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# Health and welfare assessment of beef cattle during the adaptation period in a specialized commercial fattening unit

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ABSTRACT

Beef cattle welfare and health status are influenced by housing and management systems. The present study aimed to assess the welfare and health status in the first 15 days after arrival of Limousine bulls imported from France and fattened in a commercial fattening unit in Italy. A total of 264 bulls were included in the study. Welfare, biosecurity, and major hazard and warning system were assessed on days 2 (T1) and 15 (T2) after arrival to the unit. At T1 and T2 an inspective clinical examination was performed on all bulls. At T1 and T2 blood samples were collected from 88 bulls for haematological analysis. Both at T1 and T2, the welfare, biosecurity, and major hazards and warning systems were classified with a general score of medium but with a decrease on animal-based measurements in T2. At T1 and T2 the clinical examination revealed a significant increase ($p$-value$\leq$0.05) of skin lesions and lameness in T2. A high incidence of respiratory disease was noticed in both assessed times. Leucocytes and all differentials count, and platelets were significantly increased ($p$-value$\leq$0.05) at T2, while the fibrinogen was significantly decreased. The haematological changes suggest that the bulls were under higher stress in T2 when compared with T1 linked with a difficult adaptation response to the fattening unit. A multi-factorial approach that integrates the indicators of the checklist and the clinical and haematological findings of animals can be a useful method to deepen the assessment of welfare in beef cattle.

## 1. Introduction

Animal welfare is the physical and mental state of an animal in relation to the conditions in which it lives (OIE, 2022). Consumers anticipate that their animal-related products, notably food, should be produced with consideration for the animal welfare (Welfare Quality®, 2009). There are various definitions of what constitutes animal welfare, but there is a growing consensus that farm animal welfare has to be safeguarded and enhanced. Recently, compared to other quality traits, there has been an improved recognition of animal welfare criteria. Consumers frequently believe that items that have in consideration animal welfare are more genuine, safer, tastier, and hygienic (Alonso et al., 2020). Scientific data supports the relationship between animal welfare and animal health and, thus, food safety with a strong correlation between excellent animal welfare and good animal health (de Passillé and Rushen, 2005). Housing, transportation, and management practices have a significant impact on the welfare of different species (Broom, 2009; Nannoni et al., 2022; Raspa et al., 2022; Sardi et al., 2020).

Health, comfort, and the expression of species-specific behaviours are indicators related to welfare (Botreau et al., 2007). Therefore, determining welfare requires a multidisciplinary approach through meticulous and trustworthy monitoring of the indicators related to productivity, ethology, endocrine function, immunology, and pathology (Sevi, 2009). In Italy, the farm animal welfare assessment is based on a protocol included in the ClassyFarm system. This protocol aims to support official controls, collect information, advance welfare-level implementation, and inform consumers (Mariottini et al., 2022). Routine application of the protocol offers a promising tool for the improvement of beef cattle welfare and farm profitability, in particular when a welfare certification becomes available (Gottardo et al., 2009). To perform welfare certification, accurate, reliable, and repeatable measures of welfare factors that allow quantification of welfare should be considered (Salvin et al., 2020).

When fattened in intensive systems, beef cattle are more susceptible

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to experiencing poor welfare. The main welfare issues in the beef industry are: bovine respiratory disease (BRD) linked to overcrowding, insufficient ventilation, and animal mixing; digestive problems associated with intensive concentrate feeding; and behavioural issues due to overcrowding and co-mingling (Cozzi et al., 2009; EFSA, 2012). Enhancing farming practices and management that ensure animal health is a step further to improving welfare (Alonso et al., 2020).

In the present study, we aimed to assess the welfare and health status of bulls imported from France and fattened in Italy in a commercial fattening unit in the first 15 days after arrival by applying a multidisciplinary approach.

