Associations between group sizes, serum protein levels, calf morbidity and growth in dairy-beef calves in a Finnish calf rearing unit

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Abstract

Efficient dairy-beef production relies on good quality of purchased calves, defined by breed, health, and growth characteristics. Several management factors, such as commingling of calves and large group size, predispose calves to diseases. Acute phase proteins are sensitive detectors of calf diseases. We studied the associations between group size, serum acute phase proteins, immunoglobulin G (IgG), calf morbidity and growth of dairy-beef calves in a random field trial in a calf-rearing unit in Finland.

The randomized trial was carried out at a calf rearing unit, where approximately 80 dairy or crossbred calves were allocated either into a single group of 40 calves or into four groups of 10 on arrival at the calf-rearing unit (at age 24.1 SD ± 9.2 days). The study was carried out on 6 arrival batches: 476 calves. Calves were clinically examined and blood sampled on arrival (day 0), and haptoglobin (Hp), serum amyloid A (SAA), albumin and IgG were determined. Calves were weighed on arrival (day 0, average age 24.1 days), at the end of the milk feed period (day 49), at approximately 200 days of age and at slaughter (carcass weight) at 15–18 months of age. During the rearing calves were observed by the farm workers and treated, if necessary, according to predetermined instructions of the veterinary surgeon. All NSAID and antimicrobial treatments were recorded and used as morbidity indicators in statistical analysis.

There were no differences in the numbers of antimicrobial treatments or growth among the groups. The majority (84.1%) of antimicrobial treatments were used against respiratory tract infections. Higher concentrations of albumin and IgG on arrival extended the time before the first and the second antimicrobial treatments. Complex relationships between group size, morbidity, concentrations of serum acute phase proteins and IgG at arrival, and growth of calves were explored. Group size of 10 calves did not protect calves from respiratory tract infections, when the small groups were sharing the air space with a large group. An increased SAA concentration on arrival was associated with poorer average daily gain at two rearing periods and with lower carcass weight at slaughter.

Serum proteins could be valuable health indicators for purchased calves because they have numerous and variable associations with health and growth. The mechanisms that connect increased SAA concentration and poorer average daily gain over the long term remain unclear.

Introduction

Optimal growth is essential for sustainable beef production. Growth is an aggregate of internal and external factors that promote and retard growth, including balanced nutrition, genetics, stress and disease. Beef breeds are bred specifically for particular growth and carcass quality characteristics. In the Nordic countries beef production is largely from dairy cattle, so such quality traits have also been explored in dairy bulls (Johansson et al., 2009), although as yet little used in beef production in Finland.

Diseases represent the major cause of decreased growth rate in young calves (Virtala et al., 1996; Windeyer et al., 2014), which are prone to infectious diseases (Svensson et al., 2003) due to immature immune systems, low humoral antibody concentration and exposure to a number of stressors at an early age (Hulbert and Moisá, 2016). Rearing a calf group of diverse origin increases morbidity (Step et al., 2008) increasing the profuse use of antimicrobials at the rearing unit and a risk of lower daily gain. In addition to commingled groups, large group size is another factor increasing morbidity (Svensson et al., 2003; Abdelfattah et al., 2015). Considering group size, smaller groups appear healthier (Svensson and Liberg, 2006; Abdelfattah et al., 2015), but the optimal group size with respect to health and labour has yet to be determined.

In Central Europe and North America (USA and Canada) veal calf production relies on collecting calves from various dairy farms to rearing units, resulting in high morbidity and treatment rate, often with group treatment and metaphylactic antimicrobial use (Pardon et al., 2012; Jarrige et al., 2017). Factors increasing metaphylactic use include purchase of the calves, larger group size and larger herd size (Lava et al., 2016a). The growth-promoting effect of antimicrobials in farm animals via improved feed efficiency was reported (Callaway et al., 2003; Duffield et al., 2012), but administering antibiotics as growth promoters in a prophylactic manner has since been forbidden in the European Union (EC regulation 1831/2003).

In Finland, beef production is mainly based on raising bull calves of either dairy breeds or dairy × beef breed crosses (mostly Blonde d'Aquitaine, Hereford, Aberdeen Angus, or Limousin) on separated beef farms until 15–18 months of age. Approximately two thirds of calves are delivered first to specialized calf-rearing units and after that, at approximately six months of age, to specialized beef production farms. One third is delivered from dairy farms directly to integrated beef production farms specialized in rearing, fattening and finishing the purchased cattle. After the milk or starter feeding period of up to two months, feeding is based on silage (approximately 60% of dry weight) and concentrate. All the bull calves and some surplus heifers are reared for beef production. Calves are collected from several dairy farms and transported immediately to rearing units at around 2–3 weeks of age, predisposing them to conditions similar to those for European veal production. Usually calves are not vaccinated against bovine respiratory diseases in Finland, partly because Finland is free from infectious bovine rhinotraceitis and bovine viral diarrhoea virus (Evira Finnish Food Safety Authority, 2016).

