Effect of diet supplementation with a pool of enzymes and beta-glucans deriving from bacterial and fungal fermentations on digestive efficiency, production performance and health status in fattening beef cattle

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SUMMARY

The aim of the present study was to evaluate the effect of a pool of enzymes and beta-glucans deriving from the fermentation of *Aspergillus oryzae* and *Lactobacillus*, on *in vivo* digestive efficiency, production performances and health status in fattening beef cattle under field practical conditions.

Two specific trials were set up. In the Trial I, 80 newly arrived male Charolaise beef cattle were assigned to two study groups: i) Control (391.23 ± 16.54 kg live weight), basal diet; ii) Treatment (392.54 ± 15.94 kg live weight), basal diet integrated with 5 g/head/d of the pool of enzymes and beta-glucans. The *in vivo* apparent total tract digestibility (aTTD) was evaluated comparing chemical composition of the diets and relative faeces, analysed with a portable NIR instrument, at d_{90} and d_{180} of the 185 days of fattening period. In Trial II, 306 newly arrived male Charolaise beef cattle were assigned to two study groups: i) Control (388.26 ± 17.58 kg live weight), basal diet; ii) Treatment (388.16 ± 15.82 kg live weight), basal diet integrated with 5 g/head/d of the pool of enzymes and beta-glucans. Growth performances, feed intake, feed conversion rate, carcass characteristics and health status were evaluated during the entire fattening period (205 days).

In Trial I, the Treatment has led to a significant improvement in the digestibility of neutral detergent fiber (NDF) (P= 0.0001), cellulose (P=0.02), hemicellulose (P=0.03) and starch (P<0.0001).

In Trial II, the Treatment significantly improved average daily gain (P<0.05), final weight (P=0.005), and feed conversion rate (P<0.0001). Carcass characteristics weren't affected by the Treatment. No significant differences were found between control and treated animals in health status, despite the incidence of bovine respiratory disease (BRD), digestive disorders, and animal moved to the infirmary pen, was, from a veterinary practical point of view, highly lower in treated animals.

In conclusion, supplementation of fattening beef cattle diet with a pool of enzymes and beta-glucans derived from the fermentation of *Aspergillus oryzae* and *Lactobacillus*, improved growth performance and feed efficiency, but need more investigations to better clarify his role on animal welfare.

KEY WORDS

Beef cattle; enzymes; growth performance; feed efficiency.

INTRODUCTION

The zootechnical sector must face, in the next future, several challenges, connected to both the increase in the needs of animal-derived foods as well as to the enhanced awareness of the consumers about animal welfare, antibiotic use and environmental sustainability[1]. Meet those targets is also mandatory considering the newest national and European rules on animal welfare and antimicrobials use in animal farming, and the future allocation of the PAC bonuses in relation to specific environmental targets.

In these directions, animal nutrition can have a proactive role, improving production performance, feed efficiency, and animal welfare, and reducing the risk of pathologies and the environmental impact[2-3]. Nutrition is strictly involved in maintaining a correct and optimal state of animal health. Indeed, digestive disorders (such as acidosis) are one of the leading causes of health impairment and reduction in production efficiency in beef cattle[4]. Moreover, digestive dysmetabolism are often the door opener and the trigger for other health problems, such as lameness, bloat and enterotoxaemia[4].

Thus, maintain a correct rumen functionality during the entire fattening period, starting from the adaptation to the fattening phase, is fundamental in relation to animal welfare and environmental sustainability.

Considering this last aspect, it should be emphasized that methane (CH_4), one of the main "greenhouse gases", is produced inside the rumen and represent a loss of energy of about 6 to 12% of the total energy input[5].

Rumen health and functionality can be safeguard and improved by additives, such as yeasts[6-7] and natural extracts[8-9], able to influence rumen microflora and digestive processes.

