Sanitary, environmental and nutritional management to reduce the incidence of bovine respiratory disease and the use of antibiotics in fattening beef cattle

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SUMMARY

Bovine respiratory disease (BRD) is the main health problem in fattening beef cattle. Due to its multifactorial etiology, alternative strategies to reduce its incidence and correlated antimicrobial use must consider all the main critical aspects, related to sanitary, environment and nutritional management.

A multi-year project was set up to develop a specific integrated system to counteract the incidence of BRD, while reducing the use of antibiotics, and develop a risk-assessment sheet to evaluate, at the arrival, the potential sanitary risk of each single batch. During the first period (November 2019-September 2020), the effect of four vaccination protocols, differing for the route of administration, intramuscular (IM) versus intranasal (IN), was tested, as well as the effectiveness of a proper adaptation diet. In the second period (November 2020-May 2021), the best protocol merged was tested again, but shifting vaccination to day (d) 7 and improving environmental management and the biosecurity levels. During the entire project, blood samples and nasal swabs were taken at the arrival on a sample of animals to evaluate the circulation of pathogens and the presence of antibodies. Mortality, morbidity for BRD, relapses and antimicrobial consumption were evaluated, as well as the effect of the antimicrobial mass treatment based on the risk-assessment sheet.

The results of the first period highlighted a reduction of 29.1% in morbidity, of 81.7% in the first relapse and of 71.5% in the antimicrobial use (mg/PCU) where the IN vaccination was used in combination with a proper nutrition. In the second period, the delayed vaccination, in combination with improvement in environment conditions and biosecurity, has led to reduction of 49.3% in mortality, 41.9% in morbidity, 51.9% in first relapses and of 25.5% in antimicrobial use, compared with the same vaccination protocols in the first period. Comparing the health status in batches with and without antimicrobial treatment based on the risk assessment sheet, it appeared that mandatory avoiding it, even in case of evident risk, can be a damage for animal health, with increased morbidity, mortality and antimicrobial use.

In conclusion, an integrated and multidisciplinary approach, that consider both sanitary protocols, environmental and nutritional management, and antimicrobial treatment, in relation to an evident and clear sanitary risk, is essential to limit the BRD and the antibiotic consumption and to increase the welfare of newly arrived beef cattle.

KEY WORDS

Antimicrobial resistance; bovine respiratory disease; adaptation period; beef cattle; welfare.

INTRODUCTION

The problem of antimicrobial resistance (AMR) represents a global treat for both health, welfare and economy, which needs a global and multifactorial approach to be counteracted. Indeed, on average 700,000 people die each year globally (33,000 in Europe), due to infection with antibiotic resistant organisms (AROs). Moreover, infection with AROs increase the hospitalization rate, with a stronger pressure on healthcare systems' stability, efficiency, and economy[1].

The AMR occurs when microorganisms, such as bacteria, viruses, fungi, and parasites, become resistant to the medications normally used to cure them, because of both natural selective mechanisms and processes induced by human-related factors, such as misuse and abuse of antimicrobials in human but also in veterinary medicine[2-3]. Indeed, even if the antimicrobial consumption in food-producing animals has decreased strongly over the years, it is always under scrutiny, especially when antimicrobials commonly used in human medicine are administered for prevention of disease, in the form of prophylaxis or metaphylaxis[1-4-5]. Their use should be limited, and allowed only

