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# YEAST CELL WALLS AND LIVE YEASTS DIET SUPPLEMENTATION IN BEEF CATTLE: EFFECT ON RUMEN FERMENTATIONS AND ULTRASONOGRAPHIC RUMEN WALL FINDINGS

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## 1. ABSTRACT

The objective of this thesis was to determine the effect of yeast supplements on the performance and health of beef cattle during the receiving and finishing period. Furthermore, another aim of this study was to evaluate the applicability of rumenocentesis on beef cattle and the viability of transabdominal ultrasonography of the rumen mucosa as a suitable, non-invasive diagnostic tool to identify beef cattle affected by SARA. Three trails were conducted in order to do achieve that. Trail I and Trail II were conducted simultaneously using the same animals, while the third trail used a larger sample but always in the same herd. Trail I and II assessed the effect of dietary supplementation of yeast cell wall (YCW) and live cell yeast (LY) at different dosages on rumen's metabolites. Sixty Charolaise steers were divided into two groups on the basis of their feeding phase: growing and finishing. Growing and finishing groups were each randomly divided into equal three subgroups (n = 10): no supplement (growing control), supplemented with YCW, and supplemented with LY + YCW, no supplement (finishing control), supplemented with LY and supplemented with LY + YCW. Ruminal fluid has been collected before, after 21 and 42 days of experimental period in order to evaluate the volatile fatty acids concentrations and pH values. Faeces samples were collected before (T0), after 21 (T1) and 42 (T2) days of the start of the study, which took place simultaneously and analysed for dry matter (DM), Ash, crude protein (CP), ethereal extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and Starch detection. Dietary supplementation of LY and YCW increased ($P<0.05$) DM, ADF and ADL faecal concentrations in the growing phase; DM, ADL and Starch faecal concentrations in the finishing

phase. T2/FC Diet showed a significant effect ($P$<0.001) of different diets respect to T2/FB in the finishing stage. The obtained results suggest that yeast supplementation do not have beneficial effects with all type of diet condition. Statistical analysis of VFA's data showed a significant effect of time ($P$ < .05) on all studied parameters except iso-valeric acid both in growing and finishing groups. Changes among growing subgroups ($P$ < .05) on propionic acid, acetic acid, iso-butyric acid and n-butyric acid were found, whereas no statistical significances were found among finishing subgroups. Trail III was conducted on 478 beef cattle of Charolaise breed, they were monitored three times during the livestock cycle in order to evaluating the rumen fluid pH and to assess the measures of the rumen wall: T0: 5±3 d after the arrival in farm;T1: 60±10 d after arrival; T2: 1 month before slaughter. Period effect ($P$<0.001) were found between the three periods after 10 days from the housing, rumen pH values were lower than the threshold value of 5.71 in T0 than in T1 and T2. Pearson's analysis showed interaction between pH and total ultrasound thickness of rumen wall (-0.700; $P$<0.0001) and rumen mucosa (-0.7921; $P$<0.0001). Both differentiation efficiency of mucosal and submucosal layer thickness and rumen wall thickness between healthy and ruminal acidosis affected animals, as a result of ROC curve analysis, was excellent. Using a cut-off value of 5.4 mm, sensitivity was 96.30% and specificity was 91.60% on mucosal and submucosal layer. Using a cut-off value of 8.2 mm, sensitivity was 91.36% and specificity was 91.60 % on rumen wall thickness.

The study show that transabdominal ultrasonography of the rumen mucosa has the potential to be a suitable diagnostic tool to identify fattening bulls affected by SARA.

**KEYWORDS**

Beef cattle; live cell yeast; yeast cell wall; ruminal fermentation; ruminal pH; rumen thickness ultrasound

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## LIST OF ABBREVIATIONS

ABWG average body weight gain

ADF acid detergent fibre

ADG average daily gain

ADL acid detergent lignin

BRD bovine respiratory disease

BW body weight

Cd caudal

CFU colony-forming unit

CP crude protein

Cr cranial

DFM direct fed microbial

DM dry matter

DMI dry matter intake

EE ethereal extract

FC finishing control

GC growing control

LY live yeast

LY+YCW live yeast+yeast cell wall

M medial

NDF neutral detergent fibre

SD standard deviation

TNF-α tumor necrosis factor-α

YCW yeast cell wall

# 2. INTRODUCTION

## 2.1 Receiving and finishing periods in beef cattle

The breeding of beef cattle is based on the constant pursuit of maximization of growth performance with the aim to reduce the time of spent by cattle breeding, increase the number of animals raised for year, limiting the impact of fixed costs on the production cost of each piece, increase corporate profitability and of course the production of animals in excellent of fat cover; in this respect are very important factors management food (Sgoifo Rossi et al., 2009).

The intensive production system in the livestock breeding of beef cattle is common used in Europe and in particular in Italy. Generally, the breeding method includes the importation of beef cattle of 10-15 months age and 350-400 kg weighting; those are taken to the finishing stage and slaughter.

The receiving and the finishing periods are ones of the most important phases of beef production. Those periods are a crucial time in which proper management can substantially increase efficiency of production and profitability. During the receiving period, cattle experience stress from many different sources such as weaning, transportation, feed and water deprivation, commingling, exposure to new pathogens that alter the physiological homeostasis impairing the health status (Stanton et al., 2010). As a result of this stress, cattle immunity can be compromised (Blecha et al., 1984), and performance suffers, an animal which undergoes a kind of stress condition can be find in a state of immunosuppression (Smith, 2004), reduction of motility intestinal absorption alteration of important nutrients, impaired ruminal

fermentation capacity, increased nutritional requirements, increased renal elimination of essential trace minerals.

Bovine respiratory disease (BRD) is the most common cause of cattle morbidity and mortality during the receiving period. Buhman et al. (2000) reported that most cattle are treated for BRD by day 27 of the receiving period. Treatment for BRD was consistently associated with decreased performance (Schneider et al., 2009; Bateman et al., 1990; and Gardner et al., 1999).

During the finishing period animals are fed an energy-dense diet so that they will grow rapidly and add muscle/meat to their frame and optimise fat cover in preparation for slaughter. High level of concentrate can cause metabolic disorders such as acidosis (Owens et al., 1998) and bloat (Cheng et al., 1998). These metabolic disorders are caused by the inability of ruminal microorganism to properly utilize readily fermentable carbohydrates (Brown et al., 2006). Cerchiaro et al. (2005) found lameness as one of the most important problems of beefs housed intensively, being the main causes of injury and death (about 42% of incidence). According to Blowey (1993), lameness has resulted to be especially due to environmental and/or nutritional causes.

In fact, digestive disorders, including ruminal acidosis, are second only to respiratory diseases in depressing animal performance and production efficiency (Nagaraja et al., 1998).

### 2.2 Ruminal Acidosis in Beef Cattle

Ruminal acidosis is a metabolic status defined by decreased blood pH and bicarbonate, caused by overproduction of ruminal D-lactate. It happens when animals ingest excessive amount of non-structural carbohydrates with low

neutral detergent fiber. Animals will show ruminal hypotony/atony with hydrorumen and a typical parakeratosis-rumenitis liver abscess complex, associated with a plethora of systemic manifestations such as diarrhea and dehydration, liver abscesses, infections of the lung, the heart, and/or the kidney, and laminitis, as well as neurologic symptoms due to both cerebrocortical necrosis and the direct effect of D-lactate on neurons (Hernandez et al., 2014).

Sub-acute ruminal acidosis (SARA), also known as chronic or sub-clinical acidosis, is a well-recognized digestive disorder that is frequently encountered in ruminants on high-concentrate rations and it is a condition characterized by transitory but recurrent periods of mild to moderate rumen pH depression. SARA is a disorder of ruminal fermentation that is characterized by extended periods of depressed ruminal pH below 5.5-5.6. Although SARA is commonly under-diagnosed because of lack of pathognomonic signs, diurnal fluctuations in rumen pH, and problems obtaining representative rumen fluid samples (Jorgenson et al., 1993; Nordlund and Garrett, 1994) it causes significant economic losses.

The economic impact of this condition on dairy industry has been estimated far above $500 million annually in the USA alone, these losses are mainly the result of reduced milk production, decreased efficiency of milk production, premature culling and increased death loss (Enemark, 2008).

Rumen pH is the most commonly used parameter for detecting subacute ruminal acidosis (SARA) and it can be analyzed with different methods. Rumenocentesis has become defined as an efficient large-scale diagnostic test for detecting SARA in samples of more than 100 dairy cows (Morgante et al., 2007; O'Grady et al., 2008) and avoids the sample contamination by saliva

which can occur when rumen fluid is sampled via the oesophagus (Nordlund, 2003). Moreover this technique has been demonstrated to have minimal adverse effects on health and production on dairy cattle (Gianesella et al., 2010).

Morphological changes in ruminal mucosa in response to volatile fatty acids (VFA) concentration and rumen fluid pH are well established; rumenitis is a frequent sequel to rumen acidosis; an increased production of volatile fatty acids, particularly butyrate and propionate, as well as a temporary rise in the ruminal lactate concentration and fluctuations in the osmolality of the rumen fluid lead to this condition (Enemark, 2008). The stage between parakeratosis (thickening of the stratum cornea of the rumen mucosa) and rumenitis appears undefined (Dirksen, 1985).

### 2.3 Direct fed Microbials Diet Supplementation

Management strategies must be put in place to ease receiving and finishing periods, improve and maintain the health status of fattening bulls, and increase profitability.

Antibiotics were widely used for treatment of sick individuals or groups of at risk cattle for prevention of sickness and improved performance. The use of these antibiotics has contributed to an efficient and profitable feeding industry. In spite of that, a growing concern over antibiotic resistance has resulted in a movement to limit use of antibiotics in livestock management and nutrition. The European Union banned the use of these supplemental anti-microbials in the diet because the daily feeding of various antibiotics to livestock species entering the food supply will lead to a development of antibiotic resistant pathogens. Therefore it

creates a market for other natural nutritional supplements. Bacterial direct fed microbials (DFM) are live, naturally occurring microorganisms that are supplemented to animals to improve digestive tract performance and health (Yoon and Stern, 1995). Eicher et al. (2010) reported that dietary supplements can alter the immune system and assist calves during the receiving period when stress is typically high.

Bacterial DFM are the most beneficial in terms of enhancing performance when fed in the first 14 d of the receiving period for stressed beef cattle (Crawford et al., 1980; Hutcheson et al., 1980). However, bacterial DFM have also been shown to be beneficial when fed daily for the duration of the feeding period (Galyean et al., 2000; Rust et al., 2000). Improvements in feed efficiency were observed by Swinney-Floyd (1999) for cattle supplemented with a combination of *L. acidophilus* and *P. freudenreichii*. Galyean et al. (2000) conducted a feeding trial to determine the effects of three different combinations *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on performance and carcass composition. Steer BW was higher for all DFM treatment groups compared to control ($P$ = 0.04). A trend for improvement ($P$ = 0.06) in cumulative DMI (d 0 to end) was also observed for all DFM groups compared to control, and feed efficiency was positively influenced at various time points. In terms of carcass characteristics, hot carcass weight was positively influenced by DFM supplementation ($P$ = 0.05), but no other differences in carcass quality were observed.