## 2. Materials and methods

### 2.1. Commercial fattening unit and bulls

The study was conducted in a commercial fattening unit in the province of Modena (Italy). All animals fattened in the unit were Limousine bulls imported from France. They came from several French farms distributed over the country, where most of the bulls were kept on pasture or in an indoor free stall system with straw bedding. Before arriving at the unit in Italy, the bulls were kept in transit for one day in a selection centre in France. Here, they were selected based on health status, age, and body weight, to obtain homogenous batches. At the entrance to the fattening unit, the bulls were 11 months old and weighed 380 kg. The bulls arrived once a week at the unit in Italy in numerically heterogeneous batches and were transferred in groups of six to their designated pens. Before the batch's arrival, their assigned pens were pressure washed and disinfected with sodium-P-toluen-N-chlorosulfamide. The dropping pit was emptied every 2 to 3 months.

The unit consisted of four similar barns separated by a 20-m corridor (Fig. 1A). The barns were semi-closed and well-ventilated. Each barn had 44 pens in a free-stall system with a maximum capacity of 6 bulls per pen. The pens were placed 22 × 22 in parallel with the feeder on one side. Each pen had a dimension of $18.4m^2$. Each animal had a space of $3.06\ m^2$ and a feeding front of 45 cm. The feeders were placed on one side along the feeding line. The pens were built adjacent to each other and were separated by iron bars, allowing interaction of animals (Fig. 1B, C). The flooring was slatted and underneath there was a pit for manure collection. Each barn was equipped with 46 automatic water bowls serving 264 animals and at least one automatic water bowl was present per pen. Each barn had a maximum housing capacity of 264 bulls. Therefore, the farm's total housing capacity was 1056 bulls. As each production cycle lasts between 5 and 6 months and the facility allowed the fattening of 2112 animals per year.

The current study had in consideration one barn housing 264 bulls. Bulls arrived in six batches weekly-based over the course of 6 weeks. At arrival, bulls were vaccinated using the live attenuated virus of bovine viral diarrhoea-mucosal disease (Rispoval D-Bvd®) and the live bovine herpesvirus type 1 vaccine (Bovilis IBR®). Ivermectin (Ivomec®, Boehringer Ingelheim Animal Health, Italy) was administrated for the prevention and control of parasites.

### 2.2. Dietary adaptation

At arrival, the bulls were fed an adaptation diet in order to reduce dietary stressors. The total mixed ration (TMR) was fed ad libitum and fresh clean water was always available. The TMR diet was freshly sampled in different locations (beginning, middle, and end of the feeding line) at T1 and T2 after arrival at the fattening unit. TMR ingredients and proportions are reported in Table 1. Analytical TMR analyses were performed at the University of Bologna feed analysis lab according to the methodology described in previous studies (Mammi et al., 2022).

### 2.3. Welfare, Biosecurity and Major Hazard and Warning System Assessment

For the welfare, biosecurity and, major hazard and warning system two observations were performed: at T1 and T2. An adapted version of the Italian protocol for the assessment of beef cattle welfare included in the ClassyFarm system (Bertocchi et al., 2020) was applied. The used protocol included a list of 58 items, divided into three main sections: biosecurity (items 1 to 13), welfare (items 14 to 50), and major hazard and warning system (items 51 to 58) (Supplementary TableS1 S1). The welfare section was further subdivided into three areas: A-farm management and staff training (items 14 to 28), B-housing and equipment (items 29 to 40), and C-animal-based indicators (items 41 to 50). For each item, a 2- or 3-point scale scoring system was applied (1 = insufficient; 2 = acceptable; 3 = optimal) (Mariottini et al., 2022). A value for each section was computed by summing the obtained score of each item from each section or area. For welfare, the value was calculated accounting for a contribution of 50% by areas A and B, and 50% by C. The obtained values were further converted into percentages. In particular, a result below 59% indicated a poor status (=low), a result between 60 and 80% a medium status (=medium), and a result over 80% a good status (=high) (Diana et al., 2020).

### 2.4. Clinical examination

All bulls underwent a clinical examination pen-based at T1 and T2. It consisted of a 10-min observation with the observer standing between the animals in the pen. The following parameters were assessed: mental status, cleanliness, body condition, skin lesions, gait, nasal discharge, ocular discharge, faecal consistency and other possible abnormalities. All data were recorded in a schematic table per pen (Supplementary Table S2). An animal was considered to have BRD if at least two abnormal findings related to the respiratory system were present (i.e., cough and nasal discharge; abnormal breathing and cough; abnormal breathing and nasal discharge).

