Effect of a blend of essential oils, bioflavonoids and tannins on production performance, health, immune functionality, and antioxidant status in fattening beef cattle

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SUMMARY

The aim of the study was to evaluate the effect of a blend of essential oils, bioflavonoids and tannins on production performance, health, immune response, and antioxidant status in fattening beef cattle under field conditions.
A total of 210 newly arrived male Charolaise bulls were assigned to two study groups: i) Control (417.84 ± 19.63 kg live weight), basal diet; ii) Treatment (416.37 ± 18.56 kg live weight), basal diet integrated with 5 g/head/d of the blend of essential oils, bioflavonoids, and tannins. Growth performances, dry matter intake (DMI), feed conversion rate (FCR), carcass characteristics and health were evaluated during the entire fattening period (182 days). The immune response and the oxidative status were evaluated at the arrival (d_0), and after 45 days (d_{45}) by the titration of antibodies against BHV-1, serum bactericidal activity, γ-interferon levels, free oxygen radical metabolites (ROM's), and antioxidant activity (OXY).
Growth performances and production efficiency resulted to be significantly improved by the treatment. Indeed, both weight at d_{102} and d_{182} were higher in the Treatment group (respectively 579.81 and 720.86 kg *vs* 572.69 and 709.82 kg in the Control) (P≤0.05). As a result, also the total average daily gain (ADG_{0-182}) (1.67 *vs* 1.60 kg/head/d) (P≤0.001) and the ADG between d_0 and d_{102} (ADG_{0-102}) (1.60 *vs* 1.52 kg/head/d) (P≤0.05) were improved. The DMI was lower in the Treatment group (10.59 *vs* 11.18 kg/head/d) (P≤0.001), leading to a significant improvement in the FCR (6.37 *vs* 7.04) (P≤0.001). Carcass weight was higher in the Treatment group (425.87 *vs* 418.31 kg in the Control group) (P≤0.001), while carcass characteristics weren't affected by the treatment. The incidence of bovine respiratory disease (BRD) was significantly reduced by the treatment (17.14 *vs* 28.57 % in the Control group) (P≤0.05). No differences were found in the incidence of lameness. The Treatment has led to an increase in the serum bactericidal activity (92.00 *vs* 80.40 % in the Control group) (P≤0.05) and to a better antioxidant status (365.68 *vs* 290.58 μmol HClO/ml in the Control group) (P≤0.001) at d_{45}. No differences were found in terms of antibodies titration and ROM's levels.
In conclusion, the supplementation of fattening beef cattle diet with a blend of essential oils, bioflavonoids and tannins, improved growth performance, feed efficiency and health status thanks to a better feed efficiency and immune functionality.

KEY WORDS

Beef cattle, efficiency, natural products, animal health, immunity, welfare.

INTRODUCTION

The zootechnical sector will face many challenges in the next years. The overall increase in the world population, prospected to double by 2050, together with a generalized improvement in the economic and social status, will lead to a steady growth in the demand of animal derived foods [1-5]. Moreover, those developments of the human society can increase pressure on some resources that are already scarce, such as land and water, reducing their availability for animal production [5]. Furthermore, there is a constant increase in the consumers' awareness about animal welfare and antibiotic use in farming animals, also due to the increased spread and frequency of disease caused by antimicrobial resistant bacteria. Indeed, an excessive and incorrect use of antibiotics at the farm level, frequently driven by poor welfare conditions, is one of the possible triggers for the development of those resistant strains, together with both an incorrect and excessive use in human medicine and an improper management of wastes and wastewaters [6]. Moreover, the exacerbating environmental crisis has increased the pressure about the role of zootechny on climate change and other environmental related topics, such as soil and water pollution and water and agricultural land scarcity [7-8]. The need and urgency to address, in a short time, all those targets are highlighted also by the newest national and European rules on animal welfare and antimicrobials use in animal farming, and by the new allocation of the PAC subsidies in relation to the achievement of specific environmental targets [9].
Between all the food producing animals, beef cattle are among the most criticized. Beef cattle farming is responsible of about 35% of the total livestock farming methane emissions [10], and