Supplementation of yeast and yeast culture products during the receiving period has been shown to positively influence the performance of cattle during the receiving period (Phillips and VonTungeln, 1985; Mir and Mir, 1994). Phillips

and VonTungeln (1985) observed increases in DMI and ADG for cattle fed yeast culture in the receiving period; however, statically significant results were only observed in 2 out of 4 trials.

The effects of specific yeast culture preparations on rumen environment and performance of ruminants have been well documented and have generated considerable scientific interest over the last two decades (Denev et al., 2007).

Yeast supplements used in livestock production consist of live yeast (*Saccharomyces cerevisiae*) and yeast cell wall (mannan-oligosaccaride) supplements. *Saccharomyces cerevisiae* is a probiotic yeast and it has beneficial effects on animal growth, host immune function, and inhibition of pathogen adhesion (Jurgens et al., 1997; Perez-Sotelo et al., 2005).

In dairy cattle, supplementation with yeast and/or yeast cell wall components has been associated with reduction of negative impact of heat stress on cattle that has improved milk yield, enhanced immune status, and reduced incidence of mastitis and somatic cell counts (Nocek et al., 2011; Liu et al., 2014).

Live yeast supplements work to stimulate the growth of cellulolytic bacteria in the rumen and increase the flow of microbial protein to the small intestine (Wallace and Newbold, 1992; Mould et al., 1983). This results in improvements in feed intake, efficiency, and gain. The cell wall portion of a yeast cell and yeast cell wall supplements consists of mannan and β-glucan components (Kogan and Kocher, 2007). The mannan component of the cell wall has the ability to bind and clear pathogenic bacteria from the gut (Spring et al., 2000) while β-glucans have the ability to stimulate the immune system (Ganter et al., 2003). Overall, the actions of yeast supplements work to improve ruminal fermentation and improve digestive tract health.

Yeast supplements used in animal production can typically be classified into two different forms: live yeast (viable, living cell), yeast cell wall (cell wall structure removed from the yeast cell, non-viable).

### 2.3.1 Live Yeast

Live yeast (*Saccharomyces cerevisiae*) cells are viable, living cells that are composed of an inter-cellular and extra-cellular (cell wall) matrix.

Live yeast (LY) preparations are probiotics rich in enzymes, vitamins, nutrients and co-factors, while yeast cell walls (YCWs) are largely made up of the hydrophilic polysaccharide beta-glucan. They can be beneficial to ruminant species by enhancing ruminal fermentation, as well as provide immune support in the gut (Wallace and Newbold, 1992), by the way gut immunity responses are due to yeast cell wall portion of the yeast molecule.

Wallace and Newbold (1992) suggest that live yeast additives supplementation causes a slower release of oligosaccharides, which results in decreases in lactate production, increasing the pH of ruminal contents. Higher pH conditions within the rumen provide a more ideal environment for the growth and function of beneficial ruminal bacteria, causing an increased rate of fiber digestion, as well as increased flow of microbial protein to the intestine. Increases in fiber digestion and microbial protein typically increase feed intake, resulting in increased growth rates or milk production.

Molecular oxygen present within the ruminal environment is more toxic to lactic acid utilizing bacteria (*F. succinogenes*) than other lactate producing bacterial species (Marounek and Wallace, 1994). Traces of oxygen can be toxic to lactate utilizing bacteria, resulting in a buildup of lactic acid, which decreases

pH, resulting in unfavorable conditions for the growth of bacteria (Van Soest., 1994). Yeast molecules are oxygen scavengers (Marden et al., 2005), therefore can reduce the potential negative effects oxygen being present in the rumen.

There are many suggested theories on the mode of which live yeast stimulates fiber digestion and alters ruminal fermentation. Mould et al. (1983) suggests that yeast molecules remove sugars from the rumen environment and that can suppress the function of cellulolytic bacteria. Wallace and Newbold (1992) propose that yeast cells contain, or can produce, chemical compounds that directly stimulate the growth of cellulolytic bacteria. Altered portions of volatile fatty acid and methane production can influence productivity, resulting from the ability of yeast additives to positively influence propionogenesis while suppressing methane production similar to the ionophores (Bergen and Bates, 1984).

Several studies (Bach et al., 2007; Kowalik et al., 2011; Ferraretto et al., 2012) have demonstrated the effects of dietary YC supplements on ruminal pH, ammonia-N and volatile fatty acid (VFA) patterns, reducing lactate accumulation and the concentration of oxygen in the rumen fluid and improving utilization of starch.

However, the efficacy of yeast cells supplementation on ruminant diets depends on the dose of yeast administered and the diet composition (López-Soto et al., 2013).

Yeast cultures stabilize ruminal pH, provide a more ideal environment for the growth of beneficial rumen bacteria, and improve the digestibility of cellulose. The ability of yeast to remove oxygen from the rumen increases propionate production by promoting the growth of lactate utilizing bacteria. Alerted effects

of yeast supplements on ruminal fermentation can improve animal intake and ultimately increase growth.

### 2.3.2 Yeast Cell Wall

Yeast cell wall (YCW) are feed additives containing mannanoligosaccharides, they are made by (1→3)-β-D-glucan, (1→6)-β-D-glucan, chitin, and mannan proteins (Kollar et al., 1997). The cell wall of a viable yeast cell as well as extracted yeast cell wall supplements work similarly to improve health and performance. The mode of action of yeast cell wall additives can be determinate specifically along with the function of each of its two components: mannans and β-glucans (Kogan and Kocher, 2007).

Mannan components of the cell wall improve the overall health of the digestive tract by binding and eliminating pathogens from gut, thus preventing pathogens from attaching to the gut wall, causing diarrhea (Timmerman et al., 2005). Mannan components of the yeast cell wall are able to bind to pathogens having type 1 receptors and clear them from the digestive system thus keeping them from colonizing on the gut wall (Spring et al., 2000). Mannans have also been shown to improve immunity function through the stimulation of anti-mannan antibodies (Srinivasan et al., 1999), which can provide protection against pathogen invasion.

Beta-glucan structures can have direct effects on the immune system resulting from the enhancement of innate immune cells (Ganter et al., 2003). Underhill and Ozinsky (2002) indicated that β-glucans are able activate the binding site for potential pathogens, resulting in greater aggression of the innate immune system in the engulfing and killing of pathogens. Simultaneously, cytokine

production by innate immune cells is increased, causing increased white blood cell counts (Ganter et al., 2003). Interleukin-6 and interleukin-8 mRNA levels were up-regulated in presence of killed *Saccaromices cerevisiae*, probably as a result of yeast cell wall structures such as β-glucans (Sonck et al., 2010). Interleukins 1 and 6, TNF-α, interferons, and other molecules contribute to the acute-phase immune response and can help initiate the development of specific immune responses.

The cell wall structure of both live yeast molecules and yeast cell wall extracts has been shown to stimulate the immune system and enhance the clearance of pathogens. Mannan components of the cell wall are able to bind pathogens within the gastrointestinal tract, preventing them from colonizing on the gut wall. The β-glucan components are able to stimulate the innate immune system, thus improving immune function. Overall, yeast cell wall supplementation improves total digestive tract health.

### 2.4 Rumen Wall Ultrasonography

Currently, there is no reliable, simple and non-invasive or minimally invasive diagnostic procedure to identify animals affected by acidosis that is suitable under field conditions.

Many cases of SARA may not be detected, as the current field diagnosis of SARA is not clearly defined and depend either on point ruminal pH measurements, which are invasive and due to fluctuations in pH not very accurate or sensitive for the diagnosis of a longer lasting pH depression indicative of SARA, or on continuous measurements which require costly equipment, and are primarily suited for research purposes (Plaizer et al., 2008).

Additionally, some studies suggest that a ruminal pH depression alone is not enough to result in the clinical signs related to SARA (Khafipour et al., 2009)

Ultrasonography has become widely available diagnostic tool in daily animal practice. Specifically for the diagnosis of gastrointestinal disorders, ultrasonography became a widely available and valuable diagnostic procedure in ruminants in farm animal practice (Braun 2003; 2009).

In other species, high-resolution sonomorphologic examination of the bowel allowing to identify different layers of the bowel wall and thereby to diagnose even mild inflammatory bowel disease has become standard diagnostic procedure over the last decades (Hollerbach et al., 1998; Kuzmich et al., 2009).

Braun et al. (2013) described ultrasonographic findings of the rumen in 45 healthy dairy cows. They found that the ruminal dorsal gas cap, characterised ultrasonographically by typical reverberation artifacts, was visible in all cows from the 12th intercostal space to the caudal flank. It was largest at the 12th intercostal space (20.5 ± 7.03 cm). The transition from the gas cap to the fibre mat was marked by the abrupt cessation of the reverberation artifacts. It was not possible to differentiate a fibre mat and a ventral fluid phase.

The wall of the rumen could be identified as a thick echogenic line (3.0–4.8 mm) adjacent to the left abdominal wall from left flank to 8th intercostal space (Imran et al., 2011) but the microanatomical wall layering was indistinguishable maybe due the low frequency probe used in this study (3.5 Mhz).

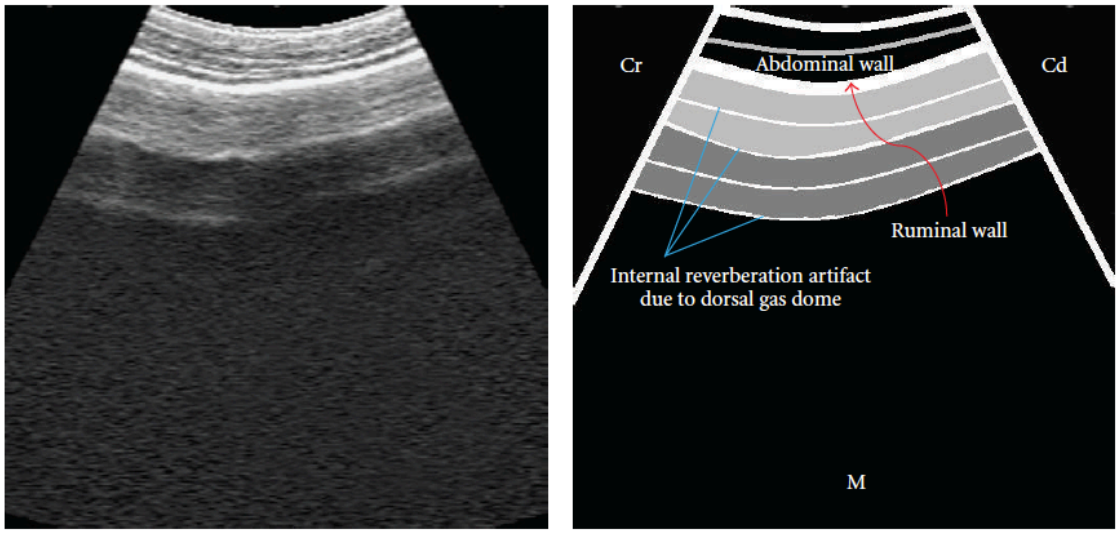


**Figure 1.** Ultrasonogram of the rumen obtained from dorsal left paralumbar fossa, by placing the transducer parallel to the longitudinal axis of the cow. Cr: cranial, Cd: caudal, and M: medial (Imran et al., 2011).