### 2.5. Blood investigation

At T1 and T2, blood samples were collected from 88 out of the 264 bulls. At T1, two bulls were randomly selected from each pen, and at T2, the same subjects were re-sampled. Blood samples were collected via coccygeal/jugular venepuncture for haematological investigations. The blood was transferred into vacuum tubes containing EDTA anticoagulant for complete blood count and into citrate tube for fibrinogen analysis. The following set of blood parameters were analysed: erythrocytes (RBC), hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte distribution width (RDW), platelets (PLT), leucocytes (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and fibrinogen.

### 2.6. Statistical analysis

Data were entered into a statistics program (JMP Pro 17). Descriptive statistics were generated mean ± standard deviation (S.D.) and/or standard error (S.E.), median and range for continuous data, and count and percentage for categorical data. For continuous variables, normality was tested by Shapiro-Wilk test and non-normally distributed variables were Box-Cox transformed before the analysis. The evaluation of differences between T1 and T2 were undertaken using the Mixed Model Procedure. Each cattle were set as an experimental unit within the arrival group and pen as nested factors. The adaptation time (T1 and T2) was implemented as fixed effect. After the analysis, normal distribution of the data was checked again for the resulting residuals. Means are reported as least square mean and pairwise multiple comparisons were performed using Tukey-test as post hoc test when a significance was