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Various microbial derivatives, as enzymes obtained from specific and controlled fermentation processes, have also been used to improve feed efficiency in various livestock species, in particular pigs and poultry[10], while in ruminants the use was limited in the past because of the risk of their inactivation by the rumen hydrolysis[11].
Currently, several studies conducted both in dairy[12] and beef[10-13] cattle have shown that some microbial derivatives, mainly consisting of fibrolytic and amylolytic enzymes, such as xylanase and/or cellulase, and compounds such as beta-glucans, can effectively improve production performance and efficiency, promoting a better ruminal functionality and stability[14-15].
For instance, derivatives of *Aspergillus oryzae* has been shown to stimulate fibre digestion both *in vivo* and *in vitro,* by stabilizing ruminal pH[16]. This condition provides a more favourable environment for the development of selected microbial populations, in particular cellulolytic bacteria and anaerobic fungi[17], increasing the digestibility of fibrous diet's components[14-15], and the flow of microbial protein and volatile fatty acids[18].
The present study aimed to determine, in two separate field trials, whether the diet supplementation with a pool of enzymes and beta-glucans deriving from *Aspergillus oryzae* and *Lactobacillus* fermentations, enhance the production performances, the health status and the digestive efficiency in fattening beef cattle.

MATERIALS AND METHODS

Trial I: Effect of the pool of enzymes and beta-glucans diet supplementation on in vivo digestive efficiency

Animals, housing, and trial groups

The study took place in an intensive beef fattening unit, located in northern Italy (Via Villaraspa 81, 37041 Albaredo d'Adige - VR, Italy), that well-represent the typical intensive beef cattle fattening farms.
A total of 80 male Charolaise beef cattle, imported from France, were enrolled at the arrival (d_0), 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)[19].
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 pool of enzymes an beta-glucans: i) Control (40 heads, 391.23 ± 16.54 kg live weight), basal diet; ii) Treatment (40 heads, 392.54 ± 15.94 kg live weight), basal diet integrated with 5 g/head/d of the pool of enzymes and beta-glucans.
The trial lasted for the entire fattening period (185 days).
The bulls were housed on permanent litter, in a total of 8 pens (4 for each group) with 10 animals each (3.5 m^2 each).

Nutritional management

The two study groups were fed with the same basal diet (Table 1), administered *ad libitum* for the entire fattening period in form of total mixed ration (TMR) 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. The TMR was studied to meet the growth needs, in agreement with the Nutrient Requirement Council [20].
The pool of enzymes and beta-glucans derived from the fermentation of *Aspergillus oryzae* and *Lactobacillus* (B-Fusion, Akron Srl, Corsico - MI, Italy), was provided by including it directly in the mineral mix used in the diet of Treatment group, while, in the mineral mix used in the Control group, a same amount (2.5%) of wheat bran was added.
Water was available *ad libitum*.

Table 1 - Trial I: Diet composition and predicted nutritional value, calculated by the rationing software (Plurimix).

Raw Materials	Kg/head/d
Corn silage 337833	7.0
Corn meal	5.5
Soybean meal 44% CP[1]	1.9
Beet pulp	0.7
Wheat straw	0.7
Mineral mix	0.2
Nutritional values, % on dry matter	
Total, kg as fed	16.00
Total, kg d.m.[2]	10.27
d.m., %	64.20
UFV/ kg d.m.	1.05
CP	14.77
Sugars	2.81
Starch	43.05
NDF[3]	27.38
ADF[4]	14.69
Lignin	2.60
CF[5]	3.51
Ash	4.95
Ca tot	0.62
P tot	0.32

[1] CP= crude protein; [2] d.m.= dry matter; [3] NDF= neutral detergent fiber; [4] Acid detergent fiber; [5]CF= crude fats.

Parameters recorded

Characteristics of the diets, faeces and apparent total tract digestibility

The characteristics of both diets and faeces were monitored at d_{90} and d_{180} of the fattening period, using a portable NIR instrument (Polispec, ITPhotonics, Via Astico, 39, 36030 Fara Vicentino -VI, Italy).
Immediately after unloading, the TMR of each pen were analysed for both Control and Treatment groups, carrying out 3 measurements along the length of the feed bunk (beginning, middle, and end), and considering the average of those 3 measurements as the reference value for each pen.

The characteristics of the faeces were analysed, in both groups, on a pool of faeces for each pen, the day after (d_{91} and d_{181}) each TMR analyses.
Each pool was made using the faeces collected directly by rectal grab from each of the 10 bulls present in each pen. Three measurements were made on each pool and the average of those 3 measurements was considered as the reference value for each pen.
The characteristics analysed by the portable NIR instrument were, dry matter (d.m.), crude protein (CP), crude fats (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), starch and ash. The hemicelluloses were obtained from the difference NDF - ADF, cellulose from the difference ADF - ADL, while sugars and pectin by calculation: 100 -(ash+fats+proteins+NDF+starch).
The apparent total tract digestibility (aTTD), for Control and Treatment group, was calculated through the following formula:

$$aTTD\ \% = \frac{\left(\frac{Xd}{ADLd}\right) - \left(\frac{Xf}{ADLf}\right)}{\left(\frac{Xd}{ADLd}\right)} \ x\ 100$$

where:
X= each analytical parameter considered (%)
ADL= acid detergent lignin (%)
d= diet
f= faeces.