Since marked morphological changes of the ruminal mucosa in response to changes in volatile fatty acid (VFA) concentration and ruminal fluid pH in cattle are well established, Mirmazhari-Anwar et al. (2013) hypothesized that transabdominal ultrasonographic evaluation of the ruminal mucosa would be a suitable noninvasive diagnostic procedure to identify tissue reactions of the ruminal mucosa caused by SARA in adult cattle (Dirksen et al., 1984; Steele et al., 2011). The main advantages of an ultrasonomorphologic examination to diagnose SARA in cattle are that this procedure is non-invasive and is not dependent on a precise timing of the examination relative to feeding as this is the case for ruminal fluid analysis. The ultrasonographic equipment used in this study is similar to equipment commonly used in food animal practice for examination of the reproductive tract (Mirmazhari-Anwar et al., 2013), an 8 Mhz probe was used.

One of the objectives of the present pilot study was accordingly to evaluate the potential suitability of the transabdominal ultrasonographic examination of the ruminal wall to diagnose SARA in beef cattle as a fast a non-invasive tool to be use in field condition.

## 3. TRAIL I

**RUMEN VOLATILE FATTY ACIDS × DIETARY SUPPLEMENTATION WITH LIVE YEAST AND YEAST CELL WALL IN FEEDLOT BEEF CATTLE**

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## 3.1 MATERIALS AND METHODS

### Animals and diets

Sixty Charolaise steers selected from a farm located in the northeast of Italy (45° 24′ N; 11° 52′ E, 12 m above sea level) were enrolled in this study. All animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart and respiratory rate, appetite, fecal consistency and hematological profile. Animals were kept under natural photoperiod (sunrise 6:30am and sunset 5:20pm) and ambient temperature (min. 9.7°C and max. 15.2°C) and housed inside concrete floor tie stalls within an enclosed barn.

Animals were divided into two equal groups (n = 30) on the basis of their feeding phase: growing (10–12 months, mean body weight (BW) 350 ± 50 kg) and finishing (17–18 months, mean BW 600 ± 50 kg). The feeding phase is defined as changing the nutrient concentrations in a series of diets formulated to meet an animal's nutrient requirements more precisely at a particular stage of growth.

Growing and finishing groups were randomly divided into three equal subgroups (n = 10), respectively: no supplement (growing control, GC), supplemented with YCW (25 g/head) of *S. cerevisiae* (outer layer of YCWs), and supplemented with live cell yeast (LY) (5 g/head) + YCW (25 g/head) of *S. cerevisiae*, no supplement (finishing control, FC), supplemented with LY (5 g/head) based on *S. cerevisiae* and supplemented with LY (5 g/head) + YCW (15 g/head).

The viable cells of *S. cerevisiae* (LY) were a strain (NCYC Sc 47) produced by batch fermentation in a growth medium typical of those used for the industrial production of yeasts and with a guaranteed concentration of 1010 CFU/g. Meanwhile the cell wall structure of a yeast cell (YCW) were mannanoligosaccharides containing (1→3)-β-D-glucan, (1→6)-β-D-glucan, chitin and mannan proteins. The cell walls of yeast were obtained by autolysis of S. cerevisiae cells (Sc 47 strain). The insoluble fraction of the cell wall is collected by centrifugation and dried according to Sanz et al. (1985).

Feed ingredients and analytical compositions of diet used for no supplement subgroups (GC and FC) are given in Table 1. The total mixed rations (TMR) were analyzed using near-infrared reflectance spectroscopy (NIRSystem 5000, FOSS ITALIA spa, Italy). The feed supplements were premixed with concentrate prior to mix of TMR. Water was available ad libitum.

**Table 1.** Feed ingredients and chemical composition of growing group diet (GC diet) and finishing group diet (FC diet) used for animals object of study

| **Feed ingredients (kg/day per head)** | **GC DIET** | **FC DIET** |
|---|---|---|
| *BULL 100 11.11** | 0.50 | 0.70 |
| *Corn gluten feed* | 0.60 | 1.00 |
| *Alfalfa hay* | 1.00 | 1.00 |
| *Hydrolyzed fat* | — | 0.10 |
| *Corn* | 2.00 | 3.50 |
| *Dry pulp* | 1.00 | 1.30 |
| *Straw* | 0.80 | 0.30 |
| *Corn silage 22* | 5.65 | 11.05 |
| *Soybean meal* | 0.30 | 0.50 |
| *Total* | 11.85 | 19.45 |
| **Chemical composition** | | |
| *Humidity (%)* | 42.83 | 39.72 |
| *Crude Protein (%)* | 13.16 | 12.89 |
| *Ethereal Extract (%)* | 3.11 | 4.79 |
| *Fiber (%)* | 14.98 | 14.04 |
| *Ash (%)* | 6.07 | 5.76 |
| *NDF (%)* | 38.93 | 36.89 |
| *Starch (%)* | 32.52 | 33.27 |
| *Ca (gr)* | 69.47 | 94.20 |
| *P (gr)* | 24.94 | 39.17 |

* Bull 100 11.11 proteic, vitamins, and minerals premix: Vitamin A (169000 UI/kg), Vitamin D3 (16900 UI/kg), Vitamin E (416 mg/kg), Vitamin B1 (42 mg/kg), Vitamin B12 (0.22 mg/Kg), Choline (845 mg/kg), Niacinamide (1793 mg/kg), Manganous sulphate (191 mg/kg), Manganous Oxide (381 mg/kg), Zinc Chelate of Aminoacids (5954 mg/kg), Zinc Oxide (742 mg/kg), Copper Sulphate Pentahydrate (216 mg/kg), Cobalt Carbonate (2.2 mg/kg) Potassium Iodide (14.7 mg/kg), Urea (49500 mg/kg).

Steers were singularly weighed at the beginning (T0) and at the end (T42) of the monitoring period. Change in average body weight gain (ABWG) and in

average daily gain (ADG) of each steers during 42 days was calculated as follows:

ABWG = Body weight at T42 − Body weight at T0,

ADG = (Body weight at T42 − Body weight at T0)/42.

All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

**Ruminal sampling and analysis**

The collection of ruminal fluid was carried out from each animal before (T0), after 21 (T21) and 42 (T42) days from the start of the experimental period. The rumenocentesis site was located 12–15 cm caudal to the costochondral junction of the last rib, on a horizontal line level with the top of the stifle. Before rumenocentesis, the area was clipped, scrubbed with a povidone-iodine scrub, wiped with 70% isopropyl alcohol, and locally anesthetized (with subcutaneous and intramuscular injection of 2 mL of 2% lidocaine). The ruminal fluid was collected by rumenocentesis (Figure 1), using a 13 G 105 mm needle, between 4 and 7 hours after TMR administration because in this range the rumen pH reaches the peak of acidity, as described by Morgante et al. (2007).

Rumen pH was determined immediately after sampling using a portable pH meter (Zetalab PC70, XSinstruments, Italy).

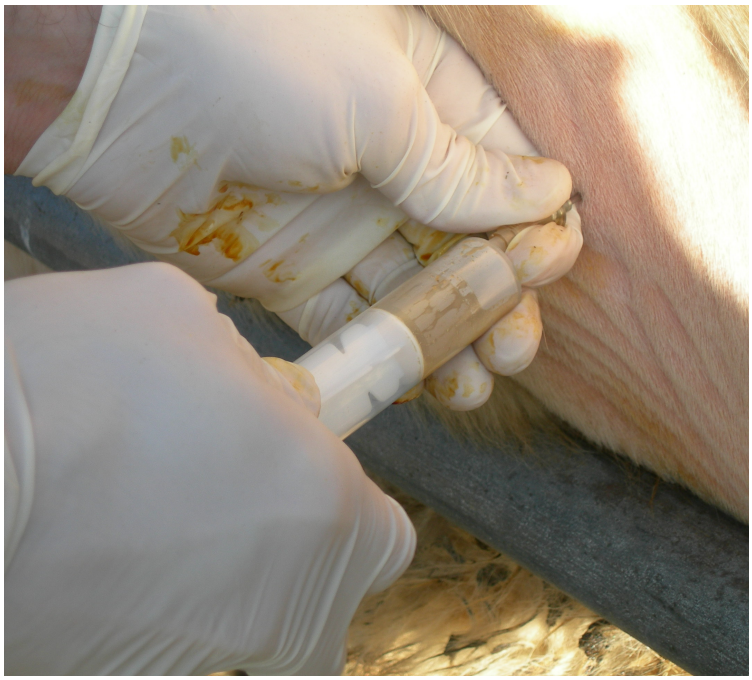

**Figure 1.** Ruminal fluid collection by rumenocentesis (courtesy of Prof. Gianesella).

Thereafter, an 8ml aliquot of ruminal fluid was placed into plastic tubes containing 2 ml of hydrochloric acid 0.6M and then stored at −20°C for later analysis. Subsequently, defrosted samples were homogenized, centrifuged at 10,000×g for 10 min at 4°C and filtered to obtaia clear supernatant, which was then analyzed for VFA using high-performance liquid chromatography, following the method of Martillotti et al. (1987). The standard solution of calibration in amount of 5 µl was analyzed and, after calibration of the instrument, the concentration of the fatty acids expressed in mg/ml−1, was calculated applying the following formula:

weight of acid = [(Area champion × Weight of the standard)/Area of the standard] × nWdilutions.

**Statistical analysis**

Two-way repeated-measures analysis of variance (ANOVA) was applied to determine significant effects of different supplementations and time (T0, T21 and T42) on the parameters studied in both groups. *P* value < .05 was considered statistically significant. The data were statistically analyzed by Prism v. 5.00 (Graphpad Software Ltd, USA, 2003). All the results were expressed as mean ± standard deviation (SD).

## 3.2 RESULTS

Animals included in the study showed no clinical signs of disease during the experimental period. Changes in BW, ABWG and in ADG of steer are given in Table 2. No statistically significant difference was found in subgroupswith the same feeding phase (Table 2).

**Table 2.** Mean ± standard deviation of body weight, average body weight gain (ABWG) and average daily gain (ADG) observed during experimental conditions (T0-T42)

| Groups | Subgroups | *Body Weight* | | *ABWG* | *ADG* |
|---|---|---|---|---|---|
| | | *T0* | *T42* | | |
| **Growing** | ***GC*** | 430.40±3.98 | 478.80±6.63 | 48.40±6.36 | 1.15±0.15 |
| | ***YCW*** | 430.80±7.11 | 478.80±7.24 | 48.00±10.54 | 1.14±0.25 |
| | ***LCY+YCW*** | 431.20±6.46 | 477.90±2.92 | 46.70±7.44 | 1.11±0.18 |
| **Finishing** | ***FC*** | 590.20±5.45 | 647.90±5.67 | 57.70±7.56 | 1.37±0.18 |
| | ***LCY*** | 590.00±6.60 | 649.60±4.88 | 59.60±6.57 | 1.42±0.16 |
| | ***LCY+YCW*** | 591.90±3.73 | 648.90±5.00 | 57.00±5.46 | 1.36±0.13 |

Two-way ANOVA showed a statistically significant effect of time ($P < .05$) on propionic acid, acetic acid, iso-butyric acid, n-butyric acid, n-valeric acid and pH both in growing and finishing groups.

In particular, Duncan's post-hoc comparison test showed a statistically significant decrease in propionic and acetic acid values at T21 and T42 compared to T0 in all subgroups. Iso-butyric acid and n-butyric acid concentrations were decreased at T21 and T42 compared to T0 in FC and LY subgroup, respectively.