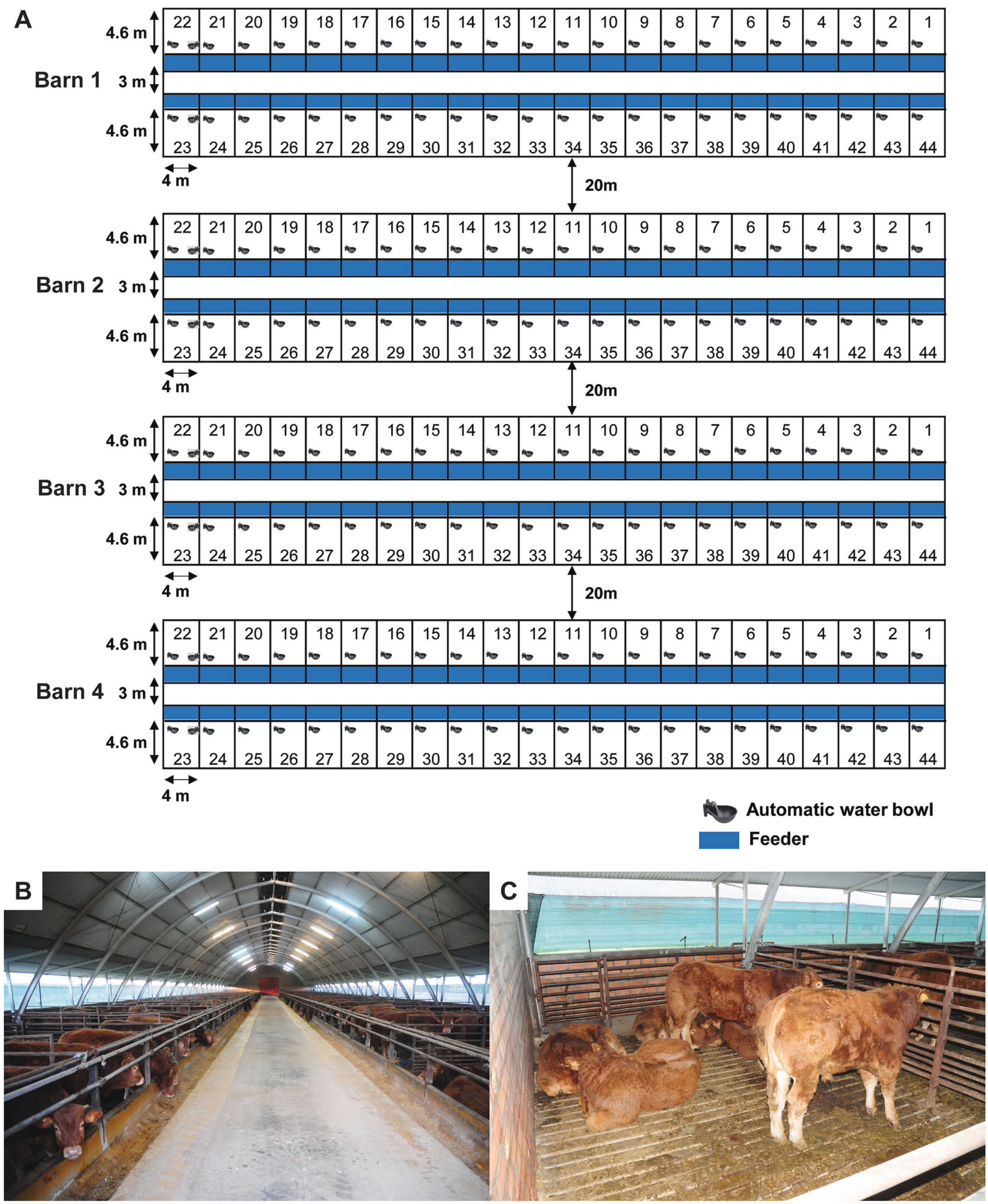


**Fig. 1.** Beef intensive commercial fattening unit. A, Schematic representation of the unit. B, Image of the barn where the study was performed. C, Image of one pen.

**Table 1**
Descriptive statistics of the adaptation TMR diet (T1 and T2) and chemical analysis.

| TMR | Feed, kg af |
|---|---|
| Wheat silage | 3.5 |
| Meadow hay[a] | 1.2 |
| Wheat straw | 1.1 |
| Beat pulp | 1.3 |
| Corn, finely ground[b] | 1.1 |
| Soybean meal | 0.5 |
| Cane molasses[c] | 0.5 |
| Min and Vit Premix | 0.3 |
| | **Nutrients, %DM** |
| DM | 70.07 |
| UFC | 0.81 |
| CP[d] | 11.25 |
| Ash | 8.78 |
| EE[e] | 2.06 |
| Starch | 13.57 |
| Sugars | 7.37 |
| NDF[f] | 39.21 |
| ADF[g] | 25.79 |
| ADL[h] | 3.45 |

Abbreviations: [a]the quality of the hay was checked to ensure the absence of molds and spores (Cavallini et al., 2022a, 2022b). [b]the corn was below the EU maxim tolerable level (Girolami et al., 2022). [c] molasses were properly characterized (Palmonari et al., 2021). [d] Crude protein. [e]ether extract. [f]neutral detergent fiber. [g]acid detergent fiber. [h]acid detergent lignin.

detected. Then a nominal logistic model was used for categorical variables using the same discriminant as before mentioned. A *p*-value≤0.10 was considered a tendency; a p-value≤0.05 was considered statistically significant; and a p-value≤0.01 was considered highly significant.

## 3. Results

### 3.1. Welfare, biosecurity and major hazards and warning system assessment

Results of welfare, biosecurity major hazards and warning system assessment at T1 and T2 are presented in Table 2. At T1, the percentages obtained for welfare, biosecurity, and major hazards and warning systems were 79.04%, 63.88%, and 76.47%, respectively. Regarding the welfare at T1, the following results were obtained: 70.45% in area A, 65.17% in area B and 90% in area C. At T2, the percentages obtained for welfare, biosecurity major hazards and warning system were 74.73%, 63.88% and 76.47%, respectively. At T2, although there was an increase in area B (68.57%), a decrease in welfare compared to T1 due to a decrease in score in area C (80%) was noticed. No differences were found between T1 and T2 in terms of biosecurity and major hazards and warning system.