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moreover beef cattle are the less efficient in converting the feeds used into final edible products, compared to other food producing species [11-13]. Indeed, beef cattle are characterized, on average, by a feed conversion rate of 6 to 10 per kg of protein produced, while the ones of pigs (2.7 to 5) and chickens (1.7 to 2) are significantly lower [11-13]. In terms of health problems and antimicrobial use, intensive beef cattle farming is in third position in terms of milligrams per population correction units (PCU) at a global level, behind swine and poultry (172 mg/PCU, 148 mg/PCU, and 45 mg/PCU, respectively) [14]. However, there are still stages, such as the arrival period, that can represent a treat for animal health and welfare, thus frequently requiring a higher use of antimicrobials [15,16]. Indeed, newly received cattle are exposed to several stressors, such as weaning, long-distance transport, mixing, feed and water restrictions, and adaptation to new environmental and feed conditions [15]. All these factors contribute to pathogen colonization and proliferation as well as to a stress-related reduced immune functionality, that can lead to a higher incidence of diseases, especially bovine respiratory disease (BRD) [15-17]. Consequently, higher quantities of antibiotics are often used in this stage, both to treat sick subjects but also to prevent the spread of the problem [15].

New strategies must be designed, tested, and implemented to maximise beef cattle health and welfare, with a consequent reflection on production efficiency and environmental sustainability, due to the strong correlations between all those parameters. As an example, the onset of BRD in the first days after the arrival can cause a strong reduction in the growth performances with a reflection on the entire fattening period [18]. This led to both a waste of feed resources, often in competition with human nutrition or already scarce, such as water, as well as to a lower quantity of final products per animal. Basically, if efficiency and health are not maximized more animals are required to produce the same amounts of final products, leading to a both a higher production of methane and to an increased need of feed products, increasing the demand of scarce and critical resources, such as water and agricultural land.

In these directions, animal nutrition can have a proactive role, improving production performance, feed efficiency, and animal welfare, and reducing the risk of pathologies, improving consequently, the environmental impact. Indeed, besides being functional to fulfil all the basic nutritional requirements, nutrition can be a helpful tool to modulate animal health and efficiency, and consequently the overall sustainability levels [19]. Firstly, through nutrition is possible to maintain and even enhance rumen functionality and efficiency [20]. A correct and balanced diet is the main tool to avoid imbalances and sudden changes in the ruminal environment, that can impair its activity, leading thus to the onset of digestive disorders, such as acidosis [18,20]. Those diseases, besides reducing the overall productivity and production efficiency of the animals, are often the door opener and the trigger for other health problems, such as lameness, bloat, and enterotoxaemia [18].

At the same time, nutrition can be a vehicle to administer bioactive compounds to support the rumen as well as the general immune system. Indeed, rumen health and functionality can be safeguard and improved by additives, such as yeasts, organic minerals, and natural extracts, able to influence rumen microflora and the digestive processes [15-19]. Moreover, modulating the ruminal environment and consequently the ruminal microflora through the feed and some bioactive molecules, it is possible to reduce the overall methane production [19]. At the same time, similar additives can also have a modulatory effect on the general immune system, providing bioactive compounds that can sustain both the antioxidant defences and the innate and adaptive immune response [15-23].

Between natural products, essential oils, bioflavonoids and tannins are showing promising result in the application in ruminant's nutrition due to their proven ability in modulate the ruminal microflora toward a more efficient environment and methanogenesis reduction [19, 24-27] and in improving health status thanks to their antimicrobial, antioxidant and immunostimulant properties [28].

Those properties are variable between the different natural compounds as well as between the different blend of them. Their effectiveness, and the specific targets for which they are used, varies depending on their molecular characteristics, their composition, and their combinations. Also, the quantities administered as well as the form and duration of the administration and other factors related to the nutritional and overall management of the animals, can affect their functionalities. Those aspects lead to different possibility of application of the different natural products, as well as to potentially controversial results.