N-valeric acid showed lower values at T21 and T42 with respect to T0 in the LY subgroup.

Statistical analysis showed an increase in pH values at T21 and T42 compared to T0 in the LY subgroup, whereas in FC and in LY + YCW were increased only at T21 and at T42, respectively. Propionic acid concentrations were decreased at T21 and T42 compared to T0 in LY + YCW subgroups, whereas in GC and YCW propionic acid showed lower values at T42 with respect to T21. Iso-butyric acid concentrations were statistically lower at T42 compared to T21 in GC and LY + YCW subgroups. N-butyric concentrations were statistically lower at T42 compared to T21 in GC and LY + YCW subgroups. pH values were higher at T42 compared to T0 in all subgroups.

Furthermore, ANOVA showed significant differences ($P < .05$) in propionic acid, acetic acid, iso-butyric acid, n-butyric acid and n-valeric acid concentrations among growing subgroups. No statistical significances were found among finishing subgroups. Statistical significances revealed by the application of Duncan's post-hoc comparison test are given in Tables 3 and 4.

**Table 3.** Mean values ± SD together with the relative statistical significance of ruminal parameters observed before (T0) after 21 (T21) and 42 (T42) days of the start of experimental period in growing subgroups (GC, YCW and LY+YCW)

| Parameters | Subgroups | Experimental Period | | |
|---|---|---|---|---|
| | | ***T0*** | ***T21*** | ***T42*** |
| **Propionic acid (mg/ml)** | ***GC*** | 1.50±0.13 | 1.50±0.19 | 1.21±0.19[ab] |
| | ***LY+YCW*** | 1.44±0.10 | 1.17±0.24[ac] | 1.12±0.29[a] |
| | ***YCW*** | 1.44±0.10 | 1.50±0.17[a] | 1.26±0.21[b] |
| **Acetic acid (mg/ml)** | ***GC*** | 3.54±0.37 | 3.53±0.18 | 3.16±0.40[ab] |
| | ***LY+YCW*** | 3.26±0.47 | 2.95±0.53[ac] | 3.02±0.81 |
| | ***YCW*** | 3.24±0.43 | 3.59±0.31 | 3.29±0.40 |
| **iso-Butyric acid (mg/ml)** | ***GC*** | 0.08±0.03 | 0.10±0.03 | 0.07±0.02[ab] |
| | ***LY+YCW*** | 0.07±0.01 | 0.09±0.02[a] | 0.06±0.01[b] |
| | ***YCW*** | 0.07±0.02 | 0.06±0.02 | 0.05±0.01 |
| **n-Butyric acid (mg/ml)** | ***GC*** | 1.16±0.17 | 1.22±0.13 | 0.94±0.12[ab] |
| | ***LY+YCW*** | 1.00±0.17 | 0.94±0.20 | 0.81±0.28 |
| | ***YCW*** | 0.98±0.18 | 1.24±0.14[a] | 0.98±0.20[b] |
| **iso-Valeric acid (mg/ml)** | ***GC*** | 0.12±0.02 | 0.12±0.02 | 0.12±0.02 |
| | ***LY+YCW*** | 0.12±0.03 | 0.12±0.03 | 0.12±0.03 |
| | ***YCW*** | 0.12±0.03 | 0.12±0.03 | 0.11±0.01 |
| **n-Valeric acid (mg/ml)** | ***GC*** | 0.12±0.03 | 0.15±0.02 | 0.12±0.02 |
| | ***LY+YCW*** | 0.14±0.04 | 0.11±0.03 | 0.11±0.03 |
| | ***YCW*** | 0.12±0.05 | 0.15±0.02 | 0.12±0.02 |
| **pH** | ***GC*** | 5.90±0.40 | 6.02±0.23 | 6.29±0.20[a] |
| | ***LY+YCW*** | 6.08±0.29 | 6.28±0.19 | 6.53±0.45[a] |
| | ***YCW*** | 6.06±0.31 | 6.17±0.29 | 6.45±0.34[a] |

Significances: (effect of time) [a] vs T0; [b] vs T21 (effect of subgroup) [c] vs GC and YCW at T21

**Table 4.** Mean values ± SD together with the relative statistical significance of ruminal parameters observed before (T0) after 21 (T21) and 42 (T42) days of the start of experimental period in finishing subgroups (FC, LY and LY+YCW)

| Parameters | Subgroups | Experimental Period | | |
|---|---|---|---|---|
| | | ***T0*** | ***T21*** | ***T42*** |
| **Propionic acid (mg/ml)** | ***FC*** | 1.41±0.19 | 1.06±0.14[a] | 1.16±0.19[a] |
| | ***LY+YCW*** | 1.37±0.24 | 1.16±0.12[a] | 1.08±0.16[a] |
| | ***LY*** | 1.49±0.14 | 1.07±0.16[a] | 1.11±0.08[a] |
| **Acetic acid (mg/ml)** | ***FC*** | 3.52±0.36 | 2.93±0.35[a] | 3.16±0.46[a] |
| | ***LY+YCW*** | 3.55±0.41 | 3.20±0.20[a] | 2.96±0.30[a] |
| | ***LY*** | 3.73±0.34 | 2.98±0.42[a] | 3.07±0.20[a] |
| **iso-Butyric acid (mg/ml)** | ***FC*** | 0.14±0.05 | 0.09±0.05[a] | 0.07±0.02[a] |
| | ***LY+YCW*** | 0.08±0.04 | 0.10±0.03 | 0.08±0.03 |
| | ***LY*** | 0.10±0.04 | 0.10±0.03 | 0.08±0.03 |
| **n-Butyric acid (mg/ml)** | ***FC*** | 0.94±0.15 | 0.80±0.11 | 0.96±0.21[b] |
| | ***LY+YCW*** | 0.99±0.21 | 0.88±0.16 | 0.86±0.19 |
| | ***LY*** | 1.13±0.13 | 0.89±0.20[a] | 0.95±0.11[a] |
| **iso-Valeric acid (mg/ml)** | ***FC*** | 0.13±0.04 | 0.09±0.03 | 0.12±0.04 |
| | ***LY+YCW*** | 0.14±0.06 | 0.11±0.02 | 0.11±0.03 |
| | ***LY*** | 0.12±0.03 | 0.11±0.03 | 0.11±0.01 |
| **n-Valeric acid (mg/ml)** | ***FC*** | 0.13±0.02 | 0.10±0.02[a] | 0.13±0.03[b] |
| | ***LY+YCW*** | 0.13±0.02 | 0.11±0.01 | 0.11±0.02 |
| | ***LY*** | 0.14±0.02 | 0.11±0.02[a] | 0.11±0.01[a] |
| **pH** | ***FC*** | 6.18±0.30 | 6.37±0.13[a] | 6.39±0.33 |
| | ***LY+YCW*** | 6.07±0.26 | 6.29±0.25 | 6.43±0.24[a] |
| | ***LY*** | 5.96±0.25 | 6.43±0.26[a] | 6.56±0.18[a] |

Significances: (effect of time) [a] vs T0; [b] vs T21

## 3.3 DISCUSSION

The rumen ecosystem is a complex microbial environment characterized by several biochemical pathways. YC has been observed (Thrune et al., 2009; Chung et al., 2011) to change patterns of VFA produced by ruminal bacteria. Yeast supplement could influence dry matter intake by altering ruminal VFA concentrations (Allen, 1997). In the LY + YCW growing subgroup we found a decrease in acetate, propionate, and butyrate levels compared with CG and YCW. Although it is known that ruminal environment needs time to adapt to the transition from the pasture diet to total mix ratio diet, the LY + YCW growing subgroup showed lower propionate and acetate levels already at T21 with respect to CG and this should be read as a faster adaptation. This effect did not occur in finishing subgroups in which animals were already on a total mix ratio diet for several months. In contrast to our results, Křížovă et al. (2011) reported higher acetate, propionate, and butyrate levels in cows fed LY.

The YCW subgroup showed higher level of all VFAs at T21 compared to T0 and T42. Forage constitutes a major portion of the diets of growing beef steers.

Feeding high-forage diets can maintain ruminal pH and improve overall ruminal functionality (Holt et al., 2010). High-forage diets result in the production of greater amounts of acetate and butyrate, while high starch diets result in the production of greater proportions of propionate, although acetate is still the dominant VFA (Beever & Mould, 2000). The effect of YCW on VFA concentration was transient, as reported in the literature (Spring et al., 2000; Franklin et al., 2005). The YCW is going to affect the body's immune response than to act directly on rumen metabolites. In all subgroups of finishing phase the concentrations of all VFA decreased during the experimental period. These

results are in contrast with Gattass et al. (2008) that reported no influence by the YC supplementation on VFA in beef cattle.

Ruminal pH values stayed within optimal levels (6–7) for cellulolysis, proteolysis and deamination (RAGFAR 2007) in both groups throughout the experimental period. In our study significant changes occurred in pH values, that increase gradually during the experimental period both in growing and finishing groups.

Although pH values did not statistically change among subgroups, the steers that received yeast supplementation showed higher pH values with respect to control subgroups at T21 and T42 both in growing and finishing groups.

These data suggested that yeast supplementation may stimulate the growth of the cellulolytic microbiota by increasing the pH (Chaucheyras-Durand & Fonty, 2001 Marden et al., 2008; Bitencourt et al., 2011) and confirm the results obtained in previous studies carried out on cows (Bach et al., 2007; Thrune et al., 2009), sheep (Chaucheyras-Durand & Fonty, 2002; Helal & Abdel-Rahman, 2010) and lamb (Mosoni et al., 2007) supplemented with the yeast product. Brossard et al. (2004) suggest that the stabilizing effect of yeast upon rumen pH could be mediated by the stimulation of ruminal protozoa, capable of engulfing starch granules, which would thus compete with amylolytic bacteria for substrate and could induce slower degradation of starch in the rumen (Bonhomme, 1990).

In conclusion, these results suggest that yeast supplementation may have beneficial effects on beef steer, improving rumen function, especially on newly restocked animals. Further studies are needed in order to better understand the mechanism by which the yeast supplementation affects rumen fermentations in beef steers and the impact on animal growth since our study showed an

average increase of two kilos on LY group of finishing phase but it was not statistically significant.

It would be also appropriate to investigate how different doses of LY and YCW can affect the animal's health and production.

## 4. TRAIL II

# EFFECT OF LIVE YEAST AND YEAST CELL WALL SACCHAROMYCES CEREVISIAE DIET SUPPLEMENTATION ON FAECES CHEMICAL COMPOSITION AND GROWTH PERFORMANCE IN GROWING AND FINISHING BEEF STEERS

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## 4.1 MATERIALS AND METHODS

### Animals and diets

All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Sixty Charolaise steers selected from a farm located in the Northeast of Italy (45° 24’ N; 11° 52’ E, 12 m above sea level) were enrolled in this study. All animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart and respiratory rate, appetite, fecal consistency, and hematological profile. Animals were kept under natural photoperiod (sunrise 6:30 AM; sunset 5:20 PM) and ambient temperature (Min. 9.7°C; Max. 15.2°C) and housed inside concrete floor tie stalls within an enclosed barn.