**Table 2**
Descriptive statistics of welfare, biosecurity and major hazard and warning system of 264 Limousine bulls.

| Item | Assessment at T1 | Classification at T1 | Assessment at T2 | Classification at T2 |
|---|---|---|---|---|
| Total welfare | 79.04% | Medium | 74.73% | Medium |
| Area A (Farm management and staff training) | 70.45% | Medium | 70.45% | Medium |
| Area B (Housing and facilities) | 65.17% | Medium | 68.57% | Medium |
| Area C (Animal-based indicators) | 90% | High | 80% | Medium |
| Biosecurity | 63.88% | Medium | 63.88% | Medium |
| Major hazard and warning system | 76.47% | Medium | 76.47% | Medium |

Abbreviations: T1 = 2 days after arrival to the unit; T2 = 15 days after arrival to the unit.

### 3.2. Clinical examination

The clinical data is provided in Table 3. At T1, 1.51% of the bulls showed integument lesions, 0.75% lameness, 0.75% diarrhoea, 27.65% signs of BRD. At T2, there was a significant increase in lameness (1.15%, *p*-value = 0.02) and in integument lesions (44.69%, *p*-value≤0.01). Most of these were alopecic lesions in the neck. In contrast, no significant changes in the percentage of animals with signs of BRD (31.81%) and diarrhoea (0%) were noticed.

### 3.3. Blood parameter analysis

Results of the blood analysis at T1 and T2 are presented in Table 4. A significant increase (p-value≤0.05) in platelets, WBC, neutrophils, monocytes, lymphocytes, eosinophil, and basophils was noticed at T2. They were within the normal reference range for bovine species with the exception of monocytes that were higher than the normal range. The fibrinogen values obtained both in T1 and T2 were above the reference range. However, a significant decrease (p-value≤0.05) of fibrinogen was noticed in T2. Indeed, there was an absence of statistically significant difference (p-value>0.05) in the RBC, HGB, HCT, MCV, MCH, MCHC, RDW and N/L between T1 and T2.

## 4. Discussion

Currently, there are different methods that enable to assess and measure beef cattle welfare (Kirchner et al., 2014a; Mariottini et al., 2022). Welfare assessment methods on farm should be implemented in a consistent modality. With this approach, the results of the assessment are expected to be representative of a longer-term farm welfare status considering that the management practices and housing conditions have not changed. Furthermore, welfare assessment methods must be reasonably free from observer influence (Kirchner et al., 2014b). Firstly, we assessed the welfare using checklist protocol approach. Secondly, we evaluated the clinical and haematological conditions of animals. The methods were applied at T1 and T2 in order to achieve consistency over a critical time. We evaluated the biosecurity and major hazard and warning system obtaining a classification of medium, without significant differences between the T1 and T2. Indeed, a decrease in welfare between T1 and T2 was noticed due to a reduction of animal-based indicators score. The observed welfare decrease could be associated with stress responses to both physical (i.e., transportation, new environment, new feed) and psychological (i.e. social-group mixing) stressors (Bassel and Caswell, 2018).

The clinical examination of animals evidenced a significant increase in integument lesions, which could have contributed to the lowering of welfare score. Crowding, inadequate feed distribution, inadequate space at the manger, mixing social group and poor pen flooring are all detrimental to the welfare of beef cattle, which in turn cause competition and

**Table 3**
Clinical investigation findings of the 264 bulls at day 2 (T1) and day 15 (T2) after arrival to the farm.

| Item | Assessment at T1 | Assessment at T2 | P-value |
|---|---|---|---|
| Integument lesions (%) | 4(1.51%) | 118(44.69%) | <0.01 |
| Lameness (%) | 2(0.75%) | 4(1.15%) | 0.02 |
| Diarrhoea (%) | 2(0.75%) | 0(0%) | 0.41 |
| Respiratory disease (%) | 73(27.65%) | 84(31.81%) | 0.54 |