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if strictly necessary, as will be underlined also in the new European regulations[6].
Between all the food-producing animals, swine and poultry farming accounted for the highest use of antibiotics, in terms of milligrams (mg) per population correction units (PCU) (172 mg/PCU, 148 mg/PCU respectively), while cattle are in third position (45 mg/PCU) at a global level[7]. However, within the intensive beef cattle farming, there are still stages of the rearing period that require antimicrobials use to reduce morbidity and mortality.
Indeed, in the beef cattle system, based mainly on the fattening of weaned cattle imported from pasture areas in foreign countries, the arrival stage represents the most critical period for animal health, welfare and antibiotics use[8].
Among all the typical health problems, bovine respiratory disease (BRD) is the most common and severe one[5-9]. Globally, the prevalence of BRD varies from about 4% to more than 80%[10]. At the Italian level, the incidence of morbidity related to BRD varies from 5% to 30% on average[11-12]. Besides, BRD is also associated with an overall reduction in productivity during the whole rearing cycle, with both welfare and economical concerns. Indeed, animals that suffer from BRD in the first days after the arrival, show poor growth performance, with a reduced average daily gain over the entire fattening period, as well as increased sanitary costs, due to a higher need of antimicrobial treatments[12-13]. For this reason, antibiotics are often used in the arrival stage, both to treat sick subjects but also to prevent the spread of BRD[12]. As a result, bacterial BRD pathogens are exhibiting more often an increased level of AMR, specifically toward macrolides, tetracyclines, β-lactams, fluoroquinolones[14-15]. Resistance to macrolides is of particular concern, due to the importance of these drugs in controlling BRD[16].
Moreover, in different studies, there was an increase in the AMR levels detected in nasal swabs after the placement in feedlots, suggesting an incorrect sanitary management in the fattening farms[17-18].
Considering those findings, a reduction in the use of antimicrobials also in beef cattle farming is required, especially in terms of mass treatments. Consequently, alternative strategies to reduce the risk, the incidence and the negative effects of BRD, while using less antibiotics, must be developed to maintain a high level of animal welfare and productivity.
Knowing the causes and predisposing factors of BRD is the basis to understand how to contain its incidence, and which are the main focal points to consider. The BRD has a multifactorial etiology, including infectious agents, host and environmental aspects[5-9]. Infectious agents of BRD are both viral and bacterial, such as *bovine herpesvirus type 1*, *bovine adenovirus*, *bovine viral diarrhoea virus*, *bovine coronavirus*, *bovine respiratory syncytial virus*, *bovine parainfluenza virus*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* and *Mycoplasma bovis*[19]. Between the host related factors, sex and arrival weight are the most influent variables, with males and lighter animals being more susceptible to BRD[20-21].
In addition, aspects related to the transport from the origin farm to the fattening units, such as length, climate, feed and water restriction and commingling of animals, could enhance the spread of BRD, due to both a stress-related reduction of the immune functionality and an increased risk of contact with viral and bacterial etiological agents[9-22]. Indeed, the prevalence, in nasopharyngeal swabs, of viral and bacterial agents, such as *Mycoplasma bovis*, *Histophilus somni*, *Bovine coronavirus* and *Manheimia haemolitica* increased after shipping from France to Italy[19]. Longer travels are strongly related to an increase in the likelihood of opportunistic infections in the lower respiratory tract, due to an increased stress-related immune suppression[20-23]. Also, weather conditions from departure to arrival, in particular sudden climate changes, very low temperatures and high humidity levels were found to be predisposing factors for the spread of BRD[22-24].
Furthermore, also the managerial procedures and facilities of the fattening farm can strongly influence both the spread of BRD agents and the immune functionality of newly arrived animals. In fact, the first weeks after arrival at the fattening unit represent a crucial period for animal health and performance. Newly arrived cattle must be managed carefully, ideally in a specifically dedicated facility[25-26]. In the arrival stage, higher stocking densities and the possibility to have closer contacts between cattle of different pens, through open fences or shared water through, are correlated with an enhanced incidence of BRD, due to an increased spread of infectious agents[27-28]. In addition, the nutritional management can act as both a prevention as well as a predisposing factor for BRD. Indeed, administering to newly arrived beef cattle a diet characterized by a high level of grains, with a lower level of rumen-active fibre, can be a predisposing factor for the development of BRD due to its relationship with lactic acidosis and subacute ruminal acidosis[27-29]. In addition, the protein content in the arrival diet must be controlled. The appropriate arrival diet should have a crude protein of less than 13%, to reduce the incidence and severity of morbidity[30].
Traditionally, beef cattle are vaccinated at the arrival with different protocols of vaccinations to counteract viral and bacterial agents involved in BRD. Indeed, prevention of disease by vaccination is the foundation of animal health management[31].
However, stress commonly associated with weaning, marketing, commingling and transport can temporarily compromise the immune function, and thereby reducing the effective response to vaccination intended to control BRD[31]. Thus, delaying respiratory vaccination may allow newly arrived "high-risk" beef cattle to overcome stress-induced immune dysfunction, preventing a blunted vaccine response and improving the efficacy of vaccination[31-32-33].
To reduce the risk of BRD and the use of antibiotics, all those different aspects must be appropriately evaluated and considered, both to understand some possible points of improvement as well as to better categorize the "risk level" of each batch of animals, to better plan the sanitary procedures.
Specifically, the aim of the study was to develop an integrated managerial and sanitary protocol for the arrival phase in fattening beef cattle, which considers all the main focal points, in order to minimize the incidence of BRD, and consequently the use of antibiotics. Moreover, another goal was to develop a risk-assessment sheet to evaluate, at the arrival, the real risk level of each single batch, to better plan a batch-specific sanitary intervention.