Animals were divided into two equal groups (n=30) on the basis of their feeding phase: growing (10-12 months) and finishing (17-18 months). The feeding phase is defined as changing the nutrient concentrations in a series of diets formulated to meet an animal's nutrient requirements more precisely at a particular stage of growth. Growing and finishing groups were randomly divided into three equal subgroups (n=10) respectively: supplemented with live cell yeast (5 g/head) + yeast cell wall (25 g/head) (GA) of *Saccharomyces cerevisiae*; supplemented with yeast cell wall (25 g/head) (GB) of *Saccharomyces cerevisiae* (outer layer of yeast cell walls); and no supplement (growing control, GC); supplemented with live cell yeast (5 g/head) + yeast cell wall (15 g/head) (FA); supplemented with live cell yeast (5 g/head) (FB) based on *Saccharomyces cerevisiae*; and no supplement (finishing control, FC).

The viable cells of *Saccharomyces cerevisiae* (LY) were a strain (NCYC Sc 47) produced by batch fermentation in a growth medium typical of those used for the industrial production of yeasts and with a guaranteed concentration of 1010 CFU/g. Meanwhile the cell wall structure of a yeast cell (YCW) were mannanoligosaccharides containing (1→3)-β-D-glucan, (1→6)-β-D-glucan, chitin, and mannan proteins.

The cell walls of yeast were obtained by autolysis of *Saccharomyces cerevisiae* cells (Sc 47 strain). The insoluble fraction of the cell wall is collected by centrifugation and dried according to Sanz et al. (1989).

Feed ingredients and analytical compositions of diet used for no supplement subgroup (GC and FC) are showed in Table 5.

**Table 5.** Feed ingredients and chemical composition of growing group diet (GC diet) and finishing group diet (FC diet) used for animals object of study

| **Feed ingredients (kg/day per head)** | **GC DIET** | **FC DIET** |
|---|---|---|
| *BULL 100 11.11** | 0.50 | 0.70 |
| *Corn gluten feed* | 0.60 | 1.00 |
| *Alfalfa hay* | 1.00 | 1.00 |
| *Hydrolyzed fat* | — | 0.10 |
| *Corn* | 2.00 | 3.50 |
| *Dry pulp* | 1.00 | 1.30 |
| *Straw* | 0.80 | 0.30 |
| *Corn silage 22* | 5.65 | 11.05 |
| *Soybean meal* | 0.30 | 0.50 |
| *Total* | 11.85 | 19.45 |
| **Chemical composition** | | |
| *Humidity (%)* | 42.83 | 39.72 |
| *Crude Protein (%)* | 13.16 | 12.89 |
| *Ethereal Extract (%)* | 3.11 | 4.79 |
| *Fiber (%)* | 14.98 | 14.04 |
| *Ash (%)* | 6.07 | 5.76 |
| *NDF (%)* | 38.93 | 36.89 |
| *Starch (%)* | 32.52 | 33.27 |
| *Ca (gr)* | 69.47 | 94.20 |
| *P (gr)* | 24.94 | 39.17 |

* Bull 100 11.11 proteic, vitamins, and minerals premix: Vitamin A (169000 UI/kg), Vitamin D3 (16900 UI/kg), Vitamin E (416 mg/kg), Vitamin B1 (42 mg/kg), Vitamin B12 (0.22 mg/Kg), Choline (845 mg/kg), Niacinamide (1793 mg/kg), Manganous sulphate (191 mg/kg), Manganous Oxide (381 mg/kg), Zinc Chelate of Aminoacids (5954 mg/kg), Zinc Oxide (742 mg/kg), Copper Sulphate Pentahydrate (216 mg/kg), Cobalt Carbonate (2.2 mg/kg) Potassium Iodide (14.7 mg/kg), Urea (49500 mg/kg).

The total mixed rations (TMR) were analyzed using near-infrared reflectance spectroscopy (NIRSystem 5000, FOSS ITALIA spa, Italy). The feed supplements were premixed with concentrate prior to mix of TMR. Water was available ad libitum.

Steers were singularly weighed at the beginning (T0) and at the end (T2) of the monitoring period. Change in average body weight gain (ABWG) and in average daily gain (ADG) of each steers during 42 days was calculated as following:

– ABWG = body weight at T2 - body weight at T0;

– ADG = (body weight at T2 - body weight at T0) / 42.

**Faeces sampling and analysis**

The collection of feces was carried out from each animal before (T0) after 21 (T1) and 42 (T2) days of the start of experimental period. Feces outputs were sampled as a proportion (15%) of total excretion of feces (by weight) and were stored at -20°C for subsequent analysis of the chemical composition (dry matter (DM), Ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and Starch) by means of near-infrared spectroscopy predictive method (NIRS, Near-Infrared Spectroscopy). Prior to NIRS analysis, fecal samples were oven-dried at 60°C for 48 h and then ground at 0.5 mm with Universal Cutting Mill Pulverisette19 (Fritsch GmbH, Idar-Oberstain, Germany). Ground samples were placed in a 50 mm diameter ring cups with quartz lens and scanned in duplicate in the region between 1100 and 2500 nm at 2 nm intervals using a NIRSystem MODEL5000 scanning NIR spectrometer (Silver Spring, MD, USA) in reflectance mode in

accordance with the methods described by the Association of Official Analytical Chemists (Horwitz, 2000). Each spectrum is the average of 32 multiple scans. Reflectance (R) data were converted into absorbance (A) data through A =log (1/R). WinISI II version 1.5 (Infrasoft International LLC, State College, PA, USA) was used to acquire spectral data.

**Statistical analysis**

All results were expressed as mean ± standard deviation (SD). Two-way repeated measures analysis of variance (ANOVA) was applied to determine significant effects of different supplementations (GA, GB, GC, FA, FB and FC) and time (T0, T1 and T2) on the studied parameters (DM, Ash; CP, EE, NDF, ADF, ADL, Starch) in faecal samples of both groups (growing and finishing). *P* value <0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post-hoc comparison. Obtained data has been analysed using the software STATISTICA 7 (Stat Soft Inc.).

## 4.2 RESULTS

Animals included in the study showed no clinical signs of disease during the experimental period. Changes in BW, ABWG and in ADG of steer are shown in Table 6. No statistically significant difference was found in subgroups with the same feeding phase.

Two-way analysis of variance (ANOVA) showed a significant effect of different diets ($P<0.001$) on NDF, ADF and Starch in the finishing group. In particular, at T2 FA and FB showed higher Starch values respect to FC, while NDF and ADF values were higher in FC compared to FA and FB.

**Table 6.** Mean ± standard deviation of body weight, average body weight gain (ABWG) and average daily gain (ADG) observed during experimental conditions (T0-T42)

| Groups | Subgroups | *Body Weight* | | *ABWG* | *ADG* |
|---|---|---|---|---|---|
| | | *T0* | *T42* | | |
| Growing | ***GC*** | 430.40±3.98 | 478.80±6.63 | 48.40±6.36 | 1.15±0.15 |
| | ***YCW*** | 430.80±7.11 | 478.80±7.24 | 48.00±10.54 | 1.14±0.25 |
| | ***LY+YCW*** | 431.20±6.46 | 477.90±2.92 | 46.70±7.44 | 1.11±0.18 |
| Finishing | ***FC*** | 590.20±5.45 | 647.90±5.67 | 57.70±7.56 | 1.37±0.18 |
| | ***LY*** | 590.00±6.60 | 649.60±4.88 | 59.60±6.57 | 1.42±0.16 |
| | ***LY+YCW*** | 591.90±3.73 | 648.90±5.00 | 57.00±5.46 | 1.36±0.13 |

A statistical significant effect of time ($P$<0.05) was found on DM, ADL and Starch in finishing group, and on DM and ADL in growing group. In particular, Bonferroni's post-hoc comparison test showed a statistically significant increase in DM values at T1 compared to T0 and T2 in all subgroups of growing phase. ADL showed higher values at T1 respect to T0 and T2 in GA and GC subgroups (Figure 2). In finishing group, Bonferroni's post-hoc comparison test showed a significant increase in DM at T2 compared to T0 and T1 in FA and FC subgroups, and at T1 and T2 compared to T0 in FB subgroup (Figure 3). ADL showed higher values at T1 respect to T0 and T2 in FA subgroup, whereas FC subgroup showed lower values at T2 respect to T1. Starch showed a statistical significant increase at T2 compared to T1 in FC subgroup.

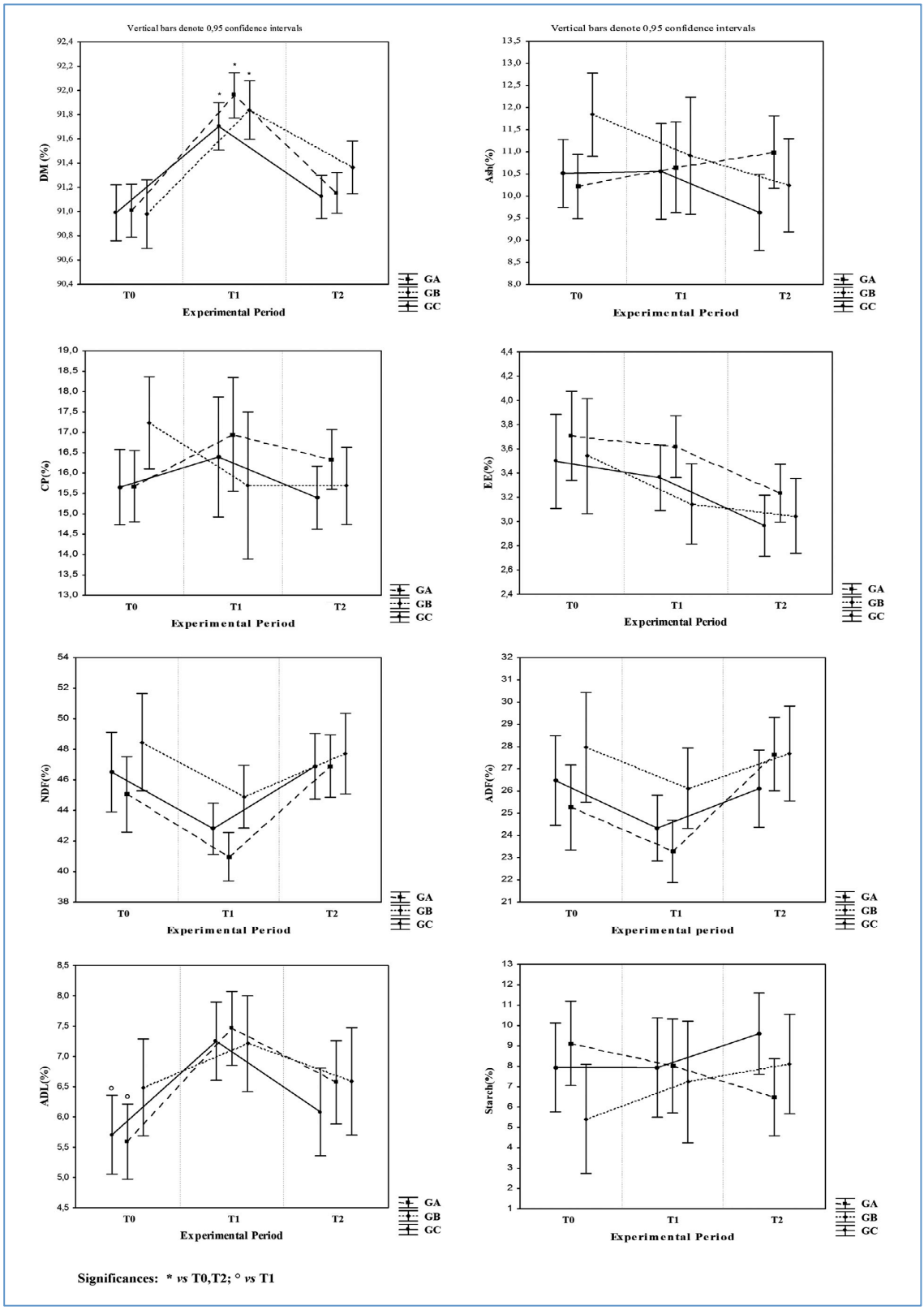


**Figure 2**. Trend of feces chemical composition (dry matter (DM); Ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and Starch) obtained from growing control (GC) and experimental subgroups (GA and GB) throughout monitoring period (T0, T1, T2) with related statistically significances found.