Statistical analysis

The statistical analysis of the data was conducted using the SAS statistical software (SAS 9.4, SAS Cary NC).
The pen was used as experimental unit. The aTTD data were analysed using a mixed model (PROC MIXED) which took into account the fixed effect of the treatment, the time of detection and the random effect of the pen.
The difference was considered significant for $P \leq 0.05$.

Trial II: Effect of the pool of enzymes and beta-glucans diet supplementation on growth performance and health 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 intensive beef cattle fattening farms.
A total of 306 male Charolaise beef cattle, imported from France, were enrolled at the arrival (d_0), and following the procedures described for Trial I, individually weighed, evaluated for body conformation and then assigned to two balanced groups: i) Control (153 heads, 388.26 ± 17.58 kg live weight), basal diet; ii) Treatment (153 heads, 388.16 ± 15.82 kg live weight), basal diet integrated with 5 g/head/d of the pool of enzymes and beta-glucans.
The trial lasted for the entire fattening period (205 days).
The bulls were housed on permanent litter in a close barn, in pens with 10-11 animals each (3.5 m^2 each).

Table 2 - Trial II: Diet composition and predicted nutritional value, calculated by the rationing software (Plurimix).

Raw Materials	Kg/head/d
Corn silage 347532	7.0
Corn meal	5.5
Sunflower meal 28% CP[1]	1.2
Soybean meal 44% CP[1]	0.8
Beet pulp	0.7
Wheat straw	0.7
Mineral mix	0.2
Nutritional values, % on dry matter	
Total, kg as fed	16.10
Total, kg d.m.[2]	10.47
d.m., %	65.10
UFV/ kg d.m.	1.00
CP	13.19
Sugars	2.31
Starch	41.68
NDF[3]	29.29
ADF[4]	17.10
Lignin	3.39
CF[5]	3.06
Ca tot	0.61
P tot	0.34

[1] CP= crude protein; [2] d.m.= dry matter; [3] NDF= neutral detergent fiber; [4] Acid detergent fiber, [5]CF=crude fats

Nutritional management

Feeding management for Control and Treatment groups (Table 2), and enzymes and beta-glucans (B-Fusion, Akron Srl, Corsico - MI, Italy), supplementation, were done following the procedures described for Trial 1.

Parameters recorded

Growth and slaughtering performances

Individual body weight was recorded before morning feeding at three timepoints, enrolment day (d_0), day 90 (d_{90}) and before slaughter (d_{205}). The individual average daily gain (ADG) was then calculated for each period, from d_0 to d_{90}, from d_{90} to d_{205} and from d_0 to d_{205}, 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
Weekly, the daily TMR intake of each pen was evaluated by weighing the TMR administered and the residue in the

manger 24 h later. Feed conversion rate (FCR) was then calculated, comparing the average TMR intake of each pen from d_0 to d_{205}, with the average daily gain of the corresponding pen in the same period.

At slaughter, cold carcass weight, dressing percentage, carcass conformation and fattening (SEUROP) scores were collected for all animals. Cold carcass weight was recorded after 24 h of chilling at a temperature of 0°C to 4°C. Dressing percentage was obtained by comparing the cold carcass weight with the final live weight.

Carcass conformation and fattening scores were assessed by an expert judge, according to EU legislation (Council Regulation EEC n. 1026/91, 22 April 1991), 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).

Health status

During the entire fattening period, individual health conditions were evaluated twice a day by the veterinary and qualified animal health care staff of the farm.

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), acidosis and lameness. 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)[21]. Sick animals were treated according to the procedures, medications, and sanitary protocols adopted by the farm veterinary staff.

Statistical analysis

The data analysis 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 took into account 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 took into account the fixed effect of the treatment.

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 $P \leq 0.05$.