Acute phase proteins such as haptoglobin (Hp), serum amyloid A (SAA) and fibrinogen (Fb) are non-specific markers of inflammation and diseases indicators (Petersen et al., 2004), whose concentration increase with respiratory tract infections umbilical diseases and diarrhoea in calves (Humblet et al., 2004; Nikunen et al., 2007; Seppä-Lassila et al., 2013; Balikci and Al, 2014). Serum Hp is useful in predicting the incidence of metritis postpartum (Huzzey et al., 2009) and the severity of calf pneumonia (Humblet et al., 2004). Increased concentrations are also associated with larger group sizes and with increased morbidity (Svensson and Liberg, 2006). Regarding SAA, increased concentrations were associated with decreased average daily gain in beef calves (Seppä-Lassila et al., 2017), reindeer calves (Orro et al., 2006) and lambs (Peetsalu et al., 2013). Albumin, in addition to its main functions in maintaining colloid osmotic pressure and transport, acts also as a negative acute phase protein, the concentration of which decreases after a lipopolysaccharide injection challenge or infection (Fayer and Lunde, 1977; Jacobsen et al., 2004; Schneider et al., 2013).

Our aim was to study the associations between group size in beef-dairy calves with calf morbidity and growth, and further explore the associations between growth and inflammatory status (measured via acute phase protein concentration) at the beginning of the rearing period.

Materials and methods

Study population and study design

The randomized trial was designed to investigate the association between group size and health and growth of the calves. It was carried out in Western Finland in two milk-feeding compartments of a voluntary calf-rearing unit comprising 18 compartments and 1,440 calves. The same two compartments were used throughout the study, three times each, and they were chosen based on convenience (easiest access when arriving). Sample size was calculated to be 248 calves for a treatment group (large or small group size) assuming 80 g (SD ± 200 g) difference in daily weight gain at the time of early rearing (50-day period before weaning). Study power was set to 0.8 and confidence level to 0.95 assuming equal variances and adjusting for clustering at pen level (mean cluster size 16, intra cluster coefficient 0.1). Six batches of calves with around 80 calves each were included in the study, one batch in one compartment at a time. Calves included in the study were transported to the rearing unit from surrounding farms, situated mainly within a 200 km radius of the rearing unit. On arrival approximately 80 calves were allocated to a single group of 40 or four groups of 10, every other to the large group and every other to a small group. The small groups were located at one end of the compartment and the large group at the other end, sharing the air space. The pens of the small groups were separated from each other and from the large group by 1.5 m high solid fences preventing contact between calves in neighbouring pens. All the groups were kept in an insulated barn, with an automated exhaust fan. All the pens were similar, with a slatted floor feeding area and a resting area with wood shaving bedding: 2.3 m^2 per calf. Calves had free access to water and to acidified milk replacer, of which they consumed on average 8–9 litres a day. Concentrates and silage were offered, but their consumption was not recorded. It was assumed that all calves were managed in the source farm according to current legislation and provided with adequate colostrum feeding and fed with milk feed or starter feed until transportation to the rearing unit. Altogether 238 calves in large groups and 238 calves in small groups were included in the study, which ran from September 2013 to April 2014.

The calves were clinically examined on arrival or the following day (first examination day = day 0). Clinical examination included auscultation of the heart and lungs, measurement of the respiratory rate, inspection for the occurrence of nasal or ocular discharge or diarrhoea, measurement of body temperature, inspection and palpation of the umbilicus and joints and inspection of general appearance of the calf (normal or dull). Blood samples were obtained after the clinical examination.

Between the clinical examinations, farm workers observed clinical signs and recorded all medications on a daily basis from arrival until the calves were moved to another compartment on approximately day 49 (SD ± 4.9 days). Farm workers treated calves with antimicrobial according to the instructions of the farm's veterinarian when two of the requirements were fulfilled: rectal temperature was ≥39.8°C, rapid breathing (the frequency was not measured exactly, but clearly more rapid than normally, approximately >60 times per minute, and often laboured) and/or calf had signs of depression (decreased interest in the surroundings, empty stomach, lying down when the group was active). Basic treatment included both antimicrobial (primary: tulathromycin; secondary: oxytetracycline; chronic case: procaine penicillin) and NSAID treatment. The latter was used alone for mild cases. The NSAID usage was included in the individual medication records. The sick and treated calves remained in their allocated pen. Antimicrobial and NSAID treatment history was used to evaluate the association of clinical disease with average daily gain during the milk-feeding period assuming that more treatment was associated with more clinical disease.

The calves were weighed using a digital cattle scale on day 0, when moved from the milk feed compartment (at approximately day 49), when moved to the finishing farm at around 200 days of age. The carcass weight of each animal was collected from the data-base of the slaughter house. The outcomes, average daily gains at EARLY (from day 0 to moving the calf to another rearing compartment at approximately day 49) and 200DAYS (from day 0 to the age of 200 days) were calculated and carcass weight at SLAUGHTER was recorded.

Clinical examination and sample collection were carried out as a part of the farm's calf health care scheme, with minimal restraint and stress to the calves.