**Table 4**
Result of the complete blood analysis at day 2 (T1) and day 15 (T2) after arrival to the farm.

| Blood Parameters | | Time of assessment | | | |
|---|---|---|---|---|---|
| | | T1 | T2 | *P* value | Reference Range |
| RBC (M/μL) | Mean ± SD | 9.8 ± 1.37 | 9.88 ± 1.11 | 0.83 | 5.1–7.6[a] |
| HGB (g/dL) | Mean ± SD | 12.09 ± 1.25 | 12.01 ± 1.16 | 0.62 | 8.5–12.2[a] |
| HCT (%) | Mean ± SD | 39.55 ± 4.36 | 39.29 ± 3.96 | 0.63 | 22-33[a] |
| MCV (fL) | Mean ± SD | 40.5 ± 2.99 | 39.9 ± 2.63 | 0.08 | 38-40[a] |
| MCH (pg) | Median [Min.- Max] | 12.2 [11.6–13.1] | 12.1 [11.6–12.8] | 0.05 | 14-18[a] |
| MCHC (g/dL) | Mean ± SD | 30.6 ± 1.23 | 30.57 ± 1.28 | 0.9 | 34-38[b] |
| RDW (%) | Mean ± SD | 24.11 ± 1.96 | 24.05 ± 1.68 | 0.52 | 15.5–19.4[a] |
| PLT (K/μL) | Median [Min.- Max] | 280 [143–338] | 315 [148–510] | <0.01 | 193-637[a] |
| WBC (K/μL) | Median [Min.- Max] | 8.69 [7.31–9.97] | 10.34 [8.53–12.95] | <0.01 | 4.9-12a |
| NEU (K/μL) | Median [Min.- Max] | 3.37 [2.65–3.97] | 3.91 [2.87–5.91] | <0.01 | 1.8–6.3[a] |
| MONO (K/μL) | Median [Min.- Max] | 1.25 [0.98–1.47] | 1.13 [0.88–1.39] | 0.04 | 0–0.6[a] |
| LYM (K/μL) | Mean ± SD | 3.84 ± 1.26 | 4.46 ± 1.79 | <0.01 | 1.6–5.6[a] |
| EOS (K/μL) | Median [Min.- Max] | 0.08 [0.03–0.2] | 0.22 [0.09–0.41] | <0.01 | 0–0.9[a] |
| BASO (K/μL) | Median [Min.- Max] | 0.06 [0.04–0.07] | 0.09 [0.06–0.12] | <0.01 | 0–0.3[a] |
| FIBR (mg/dL) | Median [Min.- Max] | 985.05 [738.15–1348.2] | 778.2 [584.18–1054.35] | <0.01 | 100-600[b] |
| N/L ratio | Median [Min.- Max] | 0.987 [0.62–1.27] | 0.95 [0.64–1.46] | 0.42 | 0.4–2.34[a] |

Abbreviations: RBC, Red blood cell; HGB, Hemoglobin; HTC; Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red blood cell distribution width; PLT, Platelets; NEU, Neutrophils; WBC, white blood cells; MONO, Monocytes; LYM, Lymphocytes; EOS, Eosinophils; BASO, Basophils; FIBR, Fibrinogen; N/L ratio, Neutrophils: Lymphocytes ratio; M/μL,$10^6$ per microliter; %, percentage; K/μL,$10^3$ per microliter; g/dL, grams per deciliter; fL, femtoliter; pg, picogram; mg/dL, milligram per deciliter; Min., Minimum; Max., Maximum, [a]George et al. (2010), [b]Cornell University College of Veterinary Medicine, 2023.

stress among pen mates and result in lesions (Cozzi et al., 2009). The clinical investigation revealed that most of the integument lesions developed between T1 and T2 were characterized by alopecia in the neck dorsal region. The development of these lesions, caused by the repeated rubbing of the animals' necks against the iron bars of the feeding structure, suggests the inadequacy of the structure, evidencing a critical point that could be addressed. Furthermore, a significant increase of lameness was noticed contributing to a decrease in welfare. Lameness can have several causes, such as social competitions, poor hygienic level and hoof care, inadequate housing facilities and flooring (e.g, unsuitable grating), and unbalanced feeding (Bertocchi et al., 2020; Nalon and Stevenson, 2019). In addition, animals selected for rapid weight gain, in conditions like those in intensive fattening units, are predisposed to develop metabolic and joint diseases (Compiani et al., 2014). Even though, an adaptation diet was provided, there is a possibility that the bulls received higher amounts of concentrates when compared to the diet provided in France. This could have increase the risk of developing ruminal acidosis that can lead to the development of laminitis and consequenty lameness (Cozzi et al., 2009; Compiani et al., 2014).