A combination of coriander, geraniol oils and bioflavonoids and tannins has been already tested in dairy cows, reporting positive effects on production performances such as milk yield, feed conversion efficiency and on *in vivo* diet digestibility [19]. This combination of natural products has still to be evaluated in terms of effect on the immune systems as well as specifically on beef cattle. However, there are some evidence that single components of this mixture can positively affect beef cattle growth as well as the immune functionality and antioxidant defences in beef and dairy cows [29-33].

On the base of these evidence, the aim of the present study was to evaluate the effect of the inclusion of a blend of coriander, eugenol, geraniol oils and bioflavonoids and tannins (Anavrin, Vetos Europe Sagl - Cadenazzo TI - Switzerland) in the diets of fattening beef cattle on production efficiency, health, immune response, and antioxidant status under field condition.

MATERIALS AND METHODS

The procedures relating to animals were carried out in compliance with the directive of the Council of the European Communities (2010/63 / EU), implemented by the Italian Ministry of Health (Legislative Decree 26, March 4, 2014).

This trial was set up to evaluate the efficacy of a blend of essential oils (EO), mainly from cloves (*Syzygium aromaticum*), coriander seed (*Coriandrum sativum*), and geranium (*Pelargonium cucullatum*), tannins (CT) from chestnuts (*Castanea sativa*) and bioflavonoids (BF) from olives (*Olea europea*) (relative concentrations of the active principles were: EO:CT:BF = 1:2.5:0.1) (Anavrin, Vetos Europe SAGL, - Cadenazzo TI, Switzerland), on beef cattle productivity, health, immune response and antioxidant status.

Animals, housing, and trial groups

The study took place in an intensive beef fattening unit, located in northern Italy (via Viola 6, 37050 Roverchiaretta, (VR), Italy), that well-represent the typical Italian intensive beef cattle fattening farms.

A total of 210 Charolaise bulls , imported from France, were enrolled at the arrival (d_0), after being individually weighed and evaluated for body conformation using a 5-point scale (1: profiles straight and poor muscle development; 2: profiles between whole straight to low convex and medium muscle development; 3: profiles low convex and good muscle development; 4: profiles on the whole convex and very good muscles development; 5: all profiles convex and exceptional muscles development) [34]. The animals were then grouped by weight and conformation, and randomly assigned to two balanced groups, differing only for the inclusion or not in the diet of the blend of natural products: i) Control (105 heads, 417.84 ± 19.63 kg live weight), basal diet; ii) Treatment (105 heads, 416.37 ± 18.56 kg live weight), basal diet integrated with 5 g/head/d of that blend.
The bulls were housed on slatted floor in a close barn, in 30 pens with 7 animals each (3.5 m^2 each).
The trial lasted for the entire fattening period (182 days).

Nutritional management

The two study groups were fed following the same nutritional plan (Table 1), characterized by two different formulations (arrival and fattening), studied to meet the specific growth needs in those different phases, in agreement with the Nutrient Requirement Council [35]. The feed was administered in the form of total mixed ration (TMR).
Two separate TMRs, equal in terms of nutritional values but differing for the inclusion or not of the treatment, were produced daily. Indeed, the pool of natural products was included directly in the mineral mix used in the TMR administered to the Treatment group, to optimize the mixing and to guarantee its inclusion at 0.5-1 g/kg d.m. (dry matter) of feed. The same amount of wheat powder was added in the mineral mix of the Control group as a placebo.
The TMRs were administered *ad libitum* for the entire fattening period and delivered once a day in the morning by a feed mixer wagon, provided with electronic scale to weigh the inclusion of each ingredient and the amount of the TMR unloaded. Water was available *ad libitum*.