Analysis of blood samples

The blood samples obtained by venapuncture of the *vena jugularis* were stored during the sampling day in a styrofoam box with cooling elements and transported to the laboratory overnight, protected from extreme temperatures. Serum was centrifuged in the laboratory the day after the sampling, frozen and stored in the freezer until analysed. Haptoglobin concentrations were determined using the haptoglobin-haemoglobin binding method described by Makimura and Suzuki (1982) and modified by Alsemgeest et al. (1994) by substituting the chromogen o-dianisidine with tetramethylbenzidine. Serum amyloid A concentrations were determined using a commercial ELISA sandwich kit (Phase SAA assay, Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland) according to the manufacturer's instructions. Albumin concentrations were determined using an automatic chemistry analyser (KONE Pro, Thermo Fisher Scientific, Vantaa, Finland)

and immunoglobulin G (IgG) samples were analyzed using commercial ELISA kit (Bio-X Diagnostics, Rochefort, Belgium).

Statistical analysis

Linear mixed regression models were used to study the association of group size, calves' inflammatory status on arrival, and medications with average daily gain for different time periods. Three mixed effect linear regression models with variables EARLY, 200DAYS and carcass weight at SLAUGHTER as outcomes were built, where batch and pen inside the batch were included as random effects. For these models, antimicrobial treatment starting dates 7 days apart were considered as separate treatment series; all the treatments starting less than 7 days apart were combined and considered as treatment of the same disease. Recurrence of a disease was recorded when an antimicrobial treatment was re-introduced ≥8 days after the previous antimicrobial treatment. Independent variables "age on arrival" in days, "group size" (large or small), albumin (g/l), globulin (g/l), SAA (mg/l) and Hp (mg/l) concentration on day 0, "number of NSAID use only" (no concurrent antimicrobial treatment), "number of antimicrobial treatments" (categories of 0–1, 2–3 or ≥4 times), gender, and breed (Ayrshire, Holstein-Friesian or dairy-beef breed mix) were inserted to the models. Age at the time of slaughter were included in the model with outcome variable as carcass weight at slaughter to control the possible confounding effect of different rearing period. Confounding was defined as a change of 15% in variable coefficients.

The final models were produced by backward elimination of the variables from the full model until all the variables had *p*-values of <0.05, or when the Wald test for categorical variables with more than two categories resulted in a *p*-value of <0.05. The variable "Age on arrival" was included in all the models where SAA was included, to control for the association of age observed in SAA (Orro et al., 2008). Group size was included in all models as the main variable of the study design. Linear relations of the outcome variables and continuous variables were checked and continuous variables were categorized if non-linear relationships were encountered (in the case of number of antimicrobial treatments in EARLY model). Biologically meaningful interactions were tested in all three models and no interactions were evident. The model fit was visually assessed using scatter and normality plots of model residuals.

A multilevel mixed-effects parametric survival Weibull distribution model was used to assess the factors associated with the time from arrival to first antimicrobial treatment. The following period started at calf arrival at the rearing unit, and ended when the event of interest had occurred or when the data were right censored when calves moved to another compartment at approximately on day 49. Two models were built; the events of interests were the first antimicrobial treatment and the recurrence after the first antimicrobial treatment. Shared frailties of batch and pen were included in the survival model, calves nested in pen and pen nested in batch. Variables included in the initial survival models were "group size" (large or small), albumin, IgG, SAA and Hp concentration on day 0, gender, and breed (Ayrshire, Holstein-Friesian or dairy-beef breed mix). "Age on arrival" was forced into the model as albumin and IgG concentrations may have undergone age-related changes. The final model was developed by backward elimination of the variables from the full model until all the variables had a $p < 0.05$. Group size was included in both survival models as a possible confounder. Because some calves were medicated on arrival day, according to instructions of the herd veterinarian, and examined one day after arrival, they had the first antimicrobial treatment on day -1. To produce positive time integers, 2 days were added to the raw data. Six calves died of unknown causes before weaning and as information about recurrence after the first antimicrobial treatment was missing for three of those calves, those data were not included in the second model. Proportional hazard assumption and fit of survival models were checked with log-log plots of survival and cumulative hazard vs. Cox-Snell residual plots, respectively. All statistical analyses were performed in Stata/MP 14.1 for Windows (StataCorp LP, Texas, USA).

Results

Descriptive statistics

The mean age for calves at arrival was 24.1 days (SD ± 9.2 days), and 75% of the calves were less than 30 days of age. Mean age at slaughter was 558.3 (SD ± 65.8) days. The calves were mostly dairy breeds; 37.4% were Ayrshire (Ay), 45.8% Holstein, Friesian or Holstein-Friesian (Hol) and 16.8% mixed dairy-beef breed. The majority of the calves (82.8%) were male. The average daily gains for three different time periods for each breed and gender are presented in Table 1. The mean weight at arrival was 55.7 kg (SD ± 10.2 kg), 58.0 kg (SD ± 10.0 kg) and 55.2 kg (SD ± 9.7 kg) for Ay, Hol and mixed dairy beef breed.

On average, calves in the small groups originated from 7.2 herds (range 5–9 herds) and calves in large groups from 23.8 herds (range 16–31 herds) per batch. There were no differences between the distribution of gender in small and large groups. Considering breeds, in batch 4 there were more mixed breed calves in small groups and more Holsteins in the large groups. Other batches showed no differences between the breeds in groups. Mean age at arrival was greater in batch 5 in calves of the small group (mean age 29.3 days) than in large groups (mean age 22.9 days), in other batches no difference was recorded. Mean weight at arrival was greater in batches 4 and 5 in small groups (60.8 kg and 59.4 kg, respectively) than in large groups (55.4 kg and 51.8 kg, respectively), but other batches showed no differences in mean weight of the groups.