MATERIALS AND METHODS

With these purposes, a multi-year project (2019-2021) has been developed, in collaboration with the Local Health Authority (ULSS n.9 Scaligera, via della Valverde, Verona), the Istituto Zooprofilattico delle Venezie (Viale Università 10, 35020 - Legnaro), and the Cooperativa Zootecnica Scaligera Società

Agricola Cooperativa (via Caterina Bon Brentoni 41/b, Mozzecane). The animals were Limousine males, raised in France in typical pasture areas and farms, and then shipped to Italy in the F.OLI.MA.N. fattening farm of the Cooperativa Agricola Scaligera located in Mozzecane (Verona).
The project was divided into two main periods. The first one (November 2019-September 2020) aimed at evaluate four different restocking protocols, in terms of both vaccinations and feeding strategies, to find the most suitable and effective one. In addition, the "risk assessment sheet" was set up in this period.
In the second period (November 2020-May 2021), the best protocol, which emerged from the previous study, was used, but varying the timing of vaccination, that was delayed from day (d) 2 to d7 and improving environment conditions.

Experimental design

First period

Between November 2019 and September 2020, a total of 91 batches were enrolled in the project and were subjected to four different restocking protocols (Table 1), in relation to the time of arrival on the farm. The protocols differed in terms of type, method and timing of the vaccine administration, use of antimicrobial, and nutrition management.
Specifically, in first protocol (28/11-18/12) all the animals were vaccinated, at d1 after restocking, against *Manheimia haemolitica* (MH), through an intramuscular vaccination (IM), and against *respiratory syncytial bovine virus* (RSBV) and *infectious bovine rhinotracheitis* (IBR), through an intranasal vaccination (IN). Moreover, a 5-days oral treatment with Doxycycline was used as prophylaxis. Animals were fed with a growing diet with, on dry matter (d.m.) basis, 0.94 UFV/kg, 14.20% crude protein, 34.2% of starch, 32% of NDF, without a specific vitamins, minerals and additives supplementation for the adaptation phase. Regarding environment, bedding wheat straw was added only "as needed", or on average once a week.
The second protocol (19/12-15/01) was the same in terms of vaccines used, nutrition and environment management, but the vaccination was delayed from d1 to d7 and the antimicrobial prophylaxis was eliminated.
In the third protocol (16/01-5/02), the animals were vaccinated at d5, adding the intramuscular vaccination against *Histophilus somni* (H. somni), fed with the previous diet and subjected to the same environment management.
In the fourth protocol (6/02-20/09), all the animals were vaccinated against *respiratory syncytial bovine virus* (RSBV) and *infectious bovine rhinotracheitis* (IBR), through an intranasal vaccination, at d2 after restocking. Also, the nutritional management was changed.
Indeed, in this protocol, the animals were fed with a specifically formulated diet, characterized by a lower level of crude protein (≤ 12% d.m.), energy (≤ 0.88 UFC - 10.60 MJ d.m.), and starch (< 26% d.m.), and a higher level of NDF (> 36% d.m.), provided by short-cut wheat straw and row materials rich in fibres (beet pulp and wheat bran).
In all the protocols, the feed was administered *ad libitum* in the form of total mixed ratio (TMR), with also availability of hay *ad libitum* during the first 7 days after arrival.
Water was always available *ad libitum*.

Table 1 - Different vaccination protocols evaluated in the project.