RESULTS AND DISCUSSION

Trial I: Effect of the pool of enzymes and beta-glucans diet supplementation on in vivo digestive efficiency

Characteristics of the diets, faeces and apparent total tract digestibility

The average values of the TMR chemical characteristics at the two different timepoints are shown in Table 3. The data highlight a good correspondence between the projection of the rationing software and the analytical results. Table 4 summarize the chemical characteristics of the faeces of both study groups, in the same timepoints.

Table 3 - Trial I: Chemical composition of the TMR in the two study groups in the two different timepoints, done with the portable NIR instrument Polispec.

Parameter	Control		Treatment	
	d90	d180	d90	d180
Humidity, %	34.90	34.30	34.38	34.45
Dry matter (d.m.), %	65.10	65.70	65.63	65.55
Ash, % d.m	4.90	4.82	4.66	4.98
Crude protein, % d.m.	14.61	14.82	14.17	14.03
Crude fats, % d.m.	3.64	3.60	3.58	3.43
NDF, % d.m.	28.84	28.92	27.44	27.68
Cellulose, % d.m.	13.01	13.21	11.53	11.86
Lignin, % d.m.	2.66	2.64	2.63	2.65
Hemicellulose, % d.m.	13.17	13.08	13.18	13.18
Starch, % d.m.	42.01	42.25	44.42	44.64
Sugars and Pectins, % d.m.	6.00	5.59	5.73	5.26

Table 4 - Trial I: Chemical composition of the feces in the two study groups in the two different timepoints, done with the portable NIR instrument Polispec.

Parameter	Control		Treatment	
	d91	d181	d91	d181
Humidity, %	79.20	79.27	79.10	79.20
Dry matter (d.m.), %	20.80	20.73	20.90	20.80
Ash, % d.m	6.24	6.90	6.23	7.22
Crude protein, % d.m.	12.37	14.25	13.33	14.45
Crude fats, % d.m.	5.25	5.96	5.45	5.60
NDF, % d.m.	62.41	65.33	60.27	62.53
Cellulose, % d.m.	31.56	32.54	28.17	29.33
Lignin, % d.m.	13.20	14.64	13.49	14.65
Hemicellulose, % d.m.	17.65	18.15	18.61	18.56
Starch, % d.m.	11.16	4.76	11.84	7.55
Sugars and Pectins, % d.m.	2.57	2.80	2.89	2.65

Table 5 - Trial I: Digestibility values in the two study groups.

Day	d90	d180	Average	P(g)[1]	P(d)[1]	P(g*d)[1]
Ash, %						
Control	74.31	73.86	74.09	0.695	0.414	0.914
Treatment	74.14	73.54	73.84			
Sem	*0.876*	*0.876*	*0.6194*			
P-value	*0.839*	*0.724*	*0.695*			
Protein, %						
Control	82.90	81.68	82.29	0.328	0.001	0.862
Treatment	82.68	81.36	82.02			
Sem	*0.422*	*0.422*	*0.299*			
P-value	*0.6156*	*0.458*	*0.382*			
Fats, %						
Control	70.91	70.39	70.64	0.686	0.922	0.586
Treatment	70.14	70.50	70.32			
Sem	*1.113*	*1.113*	*0.787*			
P-value	*0.504*	*0.919*	*0.686*			
NDF, %						
Control	56.35	57.33	56.84	0.0001	0.356	0.157
Treatment	59.19	59.97	59.08			
Sem	*0.561*	*0.561*	*0.396*			
P-value	*0.0003*	*0.012*	*0.0001*			
Cellulose, %						
Control	50.81	52.45	51.63	0.02	0.394	0.577
Treatment	54.41	54.76	54.58			
Sem	*1.587*	*1.587*	*1.122*			
P-value	*0.044*	*0.174*	*0.023*			
Hemicellulose, %						
Control	72.94	72.54	72.74	0.030	0.512	0.863
Treatment	75.07	74.38	74.73			
Sem	*1.142*	*1.142*	*0.807*			
P-value	*0.087*	*0.132*	*0.030*			
Sugars and Pectins, %						
Control	91.37	90.21	90.76	0.819	0.529	0.725
Treatment	91.23	90.89	91.06			
Sem	*1.634*	*1.634*	*1.155*			
P-value	*0.930*	*0.683*	*0.819*			
Starch, %						
Control	93.66	94.81	94.23	<0.0001	0.833	0.0017
Treatment	97.98	96.94	97.45			
Sem	*0.387*	*0.387*	*0.273*			
P-value	*<0.0001*	*0.0001*	*<0.0001*			

[1] g = group; d = day; g*d = group*day.