Use of medication

A total of 1417 records of antimicrobial treatment were collected during the 49-day rearing period (resulting in 23,132 calf days). The major indication for antimicrobial treatment was respiratory disease, representing 88.6% of the treatment indications. The second most common indication was diarrhoea, representing 3.7% of the treatments. Other indications were problems with lethargy (3.5%), feet (1.0%), colic (0.8 %), ear infections or drooping ears (0.6%), umbilical diseases (0.4%), fever (0.2%), and undefined (1.2%). Combining antimicrobial treatments administered within seven days into a single treatment resulted in 998 treatments administered to 458 calves, and 18 calves (3.8%) untreated with antimicrobials during the 49-day rearing period. For respiratory infections and other than diarrhoea tulathromycin, oxytetracycline, florphenicol and procaine penicillin were used. Trimethoprime-sulphadoxine was used for calves with diarrhoea, when necessary. Recurring infections were common, affecting 69.9% of the calves. The mean time for the first recurrence of a disease was 26 days (SD ± 10.1 days) from arrival. The numbers for antimicrobial treatment times are shown in Table 2. Mean number of antimicrobial treatments for calf was 2.10 (SD ± 1.01) times.

The calves treated with NSAIDs only (1,347 NSAID treatments) had either signs of respiratory disease (93.3%) or lethargy (5.5%), criteria for NSAID only use described in material and methods. Sporadic cases of problems with feet, colic, diarrhoea or miscellaneous indications were also treated with NSAID only. Mean number of treatments with NSAIDs only for calf was 2.77 (SD ± 2.45) and median 2 (min-max 0-14).

The factors related to the time before the first antimicrobial administration and the time to the first recurrence were examined for the survival analysis. Higher IgG and albumin concentrations on day 0 lengthened the time before the first antimicrobial treatment and the time to first recurrence (Table 3A and 3B). Calves of mixed breed had longer times to the first antimicrobial treatment and recurrence than Ay calves (Table 3A and 3B). Hp and SAA concentration on day 0, age on arrival, group size and gender were not associated with the time before the first medication or recurrence. Half of the calves (49.9%) had their first antimicrobial treatment in the rearing unit by day 8 of the study, and 89.2% of the calves by day 21 (Figure 1). By day 21, 26.8% of the calves had already had a recurrence of a disease.

APP and IgG concentrations

The mean SAA and Hp concentrations at arrival were 61.4 mg/l (SD ± 37.1), median 52.4 mg/l (min-max 2.1-296.5) and 177 mg/l (SD ± 123), median 152 mg/l (min-max 60-1468), respectively. The mean albumin and IgG concentrations at arrival were 33.8 g/l (SD ± 2.8) and 8.3 g/l (SD ± 4.5), respectively.

The concentrations of SAA at arrival were below the reference value of 178 mg/l (Seppä-Lassila et al., 2013) in most calves (91.1%), but many calves (20.8%) had concentrations of Hp that exceeded the reference value 196 mg/l (Seppä-Lassila et al., 2013) on day 0. No differences in acute phase protein concentrations were recorded between the small and large groups (results not shown). Hol calves had lower SAA concentrations (mean 55.6 mg/l ± SD 34.4 mg/l) than Ay calves (65.4 mg/l ± SD 37.8 mg/l) or mixed breed calves (68.5 mg/l ± SD 45.0 mg/l).

Factors associated with growth

The regression models for growth (Table 4) detected several factors associated with the average daily gain at different stages of calves' lives, but no difference in growth was detected between the small and large groups. Bull calves grew faster than heifer calves over all periods. Ay calves had lower average daily gains than Hol calves in EARLY and 200DAYS models, on average -60 g/day and -34 g/day, respectively. However, calves of mixed breed had the highest carcass weight at SLAUGHTER, on average +18.3 kg compared with the Ay, while there was no difference between Ay and Hol breeds in the SLAUGHTER model. The calves that were older on arrival at the rearing unit had better average daily gains in EARLY and 200DAYS models.

In the EARLY model for rearing period of 49 days (Table 4A), the use of only pain medication was negatively associated with average daily gain, with an average decrease of 8.8 g/day each time pain medication was administered without antimicrobial treatment. In contrast, antimicrobial treatments over 4 times were associated with higher daily gain (on average 84 g/day) compared with calves treated with antimicrobials 0–1 times during the milk-feed period.

Increased SAA concentrations at day 0 were associated with lower average daily gain for both periods; a 50 mg/l increase in SAA concentration would be associated with a 30 and 19 g/day decrease in average daily gain in EARLY and 200DAY models and 6 kg lower carcass weight at SLAUGHTER model, respectively (Table 4A, 4B, and Table 5). Increased IgG concentrations at day 0 were associated with better average daily gain in EARLY growth (Table 4A). Increased Hp concentration on day 0 was positively, and increased albumin concentration (day 0) negatively associated with carcass weight at slaughter (Table 5).