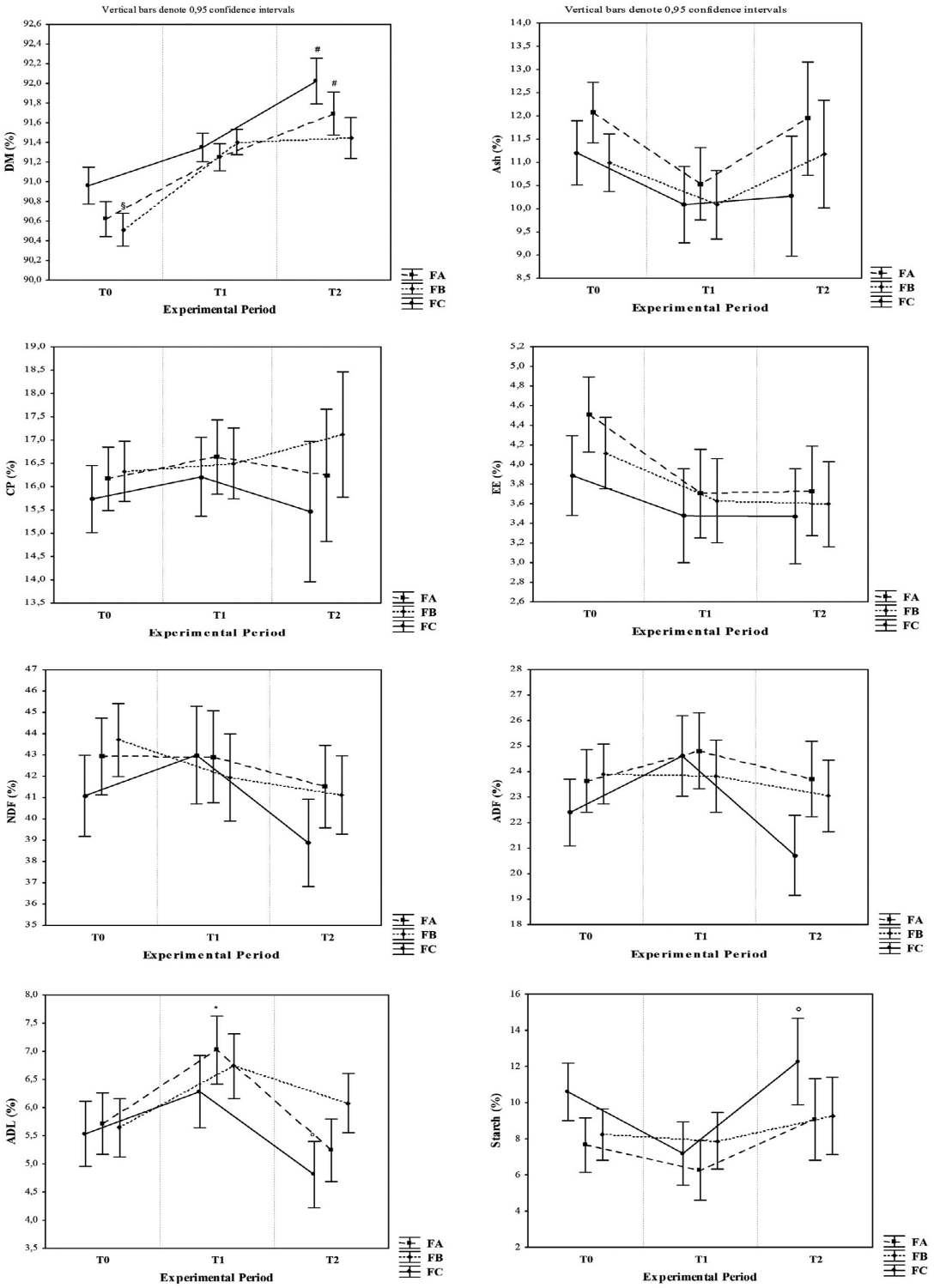


**Figure 3.** Trend of feces chemical composition (dry matter (DM); Ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and Starch) obtained from finishing control (FC) and experimental subgroups (FA and FB) throughout monitoring period (T0, T1, T2) with related statistically significances found.

## 4.3 DISCUSSION

Active yeast products have beneficial effects in ruminant livestock production as feed additives to improve feed efficiency and growth performance (Dawson et al., 1990). Saccharomyces cerevisiae supplementation was associated with an improved growth performance and an increased flow of microbial protein leaving the rumen and enhanced supply of aminoacids entering the small intestine (Tripathi et al., 2010). In this study the BW, ABWG and ADG values were similar in YC, YCW and control subgroups suggesting no effect of yeast diet supplementation. These results agree with previous studies carried out in ruminant species (Mruthunjaya et al., 1992). The inconsistency in response of yeast diet supplementation on growth performance of animals available in literature is difficult to explain as the response might depend on the specific yeast strain, type of diet and physiological stage of the animal (Singh et al., 1998). About feces chemical composition, the results obtained in the present study showed higher DM values at T1 respect to T0 and T2 in all growing subgroups and higher ADL concentration at T1 respect to T0 and T2 in GA and GC. These differences seem to be related to dietary ratio administrated to growing steers and not to yeast supplementation.

In fact, any effect of diet supplementation was found in growing group. It is known that yeast culture does not always have significant effects on ruminal fermentation with all type of diet (Singh et al., 1998). Several authors stated that minor changes in diet composition significantly alter the beneficial response of yeast culture (Mruthunjaya et al., 1992, Singh et al., 1998).

All considered parameters followed the same trend among control and experimental subgroups under finishing phase. However, experimental

subgroups (FA and FB) showed higher NDF and ADF values and lower Starch concentration respect to control group (FC) at T2. These findings highlight a positive effect of YC and YCW diet supplementation on digestibility of nutrients due to improvement of rumen function by favoring microbial establishment, stabilization of rumen pH, interaction with lactate metabolizing bacteria and yeasts, and increased fiber degradation due to more cell wall degrading microorganisms (Chaucheyras-Durand et al., 2008). Mode of action attributed to YC has been shown to modify rumen function by stimulating fermentation, increasing populations and growth rates of cellulolytic bacteria, and enhancing the initial digestion rate of forages. This is in agreement with the findings of other authors (Kung et al., 1997) reporting that dietary supplementation of yeast culture increased fiber degradation and digestion in beef cattle.

Yeast culture can stimulate the proliferation of cellulolytic bacteria, and thereby promote the decomposition of cellulose (Veum et al., 1995).

Under the condition of the present study, our results reveal that yeast supplementation has no effect on growth performance in growing and finishing steers whereas it influenced the NDF, ADF and Starch in the finishing group only suggesting that under finishing diet phase yeast supplementation improves food digestibility. These findings suggest that yeast supplementation do not have beneficial effects with all type of diet condition.

Further studies are needed to obtain more information on the mechanisms by which yeast stimulates nutrient digestibility and animal productivity in order to predict dietary conditions from which the benefits of yeast supplementation can be reasonably expected.

# 5. TRAIL III

## EVALUATION OF RUMINAL ACIDOSIS THROUGH THE USE OF ULTRASOUND MEASUREMENTS OF THE THICKNESS OF THE RUMEN WALL.

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*Article* stil under review

## 5.1 MATERIAL AND METHODS

### Animals

A total of 478 beef cattle of Charolaise breed were monitoring during the livestock cycle. Animals were selected in a farm of 1900 feedlot cattle per year in the North-East Italy. The animals were imported from France with an average body weight (B.W.) of 434.05 ± 30.44 kg and an average age of 10.8 ± 0.7 months. The arriving beef cattle were placed on a higher long fiber acclimation diet prior to the growth diet for 5 days. After this period, the feed ingredients and the chemical composition (Table 7) of the total mixed ration (TMR) with high concentrate diet were the same for all animals. The stall had a separate feed bunk and watering point.

Diet was provided daily as TMR for ad libitum intake based on 10% feed refusal (as-fed basis). Dry matter intake (DMI) mean values were recorded for all beef cows during the period of study (DMI: 18 ± 1.5 Kg per animal; DM: 9.78 ± 0.8 per animal).

**Table 7.** Feed ingredients (1a) and chemical composition (1b) of total mixed ratio

| Feed ingredients | % of total dry matter |
|---|---|
| Maize silage | 33.20 |
| Corn mash | 13.63 |
| Corn gluten feed | 9.14 |
| Maize meal | 10.78 |
| Soybean meal 44 | 2.76 |
| Sugar beet pulps | 9.29 |
| Wheat straw | 9.20 |
| Proteic, vitamin and mineral premix * | 12.00 |

| Chemical Composition | Mean ± SD |
|---|---|
| DM (%) | 54.02 ± 1.90 |
| CP (% DM) | 13.85 ± 0.20 |
| EE (% DM) | 3.29 ± 0.10 |
| Ash (% DM) | 5.84 ± 0.30 |
| NDF (% DM) | 37.22 ± 0.90 |
| NSC (% DM) | 31.15± 0.90 |
| UFV | 0.82 |

DM: dry matter; CP: Crude Protein; EE: ether extract; Ash: acid detergent fiber; NDF: neutral detergent fiber; NSC non-fibrous carbohydrates; UFV: Unitè Fouragère Viande.

* Proteic, vitamin and mineral premix: Vitamin A (45000 UI/kg), Vitamin D3 (4500 UI/kg), Vitamin E (54 mg/kg), Vitamin PP (45 mg/kg), Choline (194.60 mg/kg), Manganous sulphate (277.20 mg/kg), Copper sulfhate (141.48 mg/kg), Selenium (0.99 mg/kg), Zinc sulfate (792 mg/kg), Ferrous carbonate (372.60 mg/kg), Calcium (5.54 mg/kg), Urea (37240 mg/kg).

All animals were housed and divided in groups of 10 inside individual concrete-floor tie stalls within an enclosed barn. Health status was monitored for all animals daily.

The animals were monitored in three different periods in order to evaluating the rumen fluid pH and to assess the measures of the rumen wall during the livestock cycle: T0: 5 ± 3 d after the arrival in farm (434.05 ± 30.44 kg of B.W.; 10.8 ± 0.7 months); T1: 60 ± 10 d after arrival (569.05 ± 12.61 kg of B.W.; 13.0 ± 0.6 months); T2: 1 month before slaughter (619.19 ± 19.69 kg of B.W.; 18.5 ± 0.8 months).