Table 5 shows the digestibility values of the different nutritional parameters in both experimental groups, and in the different timepoints of the study.

The administration of the pool of enzymes and beta-glucans, derived from *Aspergillus oryzae*, and *Lactobacillus*, has led to a significant improvement in the aTTD of NDF (59.08 *vs* 56.84% in the Control group) (*P*=0.0001), that is equal to a 3.94% increase. Specifically, the Treatment has led to a significant improvement of 5.71% in the aTTD of cellulose (54.58 *vs* 51.63% in the Control group) (*P*=0.02), the more slowly degradable component of the NDF, and of 2.73% in the aTTD of hemicellulose (74.73 *vs* 72.74% in the Control group) (*P*=0.03). Moreover, the starch aTTD resulted to be increase by the Treatment (97.45 *vs* 94.23% in the Control) (*P*<0.0001), with a 3.41% growth.

The results of the present study suggest a positive role of enzymes and beta-glucans deriving from bacterial and fungal fermentations, in improving the activity of cellulolytic and amylolytic bacteria. Those results agreed with previous findings of studies related to the use of different enzymatic pools, done either *in vitro* and *in vivo*, on diet digestibility in beef and dairy cattle[10-22-23-24-25]. Specifically, Loureco et al. (2020), in an *in vitro* study with the inclusion of enzymes on standard beef cattle diets, has highlighted a significant increase in the digestibility of the fibrous fractions[10]. Despite Hristov et al. (1998)[26], didn't report significant differences in digestibility in dairy cows fed high grain diets and supplemented with a pool of amylolytic and fibrolytic enzymes, DeFrain et al. (2005)[23], and Tricarico et al. (2005)[24], on the other hand, showed a significant increase in the proportions of acetate and propionate in beef and dairy cattle supplemented with similar enzymatic pools, reflecting a greater ruminal fermentation activity. Also, Eun and Beauchemin (2005)[25], showed, *in vivo*, an increase in the overall digestibility of the various fibrous fractions in diets for dairy cows supplemented with enzymatic pools, in particular when the diets were characterized by a low level of inclusion of fibrous forages or feeds. Similarly, also in beef cattle the inclusion of fibrolytic enzymes has led to an improvement in the overall diet digestibility, as a consequence of an optimized ruminal functionality[22].

Those results are of fundamental importance specifically in fattening diets characterized by a high content of starch and a low amount of fibre, moreover represented mainly by straw.

Beauchemin et al. (2010)[27], highlight that the inclusion of fibrolytic enzymes in ruminants' diets allows to maximize the cellulolytic bacteria activity and the fermentation of the fibrous component, ensuring rumen optimal pH conditions and functionality.

Regarding the improvement in starch digestibility, the results agreed with the findings of Paloheimo et al. (2010)[28], that highlighted an increase in the development and activity of amylolytic bacteria in ruminants supplemented with enzymatic pool.

Trial II: Effect of the pool of enzymes and beta-glucans diet supplementation on growth performance and health status

Growth and slaughtering performances

Data related to growth performances are reported in Table 6.

The Treatment has led to a significant improvement in the growth performances both considering the hole fattening period and the intermediate timepoint. Specifically, the inclusion of the pool of enzymes and beta-glucans enhanced the ADG of 86 g/head/d compared to Control group (*P*=0.0003), that is equal to a 5.35% increase. Considering the intermediate timepoint, the ADG was 83 g/head/d (*P*=0.0042) and 84 g/head/d (*P*=0.025) higher respectively in the first (d_{0-90}) and in the following period (d_{91-205}) in the Treatment group.

As a result, also the final weight (725.25 *vs* 709.09 kg in the Control group) (*P*=0.005) resulted to be statistically higher in the Treatment group.

Table 6 - Trial II: Growth performance in the two study groups.