Discussion

Group size

No differences between small and large groups were detected in this study regarding the number of antimicrobial treatments or average daily gain. The small and large groups shared the same air space, resulting in similar infection pressure for the both groups. The randomization of the group increased commingling and calves from the same source farm ended up in different groups, resulting in increased stress and presentation of new pathogen strains. A common practice is to attempt to maintain calves from the same source farm together. These two aspects, together with a comparatively large group size also in the smaller groups, may be the main factors for the absence of differences.

The optimal group size for calves, economically and health-wise, has not yet been defined. In our study the groups of 4 x 10 and 40 calves were easy to form because of the compartment layout and farm routines. Group size of >10 calves is also a reported risk factor for metaphylaxis administration (Lava et al., 2016b). Frequency of coughing or clinical respiratory tract disease increases as size of the calf group increases from 2 to 6–9 and 12–18 (Svensson and Liberg, 2006; Abdelfattah et al., 2013). Dairy farms keeping calves in a group of seven or more were at greater risk of high calf mortality than farms with smaller groups (Losinger and Heinrichs, 1997). Results of the current study and the referenced literature suggest that a group of 10 calves is not small enough to protect them from diseases, at least when the calves originate from various sources and are highly mixed. The farm sizes for calf-rearing are increasing in Finland, and the results of the study can be generalized to larger scale calf-rearing unit, representing about one quarter of all the calf rearing in Finland.

Use of antimicrobials

Although metaphylactic group treatments were not used on the study farm, antimicrobials were extensively used and only a few calves were left untreated during the milk-feeding period. Very precise follow-up and measurement of the temperature of every calf, when even only slightly suspected, could have contributed to the extensive antimicrobial usage, although NSAIDs alone were used for mildly sick calves with elevated temperature. Antimicrobials were used in our study mostly to treat respiratory disease, similarly as for calves in Denmark (Fertner et al., 2016), other indications being recorded only occasionally.

Increased concentrations of IgG and albumin on arrival lengthened the time before the first antimicrobial treatment. A similar association was observed for days from arrival to recurrence. Similarly, higher immunoglobulin concentration of veal calves on arrival lengthens the time to occurrence of bovine respiratory disease (Pardon et al., 2015). The concentration of gamma globulins increases sharply after proper colostrum ingestion, and thereafter decreases until six weeks of age (Mohri et al., 2007; Tóthová et al., 2016). Low concentration of immunoglobulins in young calves indicates failure of passive transfer, due to insufficient colostrum intake (Tyler et al., 1996; Pithua et al., 2013), predisposing calves to diseases. The calves in the study were on average 24 days of age on arrival, and still heavily reliant on passive immune defence (Chase et al., 2008). The positive association of higher concentrations of globulins with health was shown in the current study, by the extended time to the first and second antimicrobial treatment. The majority of the calves were male, for which the care on source farms is sometimes questioned. However, at least a study on Canadian farmers reported that 91% of the farmers administered colostrum to all male calves (Renaud et al., 2017).

Similarly to the increased concentrations of immunoglobulins, increased concentrations of albumin were positively associated with time before the first antimicrobial treatment time and with time to recurrence. The main functions of albumin are to maintain the colloid oncotic pressure of blood and bind and transport of various compounds in the blood (Quinlan et al., 2005), and only secondary it serves as a negative acute phase protein (Petersen et al., 2004). However, increased concentration of a negative acute phase protein does not indicate better health. Lower levels of albumin could correlate with young age of a calf (Knowles et al., 2000; Tóthová et al., 2016), paired with higher risk of disease. However, the age of the calf at arrival was controlled in the survival analysis models, and age does not explain the observed association.

The concentrations of other APPs were not associated with elapsed time to antimicrobial treatments. The concentrations of SAA were moderate overall, but several increased concentrations of Hp were recorded. The treatments of the calves began on the day of arrival, indicating that the disease originated from the dairy farm, and the peak of the APPs might have already passed. The signs of disease can escalate following transport and commingling with unknown animals, promoting an increased number of antimicrobial treatments soon after arrival. The proportion of calves remaining untreated decreases steeply for the first two weeks, after which the cases become less frequent, as also the number of animals at risk diminishes. The shape of the recurrence survival curve differs from that for the first treatments in being less steep. The recurrence variable does not take into account calves that have extended periods under antibiotics, without an eight-day period untreated.

Factors associated with daily gain

Bulls showed better average daily gains at all stages. For the first months Hol calves had better average daily gains than Ay calves, which was reported previously for heifer calves (Lee et al., 1988; Pietersma et al., 2006).

However, in the current study the difference between breeds was recorded only for the bulls. The beef breeds by definition grow better than the calves of dairy breeds and this was also the case for the mixed breed calves. The calf's age on arrival at the rearing unit was positively associated with better average daily gains over all time periods, although the magnitude of association clearly diminished after the initial rearing period (EARLY model). Older calves are at an advantage in a calf group where the feed resources and rest area may be limited. In addition, the concentrations of IgG start increasing around 5–6 weeks of age, providing the calf with better resistance to diseases (Klinkon et al., 2008; Hulbert and Moisá, 2016). The relatively large variation in age of the calves on arrival was due to fluctuation in demand and supply of calves, combined with transport arrangements, when distance from farm to farm can be long.

