases and APPs. In this study there were very few of the clinical diseases that are often associated with reduced growth rate in calves (Virtala et al., 1996; Windeyer et al., 2014), so differences in the growth of the calves stemmed from other origins (e.g. subclinical infections, breed, gender, nutrition, genetics etc.). In our study we found multiple factors associated with growth in the early life (3–30 days) and in the long term growth (to 200 days weight).

Our results show that calves from higher parity dams grew better during in the early life, but other studies detected associations between increased parity of the dam and growth of calves from birth to weaning (Krupa et al., 2005) or no effect at all (Tousová et al., 2014). Primiparous dams have poorer quality colostrum (Rocha et al., 2013), which may result in lower growth in early life if calves are more susceptible to diseases. The positive association of albumin and globulin with early growth is interesting. Although albumin is a negative APP (Jacobsen et al., 2004; Petersen et al., 2004), with decreasing concentrations during inflammation, increases in albumin concentration do not indicate absence of acute phase response or healthier animals. Physiological increase of albumin is observed after birth (Knowles et al., 2000; Bertoni et al., 2009), but it remains unclear how increased albumin concentrations are associated with increased growth during the first month of life. The globulin concentration depicts the intake of colostrum and colostrum quality (Furman-Fratczak et al., 2011), which has already been associated with better growth.

The long term model showed better growth for Charolais than for Hereford calves as described by Krupa et al. (2005), who also showed different growth rates at different breeds at different ages. Bull calves grow faster and get bigger than heifer calves (Tousová et al., 2014), but in our study this held only for the Charolais breed. The parity of the dam showed no more effect on the growth rate in the long-term model.
but the dam’s BCS ≥3 after parturition was associated with better growth rate. Naturally, undernourished dams with BCS of 1.5 or 2 will not produce thriving calves, but it is surprising to see this association for 200 days is a novel finding for beef calves, although a similar rate for 200 days is a novel finding for beef calves, although a similar

Conclusions

This study investigated health and factors affecting beef calf growth in early life and during the long term. Albumin and globulin concentrations and the parity of the dam were positively associated with growth rate during the first month. The breed and gender had effects on growth only regarding long-term growth (from 3 days to 200 days). The negative association between SAA at two weeks of age and growth rate for 200 days is a novel finding for beef calves, although a similar phenomenon was observed in reindeer calves and lambs. Elevated SAA concentrations could be used as a prognostic indicator of a weaker growth rate.

Conflict of interests

Of the authors Heidi Härtel was employed by the HKScan meat company as a herd health veterinarian during the period of the study. The employment did not interfere with the scientific integrity of the study.

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