Parameters recorded

Growth and slaughtering performances
Individual body weight was recorded before morning feeding at three timepoints, enrolment day (d_0), day 102 (d_{102}) and before slaughter (d_{182}). The individual average daily gain (ADG) was then calculated for each period, from d_0 to d_{102}, from d_{102} to d_{182} and from d_0 to d_{182}, using the following formula:

$$ADG = \frac{Weight_f - Weight_i}{days\ i - f}$$

Where:
ADG= average daily gain (kg/head/day)
Weight f= final weight of each period
Weight i= initial weight of each period
Days i-f= days between the start and the end of each period
The daily feed intake of each pen in the two groups was evaluated weekly by weighing the TMR administered and the residue in the manger 24 h later. Then the feed intake was corrected for the dry matter level of the diet, to obtain the dry matter intake (DMI), The FCR was then calculated, comparing the average DMI intake of each pen from d_0 to d_{182}, with the ADG of the corresponding pen in the same period.
Data about cold carcass weight, dressing percentage, carcass conformation and fattening (SEUROP) scores were collected for all animals at the slaughterhouse. The cold carcass weight was recorded after 24h of chilling at a temperature of 0°C to 4°C. The dressing percentage was obtained comparing the final live weight with the cold carcass weight. Carcass conformation and fattening scores were assessed by an expert judge following the EU legislation (Council Regulation EEC n. 1026/91, 22 April 1991) [36] , using the SEUROP classification method, with a conformation scale ranging from S to P (S-superior: all profiles extremely convex, exceptional muscle development, double-muscled conformation; E-excellent: all profiles convex to super-convex, exceptional muscle development; U-very good: profiles on the whole convex, very good muscle development; R-good: profiles on the whole straight, good muscle development; O-pretty good: profiles straight to concave, medium muscle development; P-poor: all profiles concave to very concave, poor muscle development), and a fattening scale ranging from 1 to 5 (1-low: none up to low fat cover; 2- slight: slight fat cover, flesh visible almost everywhere; 3-medium important: flesh, with the exception of the round and shoulder, almost every-where covered by fat, slight fat deposits in the thoracic cavity; 4-high: flesh covered by fat, round and shoulder still partly visible, medium fat deposits in the thoracic cavity; 5-very high: carcass well covered by fat, heavy fat deposits in the thoracic cavity).

Table 1 - Feeding plan: composition and predicted nutritional value of the two diets used during the entire fattening period, as calculated by the rationing software (Plurimix).

Feed, kg/head/d	Arrival	Fattening
Silomais	8.00	8.00
Corn meal	2.50	5.50
Wheat bran	2.50	1.20
Soybean meal 44% CP[1]	0.70	1.00
Sunflower hulled meal 33% CP	0.60	0.60
Mineral and Vitamin Mix	0.17	0.20
	Quantities	
As fed, kg	*14.47*	*16.55*
d.m.[2], kg	*8.54*	*10.38*
	Nutritional values	
d.m., %	*59.10*	*62.74*
UFV, kg d.m.	*0.85*	*1.02*
CP, % d.m.	*11.80*	*14.28*
CP Soluble, % d.m.	*3.25*	*4.49*
CP Soluble, % CP Degradable	*44.21*	*48.96*
Sugars, % d.m.	*4.32*	*4.15*
Starch, % d.m.	*29.61*	*43.24*
NDF[3], % d.m.	*43.55*	*29.20*
ADF[4], % d.m.	*26.80*	*16.63*
ADL[5], % d.m.	*4.91*	*3.25*
Fats, % d.m.	*2.54*	*2.95*
Ca[6], % d.m.	*0.77*	*0.60*
P[7], % d.m.	*0.29*	*0.32*

[1]CP= crude protein; [2]d.m.= dry matter; [3]NDF= neutral detergent fiber; [4]ADF= acid detergent fiber; [5]ADL= acid detergent lignin; [6]Ca= calcium; [7]P= phosphorus

Health status, immune response, and antioxidant status

The individual health status was checked twice a day by the veterinary and qualified animal health care staff of the farm during the entire fattening period. Any cases of morbidity and mortality were recorded, as well as the number of animals that needed to be moved to the infirmary pen, together with the motivation, with specific attention on the incidence of bovine respiratory disease (BRD) and lameness. Sick animals were treated according to the procedures, medications, and sanitary protocols adopted by the farm veterinary staff.