**Rumenocentesis, ultrasound and evaluation of rumen fluid pH**

A sample of rumen fluid was taken for each animal by rumenocentesis method as described by Nordlund and Garrett (1994) and modified by Gianesella et al. (2010). Rumenocentesis is the technique that provides accurate pH results (Garret et al., 1999; Duffield et al., 2004; Morgante et al., 2007;). The collection of samples of rumen fluid was carried out between 4 and 7 hours after TMR administration because in this range the rumen pH reaches the peak of acidity (Garret et al., 1999; Morgante et al., 2007).

Rumen fluid was collected using a 13G 105-mm needle (Intralune PP, Vygon, France) and a 50 ml syringe from a 20 × 20 cm disinfected area in the left flank, from the ventral sac of the rumen, approximately 15–20 cm caudal and ventral to the costocondral junction of the last rib. 15 ml of rumen fluid were collected from each animal.

Ultrasonographic examination of the rumen wall was conducted on the same area used for rumenocentesis.

Ruminal wall was evaluated by ultrasonography using a portable ultrasound scanner (MyLabOne™, Esaote S.p.a., Genova, Italy) equipped with a multi-frequency (2,2-4,3-6,6 MHz) convex probe (SC3421, Esaote S.p.a., Genova, Italy) as shown in Figure 4, probe was set at 6,6 Mhz to have the best resolution on rumen wall layers.

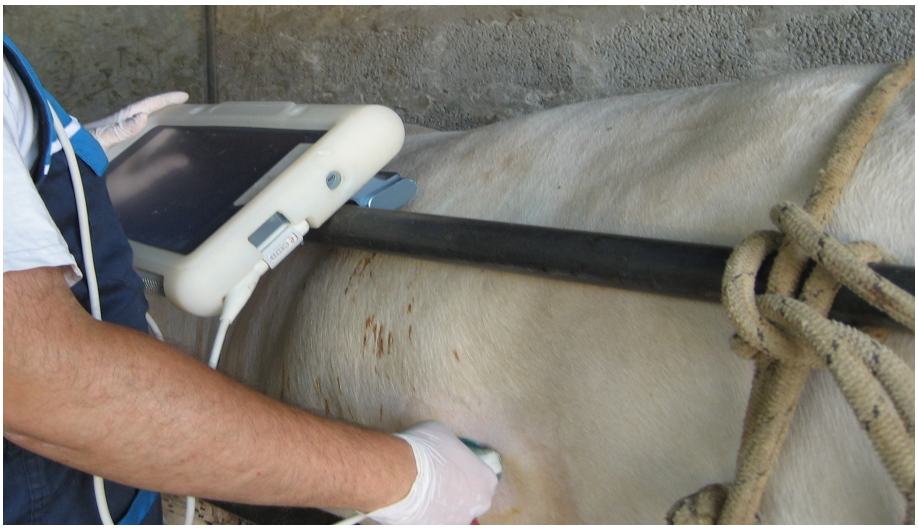

**Figure 4.** Ultrasonography of rumen wall on a Charolaise bull (Dr. Armato)

The images obtained were analyzed with the software MyLabDesk (Esaote S.p.a., Genova, Italy) as displayed in figure 5. This software provides a precise measuring tool in order to measure rumen wall layers' thickness.

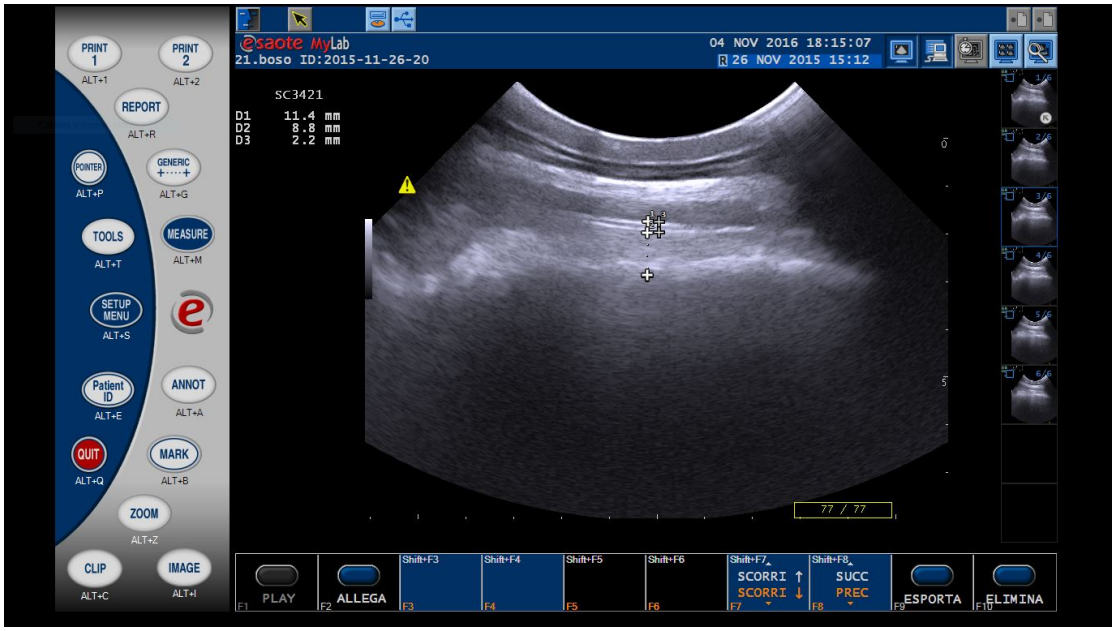


**Figure 5.** Ultrasound image of the rumen wall (Esaote MyLab Desk, Esaote S.p.a., Genova, Italy).

The rumen fluid pH was determined using a digital portable pH meter (Zetalab PC70, XSintruments).

In order to determine volatile fatty acids (VFA), an aliquot of 8 ml of rumen fluid was immediately acidified with 2ml of hydrogen chloride (HCl 0.6M) and stored at 4 °C until samples arrived at the laboratory where they were stored at − 20°C until subsequent analysis.

The quantitative determination of the VFA was performed in one run by HPLC (High Performance Liquid Chromatograpy). Volatile fatty acids determined were: acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid and n-valeric acid.

**Statistical analysis**

Data obtained were analyzed by ANOVA for repeated measures to verify the effect of rumen wall thickness on the ruminal pH and period effect using the Proc Mixed procedure of SAS (version 9.3; SAS Institute). All dependent variables measured over time periods were evaluated using the following model:

$Yijkl = \mu + Thicknessi + Rumen\ pHj + Periodk + eijkl$

where: Yijkl = dependent variable; μ = overall mean; Thicknessi = main effect of rumen wall thickness; Rumen pHj = main effect pH values on rumen wall; Periodk = main effect of time period, and eijkl = residual error term.

Significance was determined at $P \leq 0.05$, unless otherwise indicated.

Data presented are mean values ± standard deviation (SD).

Regression analysis ($R^2$) and Pearson's correlations were performed with thickness of the rumen wall, rumen mucosa as dependent and ruminal pH as independend variables.

Receiver Operating Characteristics Analysis (ROC Analysis) were conducted to identify suitable cut-off for thickness of the rumen mucosa ultrasonography.

## 5.2 RESULTS

Period effect (*P*<0.001) were found between the three periods (Figure 6) after 10 days from the housing of the fattening bulls on the rumen pH were lower than the threshold value of 5.71 in T0 than in T1 and T2.

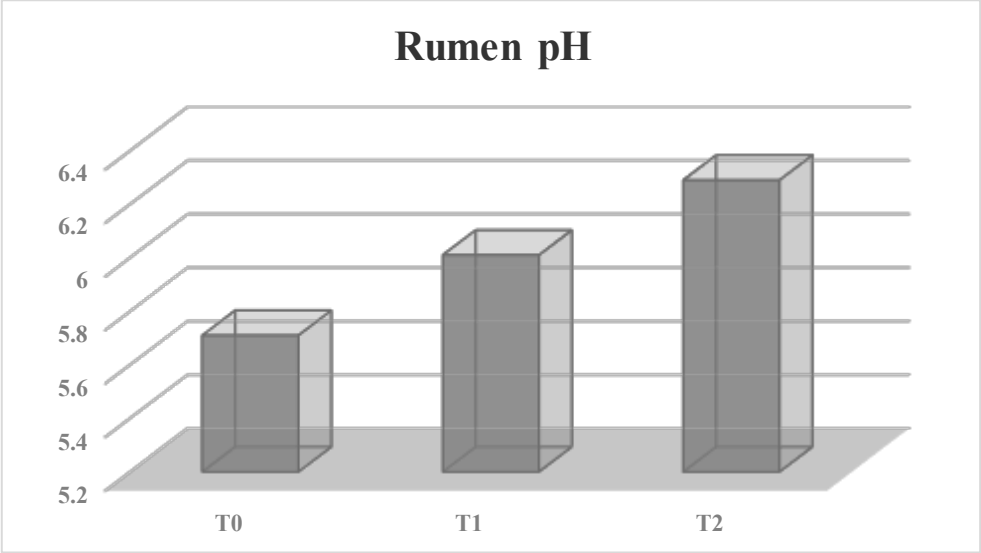


**Figure 6.** ruminal ph values of the bulls between periods of the study.

The regression analysis conducted on ruminal fluid pH and total ultrasound thickness of rumen wall and rumen mucosa showed respectively $R^2$=0.5637 and $R^2$=0.5895 (Figure 7 and Figure 8).