N° of protocol	Vaccination protocol	Antimicrobial prophylaxis	Day of vaccination
1	MH IM + RSBV + IBR IN	Doxycycline	1
2	MH IM + RSBV + IBR IN	-	7
3	MH + H. Somni IM + RSBV + IBR IN	-	5
4	RSBV + IBR IN + Feed improvement + Batch antibiotic treatment in relationship with the risk evaluation	-	2

Second period

Between November 2020 and May 2021, 62 batches were enrolled in the project. Starting from the results obtained in the first period, in terms of morbidity, relapses and antimicrobial use for BRD, the fourth protocol was selected, changing thus the vaccination timing and improving the environment conditions. Specifically, after the month of November 2020 in which the vaccination was maintained on d2 after arrival, starting from December 2020 the IN vaccination was delayed from d2 to d7 after arrival. Regarding the environment changes, the gates between the pens have been closed with solid panels to make them blind and to isolate each individual pens. Moreover, the litter management was improved adding, daily, bedding wheat straw, during the entire adaptation period, with the aim to maintain the litter completely dry and clean.
The feed management was maintained equal as in the fourth protocol of first period, with a specifically formulated TMR for the adaptation phase.
Water was available *ad libitum*.

Parameters evaluated

Microbiological exams: nasal swabs and blood samples

Between November 2019 and January 2021, a total of 108 animals undergone to nasal swabs and blood samples at d2 after the arrival. Nasal swabs were collected to evaluate the prevalence and circulation of infectious agents, both viral and bacterial ones, such as *bovine coronavirus* (BCOV), *infectious bovine rhinotracheitis* (IBR), *respiratory syncytial bovine virus* (RSBV), *bovine viral diarrhoea* (BVD), *Mannhemia haemolytica* (MH) and *Pasteurella multocida* (PM). The viral agents were analysed through biomolecular techniques, while the presence of MH and PM were identified after growth in blood agar.
Blood samples were taken to evaluate the presence of antibodies against the viral agents previously cited.
Furthermore, all the animals sampled undergone to a complete blood count and to the evaluation of blood levels of iron (Fe), copper (Cu), zinc (Zn) and glutathione peroxidase (GPX - indicator of antioxidant status), to underline possible deficiency of those essential elements.
Blood parameters were analysed using commercial ELISA kit.

Health status and antimicrobial consumption

In both periods, general health evaluations were conducted twice a day, with a direct examination of all the animals by the farm veterinary and qualified animal health care staff. Any case of morbidity and mortality were recorded, together with the mo-

tivation, with specific attention on the incidence of BRD.
Sick animals were considered affected by BRD if the rectal temperature was ≥ 40.0°C and if both depression and respiratory scores differed from the normal health status (score 0 of Baggott et al., 2011)[35]. The number of treatments for BRD were recorded. The first treatment, as well as the mass antimicrobial treatment based on the risk assessment sheet, where it was made, were recorded as "First treatment". The percentage of animals treated for the first time was calculated in reference to the totality of the animals. Furthermore, all the relapses were recorded as I°, II°, or III°, according to how many times one animal needed to be treated again for BRD after the first treatment. The percentage of animals that relapsed for the first, second and third time was calculated in reference to the number of animals treated previously, as first treatment or I° or II° relapses.
Sick animals received concurrent medications according to the facility procedures and sanitary protocols.
The antimicrobial consumption was evaluated considering the antimicrobial use, its content of active principle, the dosage and the length of the treatment. Then, the antimicrobial consumption was expressed as mg of active principle per population correction unit (mg/PCU), which in the case of beef cattle was set at 425 kg of live weight[36].
Moreover, a comparison between batches threated with antimicrobial on the base of the risk assessment sheet and batches without preventive treatments was done, in terms of health status and antimicrobial consumption.