The calves that received four to five antimicrobial treatments had better average daily gain during the milk feed period than calves with fewer treatments, but the use of NSAID without a concurrent antimicrobial treatment was negatively associated with the daily gain, although the cases were supposed to be less severe. These somewhat controversial associations can be explained by: 1) The growth-promoting effect of antimicrobials in production animals, which was reported in the 1950s, reviewed by Dibner and Richards (2005), and which may have improved growth of some animals during the high infectious pressure in the current study (Feighner and Dashkevicz, 1987; Butaye et al., 2003); 2) We did not monitor whether the well-growing and muscular animals showed more severe symptoms and thus were more likely to be treated with antimicrobials than with NSAID only; 3) Calves treated with NSAID only showed early signs of infection, also potentially requiring antimicrobial treatment. However, the study design did not allow any causal inference or evaluation of effectiveness of antimicrobial and NSAID treatments. The extensive use of antimicrobials on the farm was mainly a result of high morbidity, not excessive usage of unnecessary antimicrobial treatments. Increased concentrations of SAA in early life forecast lower average daily gain and are associated also with lower carcass weight. This negative effect was largest during the early rearing period until weaning. Although the effect was decreased in the 200 DAYS model, the carcass weights of calves with higher SAA at arrival were still lower. This is in contrary to the negative effect of morbidity (measured by numbered of NSAID treatments) during the early rearing period. Those calves with higher morbidity compensated for their weight loss and this early negative effect disappeared by age of 200 days and at slaughter.

According to regularly used farm budgeting tables the effect is in economic terms extremely important when it is causes an estimated 50 % drop in profit per calf in a calf-rearing unit. According to our results, the overall association between SAA and growth is moderate for the beef production chain: two calves differing by 50 mg/l in SAA concentrations at the beginning of rearing, slaughtered at the age of 16 months would have a carcass weight difference of 6 kg. The association of increased SAA in early life with growth rate over the long term was previously reported for healthy beef calves (Seppä-Lassila et al., 2017), healthy reindeer calves (Orro et al., 2006) and healthy lambs (Peetsalu et al., 2013). The acute phase reaction reduces growth by reducing feed intake, increasing protein catabolism and decreasing the production of insulin-like growth factor-1 (Gabay and Kushner, 1999). The concentration of insulin-like growth factor-1 decrease in response to the cytokines interleukin-1, interleukin-6 and tumour necrosis factor α, and body contributes energy and other resources to the immune system (Borghetti et al., 2009). However, the cytokines are short-lived and the normal growth rate should be restored after the infection or after inflammation ceases, leaving the mechanisms of long-term changes open. Also, the concentrations of SAA were below the reference value, thus not indicating clinical disease but probably subclinical disease. Karreman et al. (2000) showed the usefulness of serum SAA measurements as markers of sub-clinical diseases in cows.

The difference in average daily gain in Ay and Hol calves could reflect the difference in concentrations of SAA between the breeds. Pigs exhibit differences among breeds in C-reactive protein and pig-MAP concentrations (Clapperton et al., 2007; Diack et al., 2011), but in cattle no differences in APP concentrations among breeds have been reported. Even if the dairy breeds Ay and Hol would differ in mean SAA concentrations, the higher SAA concentration itself does not lead to poor average daily gain, but only reflects a background factor associated with both increased SAA concentration and poorer average daily gain.

The increased concentration of globulin on arrival was associated with good average daily gain during the milk feed period. Calves with IgG < 7.5 g/l had lower average daily gain in early growth than calves with IgG > 7.5 g/l in a Belgian study (Pardon et al., 2015), similarly to our study. A difference in IgG concentrations of 5 g/l would produce a 1.39 kg difference in weights during the milk feed period. The better growth rate in calves with higher concentrations of immunoglobulins is probably associated with better health or disease resistance, as discussed previously. However, this was not observed later. The decreased albumin and increased Hp on arrival were associated with better average daily gain at slaughter. The direction of these changes implies that changes related to inflammation would promote growth, although, as for concentrations of SAA, concentrations of Hp mostly clustered below the reference value of 196 mg/l. The authors suggest that the association of SAA and weight gain does exist, because it was recorded for the three time periods. The appearance of the Alb and Hp only in the SLAUGHTER model indicates uncertainty in the strength of the association with average daily gain. The study was initially designed to examine the association between group size and calf health and growth, thus the estimations of APPs on growth should be interpreted cautiously and the effect of additional contributing factors cannot be dismissed. Generalization of the results to veal rearing, for example, is legitimate because calf management is quite similar.

Conclusions

Smaller group size (10 vs. 40) did not have the hypothesized positive association on calf health or daily weight gain. The increase of acute phase protein serum amyloid A in the early life of a calf was associated with poorer average daily gain for all the periods measured, including at slaughter at 15 to 18 months of age. Although the mechanism for this association was not explained, it seems that events in a calf's early life can have long-lasting changes on the average daily gain. Complex relationships among morbidity, treatment protocols and daily gain of calves were described. Whether regular screening of serum proteins – serum amyloid A, albumin and IgG – would be economically profitable or not requires further exploration.