Blood samples were taken at the arrival (d_0) and at d_{45} on a subsample of animals (5 heads in each group) to evaluate some serum indicators of immune response and antioxidant status. Blood samples were collected by jugular venepuncture into 10-mL EDTA tubes and 10-mL no additive tubes (Venoject®, Terumo Europe N.V., Leuven, Belgium) and immediately placed on ice. Then, the serum was extracted by centrifugation at 3000 g for 10 min at 4 °C and stored at - 20 °C. The immune response was evaluated in terms of both specific and nonspecific immunity and in terms of oxidative status.

The titration of BHV-1 vaccination antibodies with a BHV-1 serum neutralization test was evaluated as an indicator of specific immunity modulation. The BHV-1 serum neutralization test was performed according to OIE (2012).

Non-specific immunity was evaluated with the analysis of the serum bactericidal activity and γ-interferon.

The oxidative status was evaluated through the analysis of both the amount of free oxygen radicals' metabolites (test ROM's) and of the antioxidant activity (test OXY).

The amount of free oxygen radicals in plasma samples was determined using the d-ROMs test (Diacron, Grosseto, Italy), which determines hydroperoxides (the breakdown products of lipids, as well as of other organic substrates, generated by the oxidative attack of ROS) through their reaction with the chromogen N,N-diethyl paraphenylenediamine. Results are expressed in arbitrary Carratelli Units (U CARR), where 1 (U CARR) is equivalent to the oxidizing power of 0.08 mg H_2O_2/dL.

The antioxidant activity was evaluated through the OXY-adsorbent test. This method measures the ability of a sample to oppose a massive oxidant attack, induced in vitro, by a hypochlorous acid solution and the results are expressed in terms of moles HClO/mL (hypochlorous acid).

Statistical analysis

The statistical analysis of the data was conducted using the SAS statistical software (SAS 9.4, SAS Cary NC).

For the evaluation of the growth performances the pen was used as an experimental unit. These data were analysed using a mixed model (PROC MIXED) which considered the fixed effect of the treatment and the time of detection and the random effect of the pen.

The single subject was instead used as a reference unit for evaluating the characteristics of the carcass using a mixed model (PROC MIXED) which considered the fixed effect of the treatment. Also, the single subject was used as a reference unit for the evaluation of the parameters related to the immune functionality, using a mixed model (PROC MIXED) which considered the fixed effect of the treatment and the time of detection.

For non-continuous variables, such as SEUROP classification, fattening status and health status, the difference in frequency distribution within the classes was evaluated by applying a chi-squared test (PROC FREQ).

The difference was considered significant for $P \leq 0.05$, while a tendency was set up at $P<0.1$.

RESULTS AND DISCUSSION

Growth and slaughtering performances

Data related to growth performance and production efficiency are reported in Table 2. The treatment has led to an improvement ADG, both considering the first period of the trial (ADG_{0-102}) (1.60 *vs* 1.52 kg/head/d in the Control group) ($P \leq 0.001$) and the overall fattening period (ADG_{0-182}) (1.67 *vs* 1.60 in the Control group) ($P \leq 0.05$). Specifically, this difference was equal to 80 g/head/d during the first 102 days following arrival and 70 g/head/d considering the entire rearing period. On average, at the end of the fattening cycle, there was a 4.4% of increase in terms of ADG in the Treatment group.