The "health risk assessment sheet" of a new arrival batch

During the first period of study a health risk assessment sheet was developed, with the specific aim to evaluate the real risk level of each single batch, basing on evident, practical and simple indicators. The final risk level was used to guide the veterinarians in the development of the best sanitary protocol for each batch, specifically in terms of antimicrobial mass treatments. Indeed, starting from February 2020 with the fourth protocol of the first period, the sanitary protocols and the application of mass treatments were decided according to the level of risk reached in the risk assessment sheet.
The health risk assessment (Table 2), was based on both indirect parameters, such as information regarding the transport, its duration and the environmental conditions, and direct parameters, recorded directly on the animals, such as general health status, appearance and body condition score (BCS). For each parameter, three possible risk levels were identified, with different scores (3: highest risk; 2: medium risk; 1: low risk). The final risk level was obtained by summing the scores obtained in each individual parameter and considering three risk classes (high risk: score ≥ 23; medium risk: score 18-22; low risk: score 11-17).
Information about environmental conditions during transport, transport duration, number of suppliers and the permanence in collection centres were used as indirect indicators of risk.
Climate conditions were evaluated at the departure, during transportation and at the arrival, from data collected from the local meteorological stations and calculating the temperature humidity index (THI), using the following formula reported by Brügemann et al. (2012) and Bohmanova et al. (2005)[37-38]:

$$(1.8*T_a+32) - (0.55-0.0055*Hum_a) * (1.8*T_a-26)$$

where:
T_a= average daily temperature (°C)
Hum_a= average humidity levels (%).

Table 2 - Health risk assessment form.

Parameters	Risk levels		
	High (Score 3)	Medium (Score 2)	Low (Score 1)
THI in the origin areas	≤ 32; ≥ 84	33-46; 75-83	≥ 47, ≤ 74
THI during transportation	≤ 32; ≥ 84	33-46; 75-83	≥ 47, ≤ 74
THI in the arrival farm, in the adaptation stables	≤ 32; ≥ 84	33-46; 75-83	≥ 47, ≤ 74
> Number of suppliers	≥ 5 suppliers	3-5 suppliers	< 3 suppliers
Duration of transport	> 18 h	12-18 h	< 12 h
Stay in collection centres	> 2 days or no information	2 days	≤ 1 day or no transit
Average weight	< 350 kg	350-400 kg	> 400
Batch homogeneity	> 20% of animals are inhomogeneous	10-20% of animals are inhomogeneous	< 10% of animals are inhomogeneous
Nutritional status and general appearance	> 15% animals: BCS < 3, wet, dull and bristly coats, fatigued and poorly reactive animals	5-15% animals: BCS < 3, wet, dull and bristly coats, fatigued and poorly reactive animals	< 5% animals: BCS < 3, wet, dull and bristly coats, fatigued and poorly reactive animals
ARRIVAL: % of animals with general physical examination as an indicator of health risk	> 10% at risk[1]	5-10% at risk[1]	< 5% at risk[1]
FOLLOWING DAY: % of animals with general physical examination as an indicator of health risk	> 10% at risk [1]	5-10% at risk[1]	< 5% at risk [1]

[1] Probable onset of BRD, with nasal and ocular discharge, cough and respiratory failure.

T° / UR %	20	30	40	50	60	70	80	90	100
-7	36	34	32	30	28	26	24	22	19
-5	38	36	35	33	31	29	27	25	23
-2	41	40	38	37	35	33	32	30	28
0	43	42	41	39	38	36	35	33	32
2	45	44	43	42	41	39	38	37	36
4	47	46	45	44	43	42	41	40	39
6	49	49	48	47	46	45	44	44	43
8	52	51	50	50	49	48	48	47	46
10	54	53	53	52	52	51	51	50	50
12	56	55	55	55	55	54	54	54	54
14	58	58	57	57	57	57	57	57	57
16	60	60	60	60	60	60	60	61	61
18	62	62	62	63	63	63	64	64	64
20	64	64	65	65	66	66	67	67	68
22	66	66	67	68	69	69	70	71	72
24	68	69	70	70	71	72	73	74	75
26	70	71	72	73	74	75	77	78	79
28	72	73	74	76	77	78	80	81	82
30	74	75	77	78	80	81	83	84	86
32	76	77	79	81	83	84	86	88	90
34	78	80	82	84	85	87	89	91	93
36	80	82	84	86	88	90	93	95	97
38	82	84	86	89	91	93	96	98	100

LOW RISK
MEDIUM RISK
HIGH RISK

Figure 1 - Temperature Humidity Index (THI) and correlated risk levels.