Parameter	Groups		SED	*P-value*
	Control	Treatment		
Weight, kg				
Weight d_0	388.42	386.16	5.74	0.695
Weight d_{90}	538.54	545.15	5.74	0.251
Weight d_{205}	709.09	725.25	5.74	0.005
P(g)[1]	0.041			
P(d)	<0.0001			
P(g*d)	0.079			
ADG[2], kg/head/day				
ADG_{0-205}	1.571	1.657	0.025	0.0003
ADG_{0-90}	1.670	1.753	0.036	0.0042
ADG_{90-205}	1.484	1.568	0.036	0.0225
P(g)	0.0003			
P(d)	<0.0001			
P(g*d)	0.663			
Feed intake kg d.m./day				
$Intake_{0-205}$	11.77	11.57	0.166	0.239
FCR[3]				
FCR_{0-205}	7.51	7.02	0.079	<0.0001

[1] g=group, d=day, g*d=group*day. [3] ADG= average daily gain. [4] FCR= feed conversion rate.

The better growth performances are supported by a significant improvement in the feed conversion rate (FCR) (P <0.0001), found in the Treatment group, demonstrating a higher efficiency of nutrient utilization.
Feed intake was statistically unchanged in the two groups.
These results agree with the findings of Neumann et al. (2018)[29] which, in an *in vivo* study on fibrolytic and amylolytic enzymes in the diet of fattening Angus cattle, found an overall improvement in growth performance, attributed to greater feed efficiency. Also, Beauchemin et al (1999)[30], in a previous *in vivo* study in fattening beef cattle, highlighted a growth improvement, attributable to a better feed conversion rate and nutrients utilization efficiency at ruminal level.
Data related to slaughtering performance are reported in Table 7. No significant differences have been found between the two study groups. Conversely, Neumann et al. (2018)[29], have found a significant improvement in marbling and *Longissimus dorsi* area in beef cattle supplemented with a pool of fibrolytic and amylolytic enzymes.

Health status

Data about mortality and incidence of the different pathologies are reported in Table 8. No statistically significant differences emerged between the two groups. However, the results are positive and interesting from a practical point of view. Indeed, the inclusion of the pool of enzymes and beta-glucans in the TMR has led to a numerical reduction of all the health problems.
Those result can be related to the improvement in ruminal activity and efficiency, highlighted both in Trial I, by a better digestibility of NDF and its fractions, and in the present one, by the higher growth performances, observed in the Treatment group. Specifically, the lower incidence of bloat and lameness can be explained by a reduction of the risk of acidosis, due to a higher and more stable ruminal pH promoted by the improved activity of cellulolytic bacteria[31].
It is recognized that all fermentation patterns are positively influenced by a more stable ruminal environment, resulting in higher volatile fatty acids production and absorption, and, consequently, in better growth performance and health conditions[32].

CONCLUSIONS

The results of the present study, composed of two separated trials, have highlighted that the inclusion of the pool of enzymes and beta-glucans, deriving from the fermentation of *Aspergillus oryzae* and *Lactobacillus*, improve growth performance and feed efficiency. The improvement found in NDF and starch digestibility seems to promote a higher stability of rumen environment with positive effects on fermentation processes and nutrients absorption. The lower incidence and severity of health problems found in the Treatment group, even if important from a practical point of view, didn't reach the statistical significance. More investigations are needed to better clarify the role of enzymes and beta-glucans diet supplementation on beef cattle health status and welfare. Enzymes and beta-glucans can also have an interesting role in terms of environmental sustainability purposes, reducing the impact related to the production of 1 kg of meat by increasing feed efficiency, digestibility, and growth performance.

Table 7 - Trial II: Slaughtering performances in the two study groups.

Parameter	Groups		SED	*P-value*
	Control	Treatment		
CCW[1] weight, kg	422.65	424.30	5.355	0.759
Yield %	59.25	59.31	0.116	0.625
SEUROP Classification				
% carcasses conformation U	20.26	19.61	-	0.886
% carcasses conformation E	79.74	80.39	-	0.886
% carcasses fattening 2	33.99	34.64	-	0.904
% carcasses fattening 3	66.01	65.36	-	0.904

[1] CCW= cold carcass weight.

Table 8 - Trial II: Health status in the two study groups.

Parameter	Groups		*P-value*
	Control	Treatment	
BRD[1], % (n)	17.65 (27)	11.76 (18)	0.146
Lameness, % (n)	1.98 (3)	0.65 (1)	0.314
Digestive disorders - bloat, % (n)	1.307 (2)	0.00 (0)	0.155
Mortality, % (n)	1.307 (2)	0.654 (1)	0.561
Infirmary, % (n)	4.575 (7)	1.961 (3)	0.886

[1] BRD= bovine respiratory disease.

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