Conflict of interest

During the study Tuomas Herva was working as a veterinarian of Atria Ltd, to which the calves were finally sold and slaughtered. This did not affect aims or performance of the study or interpreting the results. The rest of the authors have no conflict of interest.

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TABLES AND FIGURES

Table 1

Average daily gains (g/day) for calves at two different time periods and carcass weights (kg) at slaughter by breed and gender.

		EARLY[1]		200 DAYS[2]		SLAUGHTER[3]	
		n	Mean (±SD)	n	Mean (±SD)	n	Mean (±SD)
Ayrshire	Heifer	15	686 (141)	15	1005 (111)	15	214.9 (27.5)
	Bull	160	731 (237)	158	1121 (123)	150	337.9 (37.3)
Holstein Friesian	Heifer	17	674 (132)	17	1002 (106)	17	224.7 (25.5)
	Bull	196	808 (209)	196	1147 (108)	187	333.9 (35.3)
Mixed[4]	Heifer	50	689 (225)	48	1019 (112)	49	245.3 (31.8)
	Bull	30	676 (265)	30	1064 (139)	29	349.7 (51.7)
All breeds	Heifer	82	685 (194)	80	1013 (110)	81	235.4 (32.1)
	Bull	386	766 (239)	384	1130 (119)	366	336.9 (37.8)
Total	Animals	468	752 (233)	464	1110 (125)	447	318 (53.7)

[1] Average daily gain during 49 days following arrival (average 24.1 days of age) at milk feed compartment.
[2] Average daily gain from arrival (average 24.1 days of age) to approximately 200 days of age.
[3] Carcass weight (kg) at slaughter.
[4] Mixed dairy-beef breed calves.

Table 2

Number of antimicrobial treatments of calves during the 49-day rearing period in milk feed compartment.

Number of antimicrobial treatments	Small groups		Large groups		Total	
	n	%	n	%	n	%
0	10	4.2	8	3.4	18	3.8
1	61	25.6	63	26.5	124	26.1
2	89	37.4	81	34.3	170	35.7
3	61	25.6	64	26.9	125	26.3
4	15	6.3	21	8.8	36	7.6
5	2	0.8	1	0.4	3	0.6
Total	238	100	238	100	476	100

Table 3 A, B

Survival analysis for risk factors for the time of first antimicrobial (ab) treatment in the rearing unit and the time to first recurrence of a disease. The batch and pen inside the batch were included as random variables in both models.

A. Risk factors for days to first ab treatment

Variable		n	Hazard ratio	95% confidence interval	*p*-value	Wald test *p*-value t
IgG[1] g/l		476	0.957	0.931; 0.983	0.002	
Albumin[1] g/l		476	0.907	0.869; 0.947	<0.001	
Age on arrival (days)		476	1.008	0.997; 1.019	0.152	
Breed:	Ayrshire	178	1			0.067
	Holstein Friesian	218	0.954	0.771; 1.180	0.662	
	Mixed[2]	80	0.724	0.547; 0.959	0.024	
Group size:	Small	238	1			
	Large	238	0.979	0.740; 1.297	0.152	

Shape parameter p = 1.19, scale parameter λ = 1.598. Variance parameter for batch was 0.04 (95% CI 0.005; 0.30) and for pen 0.05 (95% CI 0.01; 0.26). Likelihood ratio test p < 0.001 for random effect.

B. Risk factors for days to first recurrence

Variable		n	Hazard ratio	95% confidence interval	*p*-value	Wald test *p*-value
IgG[1] g/l		473	0.932	0.903; 0.962	<0.001	
Albumin[1] g/l		473	0.938	0.894; 0.983	0.008	
Age on arrival (days)		473	1.002	0.988; 1.015	0.820	
Breed:	Ayrshire	177	1			0.046
	Holstein Friesian	216	0.858	0.677; 1.088	0.206	
	Mixed[2]	80	0.659	0.473; 0.919	0.014	
Group size:	Small	238	1			
	Large	235	0.984	0.792; 1.222	0.883	

Shape parameter p = 2.24, scale parameter λ = 0.0036.Variance parameter for batch was 0.09 (95% CI 0.02; 0.38) and for pen 0.0 (95% CI 0.0; 0.00). Likelihood ratio test p < 0.001 for random effect.

[1] Immunoglobulin (IgG) and albumin concentrations measured at beginning of study period (average 24.1 days of age).
[2] Mixed dairy-beef breed calves.

Table 4

Linear mixed models assessing factors associated with calf growth (g/day) for three rearing periods. The batch and pen inside the batch were included as random variables.