The risk thresholds, reported in Figure 1, were set for both hot and cold environmental conditions. In fact, excess, both in one sense and another, can be a source of stress for the animals, reducing their immune functionality. The thresholds for heat stress were set basing on pre-existing data (Brown-Brandl et al., 2018)[39]. Conversely, cold stress thresholds are not present in bibliography for beef cattle and were set up starting from the pre-existing data related to dairy cows (Brügemann et al., 2012; Xu et al., 2018)[37-40]. Taking into account the different sensitivity between beef and dairy cattle, with beef cattle being more sensible to cold stress due to a lower endogenous production of metabolic heat (St-Pierre et al., 2003; Nardone et al., 2010)[41-42]. Consequently, cold stress THI thresholds for beef cattle has been considered higher than those reported for dairy cows (Brügemann et al., 2012; Xu et al., 2018)[37-40].
Three different risk levels were identified, considering the THI. Data about the number of suppliers and the permanence in the collection centres were obtained from the final retailers of the animals, while the duration of the transport was obtained from transport's document.
Considering the direct indicators of risk, the average arrival weight was the first parameter considered, with lighter animals being at higher risk.
Also, the batch homogeneity was included, considering age, weight, and conformation of the animals, with more homogeneous batches being less risky. General appearance and nutritional status in terms of percentage of animals that showed a low body condition score (BCS<3) and sign of fatigue and distress were evaluated. Moreover, the percentage of animals that showed clear signs of probable onset of BRD, with nasal and ocular discharge, cough and respiratory failure, were evaluated both at the arrival and the following day, after a 24h rest period.
The percentage thresholds used for homogeneity of the batches, general appearance, nutritional and health status were derived from field studies[8-11-43-44].

RESULTS AND DISCUSSION

Microbiological exam: nasal swabs and blood samples

The results on circulation and the percentage of seropositivity to different infectious agents are summarized in Table 3 and 4. Between the bacterial agents, positivity to *Mannhemia haemolytica* (MH) and *Pasteurella multocida* (PM) were found, respectively with a prevalence of 8.33% and 4.63%. Conversely, Cirone et al., 2019 reported a higher prevalence of both *Mannhemia haemolytica* (MH) and *Pasteurella multocida* (PM), respectively of 21.4% and 57.1%. Moreover, Cirone et al., 2019, found also a 100% percentage of positivity to *Histophilus somni*, that in the present study wasn't detected[19]. Considering the viral components, 87% of the animals were positive for *bovine coronavirus* (BCOV), while the positivity for *respiratory syncytial bovine virus* (RSBV) was negligible. The positivity for IBR was completely absent thanks to the France eradication plan. The present results on *bovine coronavirus* positivity are in line with the finding of Cirone et al., 2019, that report a positivity of 75% in limousine cattle shipped from France to Italy[19].
Regarding the seropositivity, a high percentage (72.22%) of the animals tested were positive for BCOV, resulting to be already protected from this virus. Conversely, only 33.33% of the animals were positive for RSBV. Moreover, the seropositivity was absent for IBR and BVD. Consequently, the vaccination in the fattening farm against RSBV, IBR, and BVD, is mandatory. Indeed, only a small part of the animals from France were already protected for these viruses.
The blood mineral levels are reported in Table 5. A high percentage of the animal tested resulted to be in a deficient or sub-deficient status in terms of concentration of Fe, Cu, Zn, and GPx, components fundamentals for the immune system. Those result are in line with the findings of Mottaran et al.

Table 3 - Percentage of positivity in nasal swabs between different viral and bacterial agents at the arrival.

	BCOV[1], %	IBR[2], %	RSBV[3], %	BVD[4], %	MH[5], %	PM[6], %
Positives	87.04	0.00	4.63	0.926	8.33	4.63
Negatives	12.96	100.00	95.37	99.074	91.67	95.37

[1]BCOV = bovine coronavirus; [2]IBR = infectious bovine rhinotracheitis; [3]RSBV = respiratory syncytial bovine virus; [4]BVD = bovine viral diarrhoea; [5]MH = Mannheimia haemolitica; [6]PM = Pasteurella multocida.

Table 4 - Seropositivity to different viral agents at the arrival.