Those improvements in the daily gain have led to higher weights in treated animals, both at d_{102} (579.81 *vs* 572.69 kg in the Control group) ($P \leq 0.05$) and at the end of the fattening period (720.86 *vs* 709.82 kg/head/d in the Control group) ($P \leq 0.05$). Moreover, a lower DMI was reported in the Treatment group (10.59 *vs* 11.18 kg in the Control group) ($P \leq 0.001$) during the entire fattening period. This reduction, together with the improvement in the growth performances has led to an optimization of the feed efficiency (FCR: 6.37 *vs* 7.04 in the Control group) ($P \leq 0.001$), resulting in an overall 9.52% improvement in FCR in treated animals.

This increase in production efficiency can be explained by a pos-

Table 2 - Growth performance in the two study groups.

Parameter	Control	Treatment	*P value*
n° of animals	105	105	
Weight d_0, kg (± ds[1])	417.84 (±19.63)	416.37 (±18.56)	*ns*
Weight d_{102}, kg (± ds)	572.69 (±20.87)	579.81 (±21.71)	*<0.05*
Weight d_{182}, kg (± ds)	709.82 (±30.41)	720.86(±29.97)	*<0.05*
ADG_{0-102}[2], kg/d (± ds)	1.52 (±0.10)	1.60 (±0.14)	*<0.001*
$ADG_{102-182}$, kg/d (± ds)	1.71 (±0.39)	1.76 (±0.37)	*ns*
ADG_{0-182}, kg/d (± ds)	1.60 (±0.16)	1.67 (±0.16)	*<0.05*
DMI[3] intake, kg dm/d (± ds)	11.18 (±0.17)	10.56 (±0.15)	*<0.001*
FCR[4] (± ds)	7.04 (±0.32)	6.37 (±0.41)	*<0.001*

[1] ds= standard deviation; [2] ADG= average daily gain (kg/head/d); [3] DMI= dry matter intake; [4] FCR= feed conversion rate

Table 3 - Carcass characteristics and slaughtering performances in the two study groups.

Parameter	Control	Treatment	*P value*
Carcass weight, kg (± ds)[1]	418.31 (±21.19)	425.87 (±19.20)	*<0.001*
Dressing percentage, %	58.92	59.07	*ns*
Conformation			
E, % (n)	95.24 (100)	96.19 (101)	*ns*
U, % (n)	4.76 (5)	3.81 (4)	*ns*
Fattening status			
2, % (n)	56.19 (59)	59.05 (62)	*ns*
3, % (n)	43.81(46)	40.95 (43)	*ns*

[1] ds= standard deviation.

itive and stimulating action of the blend of natural compounds on the ruminal microflora, resulting in a more efficient ruminal environment. This aspect can improve the efficiency of utilisation of the feeds by the ruminal microflora, leading to a higher percentage of volatile fatty acids, especially of propionate, produced in the rumen [19,37,38]. Sgoifo Rossi et al. [19], in a study done in dairy cows, have found a significant improvement of the *in vivo* diet digestibility of starch and cellulose in cows treated with the same blend of natural products, explained by a more viable and functional ruminal microflora. Indeed, several studies based on similar natural compounds have shown an increase in the main ruminal populations involved in both structural and non-structural carbohydrates degradation, such as *Ruminococcaceae* and *Propionic acid* bacteria [39,40], with an improvement in propionate production and the acetate:propionate ratio [40]. As an example, Klop et al. [40], in an *in vitro* incubation test reported a higher proportion of propionate in the ruminal fluid of donor cows, that received a blend of similar natural compounds, compared to control cows that did not receive the integration. Moreover, different bibliographical studies, done both *in vitro* and *in vivo*, have reported a potential inhibitory activity of some of the natural compounds present in the blend used in the present study on ruminal methanogenic bacteria, highlighting how this can increase the bioavailability of substrates for other microbial populations, leading to a greater production of volatile fatty acids [19,27,39-42]. Consequently, those increased quantities of volatile fatty acids available for the animal can explain the increase in the overall growth and productive parameters as well as the overall reduction in the feed intake, since a higher amount of energy is already available at the ruminal level.