A. Average daily gain EARLY (From arrival to end of milk feeding period/to weaning of milk)

Variable		n	Coeff.	95% confidence interval	*p*-value	Wald test *p*-value
SAA[1] (mg/l)		468	-0.60	-1.05; -0.15	0.009	
IgG[1] (g/l)		468	5.67	1.94; 9.40	0.003	
Age on arrival (days)		468	10.92	8.97; 12.87	<0.001	
Ab treatments[2]	0-1	139	0			0.061
	2-3	292	17.66	-19.50; 55.02	0.352	
	≥4	37	80.60	14.67; 147.53	0.018	
NSAID treatments[3]		468	-8.80	-16.14; -1.47	0.019	
Gender:	Heifer	82	0			
	Bull	386	48.49	-1.62; 98.61	0.058	
Breed:	Ayrshire	175	0			0.003
	Holstein Friesian	213	61.29	25.16; 97.44	0.001	
	Mixed[4]	80	9.54	-45.26; 64.33	0.733	
Group size:	Small	237	0			
	Large	231	22.51	-29.12; 74.14	0.393	
Constant			451.09	353.20; 548.98	<0.001	

Intra-class correlations coefficient (ICC) for batch = 0.096 and for pen = 0.063. Likelihood ratio test $p < 0.001$ for random effect.

B. Average daily gain 200DAYS (From arrival to moving to another compartment at approximately 200 days of age)

Variable		n	Coeff.	95% confidence interval	*p*-value	Wald test *p*-value
SAA[1] (mg/l)		464	-0.38	-0.66; -0.10	0.008	
Age on arrival (days)		464	2.09	0.91; 3.27	0.001	
Gender:	Heifer	80	0			
	Bull	384	92.44	61.08; 123.80	<0.001	
Breed:	Ayrshire	173	0			0.059
	Holstein Friesian	213	19.26	-3.01; 41.52	0.090	
	Mixed[4]	78	-16.86	-50.81; 17.08	0.330	
Group size:	Small	236	0			
	Large	229	5.53	-17.18; 28.22	0.633	
Constant			1089.81	1041.19; 1138.43	<0.001	

Intra-class correlations coefficient (ICC) for batch = 0.048 and for pen = 0.012. Likelihood ratio test $p < 0.001$ for random effect.

[1] Immunoglobulin G (IgG) and serum amyloid A (SAA) concentrations measured at beginning of the study period (average 24.1 days of age).
[2] Number of antimicrobial treatments during the 49-day rearing period.
[3] Number of NSAID administrations during the 49-day rearing period.
[4] Mixed dairy-beef breed calves

Table 5

Linear mixed model assessing factors associated with calf carcass weight (kg) at slaughter. The batch and pen inside the batch were included as random variables.

Variable		n	Coeff.	95% confidence interval	***p*-value**	Wald test ***p*-value**
SAA[1] (mg/l)		445	-0.12	-0.21; -0.04	0.006	
Haptoglobin[1] (mg/l)		445	0.03	0.0005; 0.05	0.046	
Albumin[1] (g/l)		445	-1.41	-2.69; -0.13	0.031	
Age at slaughter (days)		445	0.28	0.22; 0.35	<0.001	
Age on arrival (days)		445	0.46	0.10; 0.83	0.013	
Gender:	Heifer	80				
	Bull	365	77.67	65.44; 89.91	<0.001	
Breed:	Ayrshire	164	0			<0.001
	Holstein Friesian	204	-1.81	-8.61; 4.99	0.602	
	Mixed[2]	77	18.31	8.11; 28.52	<0.001	
Group size:	Small	222				
	Large	223	-5.95	-12.05; 0.16	0.056	
Constant			215.61	161.10; 270.12	<0.001	

Intra-class correlations coefficient (ICC) for batch = 0.000 and for pen = 0.000. Likelihood ratio test $p < 0.996$ for random effect.

[1] Serum amyloid A (SAA), haptoglobin and albumin concentrations measured at beginning of the study period (average 24.1 days of age).

[2] Mixed dairy-beef breed calves.

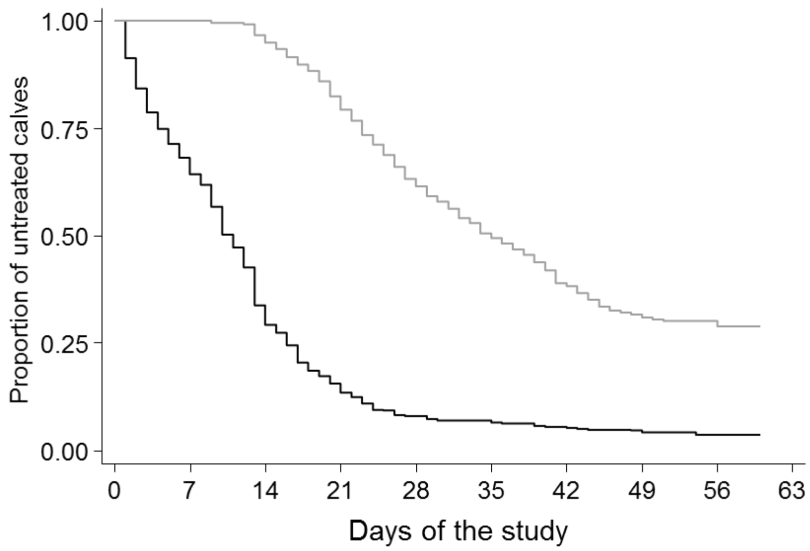

Figure 1. The Kaplan-Meyer curve for the calves showing the proportion of untreated (for the 1st or 2nd time) calves before the first antimicrobial treatment time (black line) and before the first recurrence (grey line) during the follow-up period, based on the survival models (Table 3A, B).