	BCOV[1], %	IBR[2], %	RSBV[3], %	BVD[4], %
Positives	72.22	1.85	33.34	2.77
Negatives	27.77	98.1	66.66	97.22

[1]BCOV = bovine coronavirus; [2]IBR = infectious bovine rhinotracheitis; [3]RSBV = respiratory syncytial bovine virus; [4]BVD = bovine viral diarrhoea; [5]MH = Mannheimia haemolitica; [6]PM = Pasteurella multocida.

Table 5 - Concentration of minerals in the serum.

		Reference interval	Animals, % (n)
Fe (µg/dL)	*Deficiency*	< 69	45.37
	Adequate	69 - 196	54.63
Cu (µmol/L)	*Deficiency*	< 8	11.11
	Sub-deficiency	8 -12.9	72.22
	Adequate	13 -18	16.67
Zn (µmol/L)	*Deficiency*	< 8	39.81
	Sub-deficiency	8 - 13.9	49.07
	Adequate	14 - 21	11.12
	Excess	> 21	0.00
GPx (IU/gHb)	*Deficiency*	< 75	6.48
	Sub-deficiency	75 - 220	34.26
	Adequate	220 - 600	59.26

(2015), that reported similar percentage of deficient animals in terms of Fe, Cu, Zn and also GPx[45].

A deficiency in those minerals can result in an impaired immune functionality during the adaptation phase, and need to be resolved as soon as possible, especially through a specifically balanced nutritional integration.

First period

Data observed during the first period of the study are reported in Table 6. The fourth protocol, in which the intranasal vaccination was matched with the specifically formulated arrival diet, has led to a general improvement in the health status. Indeed, all the health indicators (mortality, morbidity and relapses) were lowered as well as the antimicrobial consumption. On average, between the fourth and the other three protocols, there was a reduction of 29.1% in the percentage of animal treated for the first time, of 81.7% in the first relapse and of 71.5% in the antimicrobial use (mg/PCU).

Those results confirm the effectiveness of the intranasal vaccination, as well as the importance of the match between sanitary protocols and a proper nutrition. In fact, the better health status can be explained by both a better and quicker stimulation of the immune functionality and lower vaccine-induced stress, attributable to the intranasal vaccination, and to a proper energy, protein, fibre, minerals, vitamins and additives administration through the diet. Intranasal vaccines induce both an adequate systemic response, with rapidly increased levels of antigen specific IgA and greater activation of the type I interferons, and, at the same time, a better local antibody response. Indeed, when a vaccine is delivered to the nasopharyngeal mucosal surface, the animal rapidly produces a mucosal immune response that provides a first line of defence against respiratory pathogens[46]. The positive effect on health status of a proper adaptation diet, formulated to preserve the ruminal health, the functionality of the microbiota and its adaptation to a new nutritional management, was highlighted also in previous studies. Indeed, digestive disorders, such as acidosis, can cause an inflammatory status that can lead to an overall reduction in the immune functionality, increasing the risk of BRD[27-29-30].

Second period

In the second period a modified version of the fourth protocol was applied. Specifically, the vaccination was delayed from d2 to d7, the gates between the pens have been closed with solid panels, and the litter management was improved adding, daily, wheat straw to keep the litter completely clean and dry, during all the adaptation period. Data showed that those improvements has led to a clear reduction in the incidence and severity of sanitary problems, and, as a consequence, in the use of antibiotics. Indeed, the results highlighted in Table 7, that shows the comparison between the periods February-May 2020

Table 6 - Morbidity, relapses and mortality in relation to the restocking protocol (period November 2019 - September 2020).

Protocol	N batches	N heads	Treated %	Relapse I %	Relapse II %	Relapse III %	Mortality %	mg/PCU[1]
1[2]	8	208	68.26	39.43	35.71	15.00	2.88	69.02
2[3]	6	146	70.55	25.24	23.08	0.00	2.74	13.34
3[4]	8	194	81.96	32.08	9.80	0.00	6.18	13.57
4[5]	69	1624	51.8	5.71	4.17	0.00	1.47	5.23

[1]PCU: population correction unit (425 kg for fattening beef cattle).
[2]1= MH IM+ RSBV+IBR IN + doxycycline, d1; [3]2= MH IM+ RSBV+IBR IN, d7; [4]3= MH+ H. Somni IM + RSBV+IBR IN, d5; [5]4= RSBV+IBR IN, d2 + Feed improvement+ Batch antibiotic treatment in relationship with the risk evaluation.