Because of the better rumen functionality and diet digestibility, Sgoifo Rossi et al. [19] found a 3.8% increase in daily milk, together with a 7.2% of increase in terms of FCR production in dairy cows fed with the same blend of natural products. Those results are also confirmed in other studies, that also highlighted that shorter administration periods can affect the results, with lower efficacy in shorter studies [27]. Those results obtained in dairy cows are in line with the finding of the present study, even if it is necessary to consider the differences between dairy and beef cattle, as well as the differences in their management, especially considering the nutritional plans.

Nowadays, there are not studies done in beef cattle with the same blend of essential oils, that evaluate comprehensively the effectiveness of this specific combination.

However, there are some evidences that its components, alone or in combination with other natural products, can positively affect growth performance and feed efficiency in beef cattle. Alves de Souza et al. [30] found an increase in the ADG (+0.240 g/head/d) in fattening beef heifers that received a mixture of clove and different natural products compared to control heifer without any integration [30]. Conversely, in that study the DMI resulted to be increase, partially explaining the increase in the growth performance [30]. Also, Compiani et al. [28], found a significant increase in the ADG and overall growth performances in beef cattle treated with a pool of different natural compounds, also containing eugenol, one of the main bioactive compounds present in clove. Moreover, different studies have reported some positive effects of tannins on growth performance of beef cattle fed with corn-based diets [30-33]. Indeed, even if high concentrations of tannins may be toxic, reducing voluntary feed intake and nutrient digestibility, however at low to moderate concentrations, tannin supplementation may shift site of protein degradation increasing metabolizable amino acid flow to the small intestine [43-44]. This tannin effect may explain the improvements observed in performance of feedlot cattle [32-33]. Conversely, Tabke et al. [45] did not found any improvement in terms of growth performance in beef steers that received an integration of tannic acid. Conversely, a reduction in the apparent total tract digestibility of starch and protein was found in treated animals [45].

Data related to slaughtering performances are reported in Table 3. Carcass weight resulted to be higher in the Treatment group (425.87 *vs* 418.31 kg) ($P \leq 0.001$), as a reflection of the higher live weight achieved at the end of the fattening period. No significant differences were found in terms of dressing percentage, conformation, and fattening score between groups. No specific studies are available in the bibliography that evaluate the effect of the same blend of natural products on carcass quality and characteristics. However, there are some studies that tested the effect of some of its components, alone or in combination with other natural products, on carcass characteristics. As in the present study, Mottin et al. [46] have found no effect of the inclusion of a blend of natural products, containing clove and other compounds, on the main parameters related to the carcass evaluation, such as carcass yield, subcutaneous fat thickness, muscle area and marbling. Also, Tabke et al. [45] did not found any effects of the inclusion of tannic acids on carcass yield and quality, expressed as quality grade and yield grade.

Health status, immune functionality, and antioxidant status

Data related to the health status, recorded during the entire trial, are reported in Table 4. No cases of mortality were record-

Table 4 - Health status in the two study groups during the entire fattening period.

	Control	Treatment	*P value*
n° of animals	105	105	
BRD[1]			
First episode, % (n)	*19.05 (20)*	*13.33 (14)*	*0.08*
First relapse, % (n)	*8.57 (9)*	*3.81 (4)*	*0.08*
Second relapse, % (n)	*0.95 (1)*	*0.00 (0)*	*ns*
Total, % (n)	***28.57 (30)***	***17.14 (18)***	***<0.05***
Lameness, % (n)	1.90 (2)	1.90 (2)	*ns*