In beef cattle BRD, is one of the major health and welfare issues that negatively impacts productivity. A large spectrum of stressors, in particular transportation, contribute to higher disease susceptibility, such as BRD (Chen et al., 2015). BRD, is frequently developed in the first weeks after arrival on the farm (Pratelli et al., 2021; Valadez-Noriega et al., 2022). In particular, even when antimicrobial metaphylactic treatments and vaccines for BRD are administered, the first two weeks following the introduction of cattle to beef-fattening facilities appear to be the most vulnerable time for the development of BRD (Pratelli et al., 2021). In our study, the clinical examination at T2 did not revealed significant change in the number of animals with BRD compared to T1. The first two months after arrival to the farm seem to be the most critical period that impacts negatively on animal health and consequently welfare, and that the signs of BRD can persist during these months (Valadez-Noriega et al., 2022). Finally, our clinical investigation did not evidenced animals with diarrhoea at T2. This finding suggests that an adequate adaptation diet was provided, according to the literature (Fusaro et al., 2022). Ration was provided as total mixed ration (TMR) to promote a synchronized intake of roughage and concentrates which decreases, for example, the risk of the occurrence of ruminal acidosis (Cavallini et al., 2022a, 2022b).

ClassyFarm protocol considers a window of 8 days after arrival during which the welfare assessment should not be performed because all the health and welfare impairments detected during this period may be affected by the stress caused by the transport (Bertocchi et al., 2020). Indeed, immediately after transportation, haematological parameters increase from the baseline due to transportation stress followed by a significantly decreased in haematological parameters after 4 to 7 days, suggesting that animals recover from transportation stress (Zulkifli et al., 2019). In our study, even though mean and median values of the WBC and their differential counts, and platelets were within the reference range for cattle, they were significantly higher 15 days after arrival to the fattening unit when compared to those parameters two days after arrival. We can speculate that the animals two days after transportation were still under transportation stress (Zulkifli et al., 2019). Interestingly, we observed a significant increase of all measured white blood cells and platelets 15 days after arrival suggesting that stressor factors such as new environment, feeding, housing, and management practices have a greater important impact than transportation. Additionally, these increase in white blood cells and platelets observed 15 days after arrival suggest an unsatisfactory adaptation to the fattening unit. In fact, it is well known that increased neutrophil and white blood cell counts are signs of inadequate adaptation (Tarantola et al., 2020). Moreover, ruminants can have increased platelets because of stress and/or inflammatory diseases (Jones and Allison, 2007). Herein, fibrinogen was significantly lower 15 days after arrival at the fattening unit, but it was still higher than the normal range for bovine. Fibrinogen is a marker of acute inflammation and stress in cattle (Ansiliero et al., 2019). In acute inflammatory conditions, fibrinogen reaches the highest peak and then declines, while in chronic inflammatory conditions the fibrinogen generally remains high as long as the disease is present and active (McSherry et al., 1970). Thus, we can speculate that at T1, where the highest values of fibrinogen were observed, the bulls were under an acute inflammation (e.g. associated to BRD) and/or stress. At T2, even though there was a decrease of the fibrinogen it was still higher than the normal range indicating the presence of a chronic inflammation.

Our results suggest that the welfare assessment during the first two week after arrival present several critical aspects but can already evidence some risk factors that can cause welfare issues, if present. The repetition of welfare assessment with a consistent method over time is fundamental to assess the long-term welfare of animal in constant housing and management conditions (Kirchner et al., 2014a).

## 5. Conclusions

Our study suggests that in the context of beef-intensive fattening systems, in the first 15 days after arrival, adaption to a new environment, feeding and management represents an important challenge for the immune system. Consequently, during this period, a reduction in welfare and health is noticed. White blood cells analysis could be a useful tool as warning sign when measuring the welfare status of beef cattle. Clinical investigations can help to evidence critical points in management and housing system that could threat health and welfare of animals. Protocols for welfare assessment with the integration of different assessment indicators, including health, biosecurity, major hazard and warning system and a complete blood cells count, could provide more information of the welfare status and of the critical points in the housing and management system.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.03.008.

## Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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