Table 7 - Morbidity, relapses, mortality and antibiotic use in the periods February 2020 - May 2020 and February 2021 - May 2021.

Period	n heads	Treated %	Relapse I %	Relapse II %	Relapse III %	Mortality %	mg/PCU[1]
February - May 2020	894	81.99	11.05	2.46	0.00	1.56	8.08
February - May 2021	790	47.6	5.32	5.00	0.00	0.76	6.02

[1]PCU: population correction unit (425 kg for fattening beef cattle).

Table 8 - Morbidity, relapses, mortality and antibiotic consumption: comparison between batches with and without antibiotic mass treatment in relationship with the risk evaluation form.

Antimicrobial mass treatment	Treated %	Relapse I %	Relapse II %	Relapse III %	Mortality %	mg/PCU[1]
YES	100.00	11.37	1.29	0.00	0.62	5.97
NO	30.63	9.72	14.28	0.00	0.85	7.46

[1]PCU= population correction unit (425 kg for fattening beef cattle).

and February-May 2021, underline the positive effect of delaying the vaccination, reducing the contacts between animals in different pens, and improving the litter conditions, on animal health and welfare. Mortality was reduced by 49.3% and morbidity by 41.9%, considering animals that needs a first treatment for BRD. Also, the percentage of animals that relapsed one time after the first treatment was reduced by 51.9%. Moreover, the antimicrobial consumption resulted to be lowered by 25.5%.
In agreement with the findings of previous researches, delaying the vaccination, in combination with the rapid antibody response times of nasal vaccination, allows the animals to fully recover the stress of transportation and to exert a better antibody response[31-32-33]. In addition, the results of the present study agreed with the statements of Stoksad et al. (2020), and of Diana et al. (2020), that largely discuss the importance of implementing the internal biosecurity, both through a higher hygiene of the stables as well as trough practices to reduce the spread of pathogens, to counteract the incidence of BRD in newly arrived beef cattle[47-48].
In Table 8, is reported a comparison between batches treated or untreated with antimicrobials at the arrival on the base of the risk assessment sheet, in terms of mortality, morbidity, relapses and antimicrobial use. The results show that mandatory avoiding it can lead to a severe worsening of animal welfare and health, with an important increase in mortality (+ 37%), morbidity (+ 90%), antibiotics consumption (+25%) (mg/PCU). Therefore, the antimicrobial mass treatment must not be demonized, but instead must be considered as a key tool to limit the overall consumption of antibiotics and to improve the animal welfare, in situations of evident and visible risk. To this end, the application at the farm level of a system for assessing the level of risk upon arrival, based on evident and scientific parameters, can serve as a basis for the veterinarian to decide whether to apply, or not, the antibiotic mass treatment to safeguard animal welfare and health.

CONCLUSIONS

The spread of antimicrobial resistance, and its implication in human health, has led the zootechnical producers to rethink about their standard management and sanitary practices, to find out how to reduce this phenomenon. In beef cattle farming, the main sanitary problem that require a high use of antibiotics is the bovine respiratory disease, the incidence of which is mainly concentrated in the adaptation period. Due to its multifactorial etiology, a combination of infectious agents, host and environment related factors, alternative strategies must follow an integrated managerial, nutritional and sanitary protocol that includes all the main critical points.
Indeed, the evidence emerging from this study, underline how an integrated and multidisciplinary approach is essential to limit health problems and antibiotic consumption in newly arrived beef cattle, in particular, in the most critical period, from autumn to spring. In addition to a correct vaccination protocol, in terms of type, administration and timing, it is crucial to ensure the absence of contact between the animals of different pens, even if they are of the same batch, to ensure a clean and dry litter added daily, and to use a non-selectable specific and properly integrated diet, characterized by low protein and energy contents and rich in nutrients able to improve digestive process and immunoreaction, and reduce inflammation and pro-oxidative status.
The use, by the veterinarian, of the health risk assessment form developed in this study, is useful to decide whether or not an antibiotic mass treatment is needed, in order to optimize animal welfare and, at the same time, reduce the consumption of antibiotics.

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