[1] ds= standard deviation; [2] ADG= average daily gain (kg/head/d); [3] DMI= dry matter intake; [4] FCR= feed conversion rate

ed. Moreover, no animals in both groups needed to be moved to the hospital pens due to severe health issues that required isolation and specific care.
In terms of pathological issues, the treatment has led to an overall reduction of the incidence of BRD (28.57% vs 17.14%) (P ≤0.05), because of a tendency toward a reduction of both the incidence of first cases (13.33 *vs* 19.05 in the Control group) (P=0.08) and in the number of relapses (3.81 *vs* 8.59 in the Control group) (P=0.08).
Conversely, no differences were found in terms of lameness incidence, that was similar in the two groups and in line with a value corresponding to a low incidence [47].
The positive effects on health registered during the overall fattening period in the Treatment group may be justified by a better non-specific immune reactivity and antioxidant status, as highlighted by the immunological results reported in Table 5, especially during the arrival phase, that is the most critical period of fattening cycle, and the one in which most of the health issues are registered.
Specifically, the treatment has led to a higher serum bactericidal activity at d_{45} (92.00 *vs* 80.40 in the Control group) (P≤0.05), resulting significantly above the 90% threshold, a value considered to be the limit for healthy cattle [48]. Moreover, the treatment has led to an improvement in the antioxidant defences, as highlighted by the significantly higher results obtained in terms of antioxidant activity (365.68 *vs* 290.58 µmol HClO/ml in the Control group) (P≤0.05). No difference was found in specific immune response to BHV-1 vaccine, and on γ-interferon and ROMs values.
Even if, also in terms of the evaluation of health and immune parameters, no studies that tested the same combination of natural products are available in bibliography, there are still some evidences related to some of the single compounds. Compiani et al. [28] found that the inclusion of a mixture on natural compounds, also containing eugenol, has led to a significant increase in the bactericidal activity during the first period of the fattening cycle [28]. Indeed, different studies have reported a strong bactericidal activity of clove against different pathogens, including some related to BRD, such as *Pasteurella multocida* [48-49]. Also, Santillo et al. [29] found a positive effect of feeding tannins to lactating diary cows on the main parameters related to the antioxidant status. As in the present study, in which the highest levels reached in the OXY test highlight a better efficiency of the antioxidant defences, Santillo et al. [29] found an increased biological antioxidant potential in the treated animals. The inclusion of tannins in the dairy cows' diet was also able to reduce the presence of ROMs in the serum of treated animals. Due to the low percentage of direct absorption of tannins, the effect on the antioxidant status and antioxidant defences can be explained by a local activity in the gastrointestinal tract, that can lead to an increase in the expression and activity of some endogenous antioxidant enzymes [50-51].

Table 5 - Immunological parameters in the two study groups evaluated at d_0 and d_{45}.

Parameter	Control	Treatment	*P value*
BHV-1 serum neutralization, log(dilution)			
d_0	0.00	0.00	*ns*
d_{45}	0.78	0.78	*ns*
Serum bactericidal activity, %			
d_0	68.60	67.80	*ns*
d_{45}	80.40	92.00	*<0.001*
γ-interferon, pg/ml			
d_0	15.00	15.60	*ns*
d_{45}	14.60	14.80	*ns*
ROM, U/Carr			
d_0	51.64	50.99	*ns*
d_{45}	68.62	68.39	*ns*
OXY, µmol HClO/ml			
d_0	257.52	255.30	*ns*
d_{45}	290.58	356.68	*<0.001*

CONCLUSIONS

The results of the present study highlight that inclusion of a blend of essential oils, bioflavonoids, and tannins in the diet of fattening beef cattle can improve the overall production efficiency. Indeed, this blend of natural product can exert a proactive role on both the ruminal efficiency and health, modulating the fermentation kinetics and increasing thus the synthesis of volatile fatty acids used for energy purpose. Moreover, the inclusion of that blend of natural products can improve the immune functionality and in the antioxidant defenders, factors that may have influenced the health status of the treated animals, leading to a lower incidence of bovine respiratory disease. The combination of those aspects increase the production efficiency of fattening beef cattle, also reducing the need of antimicrobials due to an improved welfare status. On average, the inclusion of this blend of natural products can led to a more sustainable way to raise beef cattle.

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