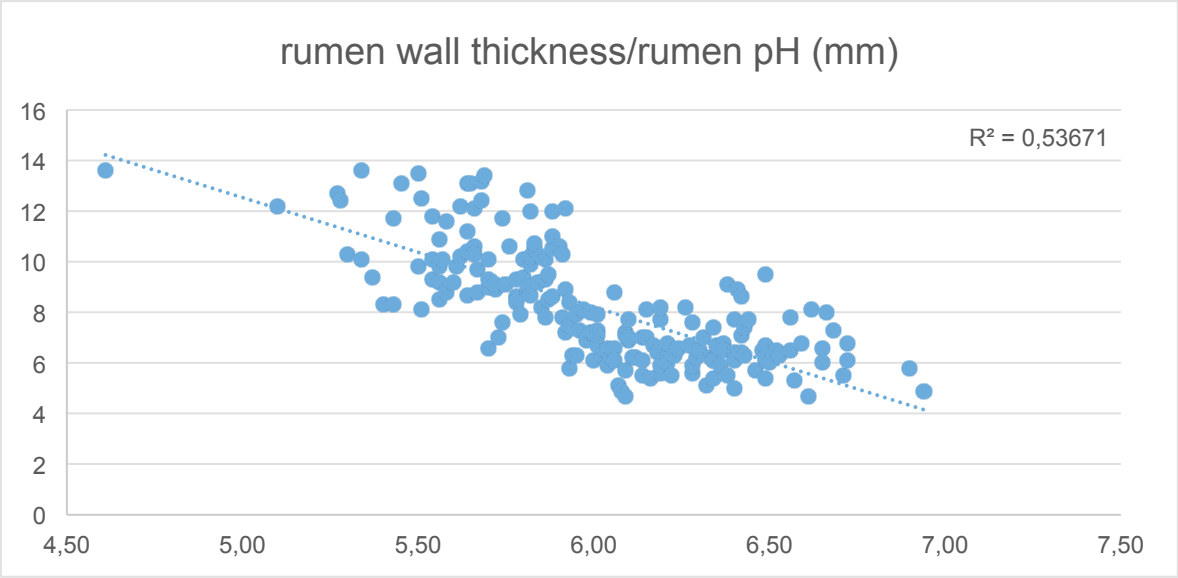


**Figure 7.** regression analysis of rumen wall thickness over rumen pH.

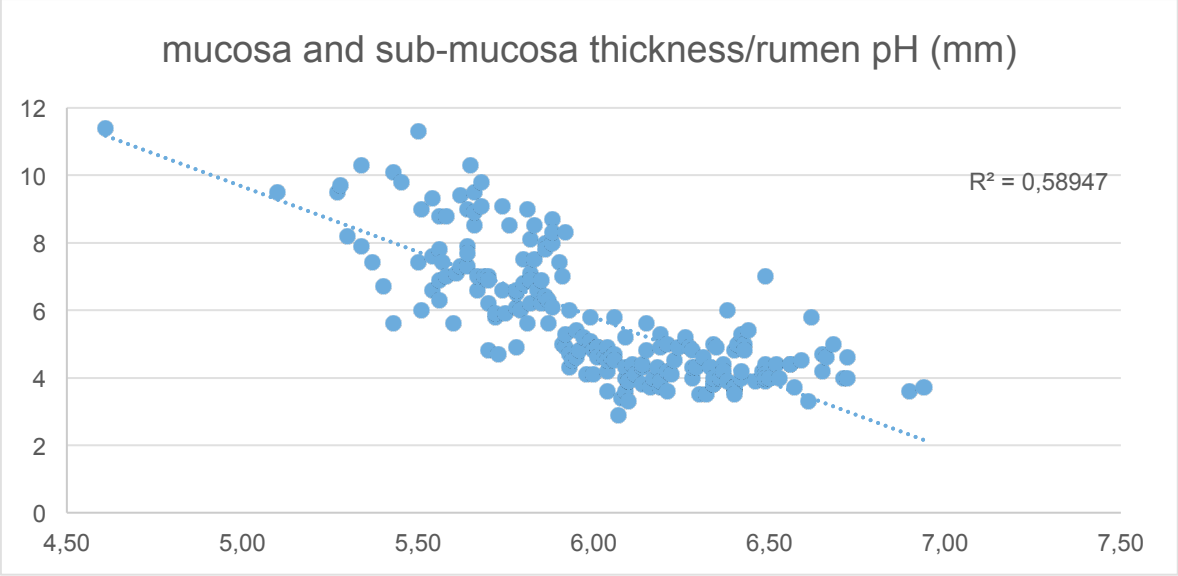


**Figure 8.** regression analysis of mucosa and sub-mucosa thickness over rumen pH.

Pearson's analysis (Table 8) showed interaction between pH and total ultrasound thickness of rumen wall (-0.700; *P*<0.0001) and rumen mucosa (-0.7921; *P*<0.0001).

**Table 8.** Significant Pearson's correlations between parameters investigated.

| Parameters | | r | P |
|---|---|---|---|
| Thickness of the rumen wall | Rumen pH | -0.705 | <0.0001 |
| | Acetic Acid | +0.407 | <0.0001 |
| | Propionic Acid | +0.543 | <0.0001 |
| | iso-Butirric Acid | -0.428 | <0.0001 |
| | n-Butirric Acid | +0.471 | <0.0001 |
| | Thickness of Mucosa and Sub Mucosa of the rumen wall | +0.949 | <0.0001 |
| | Thickness of Muscle and Sierosa of the rumen wall | +0.575 | <0.0001 |
| Thickness of Mucosa and sub mucosa of the rumen wall | Rumen pH | -0.789 | <0.0001 |
| | Acetic Acid | +0.442 | 0.005 |
| | Propionic Acid | +0.553 | <0.0001 |
| | iso-Butirric Acid | -0.346 | 0.001 |
| | n-Butirric Acid | +0.301 | <0.0001 |
| Thickness of Muscle and Sierosa of the rumen wall | Rumen pH | -0.282 | 0.002 |
| | Acetic Acid | +0.304 | <0.0001 |
| | Propionic Acid | +0.232 | 0.0015 |
| | iso-Butirric Acid | -0.156 | <0.001 |
| | n-Butirric Acid | +0.346 | <0.001 |
| Rumen pH | Acetic Acid | -0.643 | <0.0001 |
| | Propionic Acid | -0.758 | 0.001 |
| | iso-Butirric Acid | +0.541 | <0.0001 |
| | n-Butirric Acid | +0.576 | <0.0001 |

The differentiation efficiency of mucosal and submucosal layer thickness between healthy and ruminal acidosis affected animals, as a result of ROC curve analysis, was excellent with an area under the receiver operator curve (AUROC) of 0.970: *P*<0.0001; 95% CI: 0.935 - 0.989, positive likelihood ratio = 11.46, negative likelihood ratio = 0.04. Using a cut-off value of 5.4 mm, sensitivity was 96.30% and specificity was 91.60% (Figure 9).

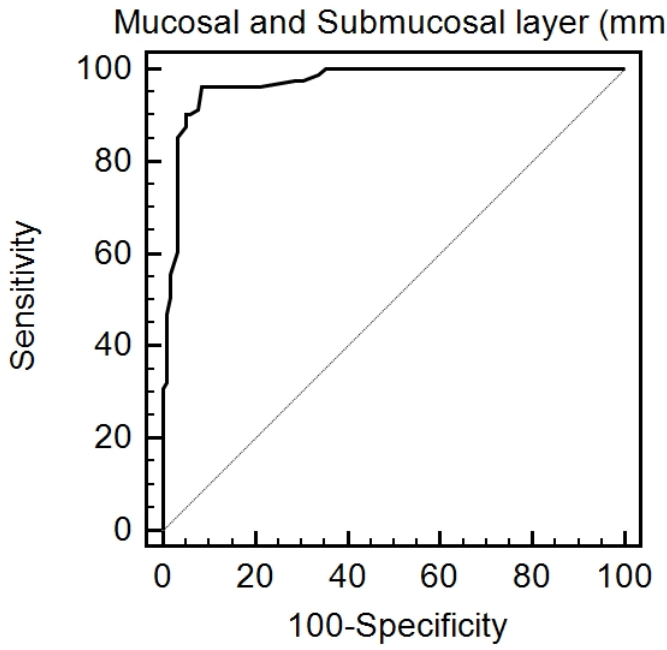


**Figure 9.** ROC curve analysis of mucosal and submucosal thickness.

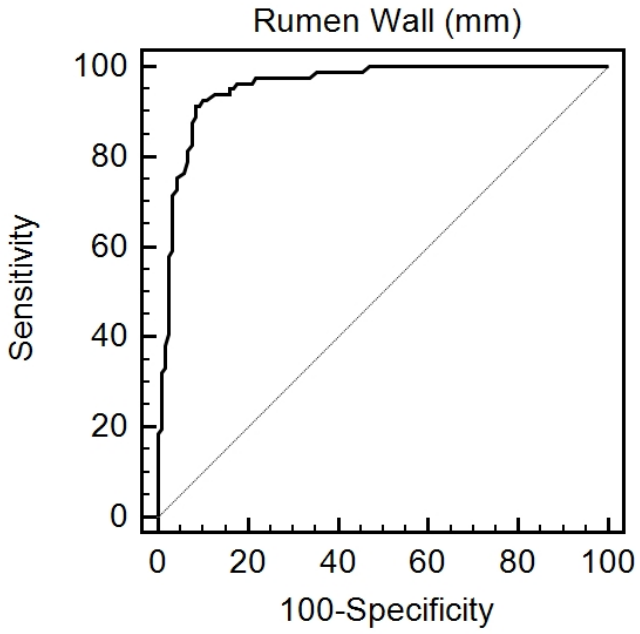


**Figure 10.** ROC curve analysis of rumen wall thickness.

The differentiation efficiency of rumen wall thickness between healthy and ruminal acidosis affected animals, as a result of ROC curve analysis, was

excellent with an area under the receiver operator curve (AUROC) of 0.956: *P*<0.0001; 95% CI: 0.918 - 0.980, positive likelihood ratio = 10.87, negative likelihood ratio=0.094. Using a cut-off value of 8.2 mm, sensitivity was 91,36% and specificity was 91.60 % (Figure 10).

## 5.3 DISCUSSION

The aim of this study was to assess the suitability of the transabdominal ultrasonographic examination of the ruminal wall to diagnose different stages of Acidosis in beef cattle. Our results show that the effect of increasing dietary concentrate on the ruminal mucosa during the fattening cycle. The first result reported after 10 days from the housing 41.55% of the fattening bulls had a rumen pH lower than the threshold value of 5.71. This lowering of the pH may be attributed to the sudden change in diet and the difficulty that the bulls may have to adapt. Diets with high-concentrate content leading only to moderate decline in ruminal fluid pH were consistently reported to trigger an increase in length of ruminal papillae, dilatation and hyperemia of mucosal a submucosal capillaries as well as submucosal edema, thereby causing a marked increase in thickness of ruminal mucosa (Dirksen et al., 1984; Zitnan et al., 2003; Cernik et al., 2011). The thickening of the ruminal mucosa is also well correlated with the lowering of ruminal pH. Increased concentrations of VFA in the rumen are widely accepted to be the main trigger for ruminal papillar growth wherever the mucosa is directly exposed to these acids (Clauss et al., 2009). It is suggested that the adaptation of morphological dimensions of ruminal epithelia can be

functionally fast and strong, because the epithelia have to cope with the rapid increase in VFA load to which they are exposed.

Morphological changes in ruminal mucosa in response to volatile fatty acids (VFA) concentration and rumen fluid pH are well established (Hernández et al., 2014); rumenitis is a frequent sequel to rumen acidosis; an increased production of volatile fatty acids, particularly butyrate and propionate, as well as a temporary rise in the ruminal lactate concentration and fluctuations in the osmolality of the rumen fluid lead to this condition (Enemark, 2008). The stage between parakeratosis (thickening of the stratum cornea of the rumen mucosa) and rumenitis appears undefined (Dirksen, 1985). The growth of ruminal epithelium has been shown to be directly linked to the non-structural carbohydrates presence in the tissue. Propionic and butyric acid are promoting the growth of the ruminal papillae, thus providing a higher absorption from the rumen by the mucosa, but, in a low ruminal pH, with excessive amount of VFA, will lead to a parakeratosis of the ruminal epithelium, and this parakeratosis will lead to rumenitis, particularly the presence of micro abscesses within the ruminal mucosa, favoring to incorporate with the bloodstream of the different ruminal bacteria, especially among others, with *Fusobacterium necrophorum* and *Arcanobacterium* pyogenes, colonizing the liver tissue and from there spreading to other organs like kidneys, heart, and lungs and promoting the parakeratosis-rumenitis liver abscesses complex (Joaquín Hernández, 2014).

Transabdominal ultrasonography of the rumen mucosa has the potential to be a suitable diagnostic tool to identify fattening bulls affected by SARA. This study provides encouraging preliminary data warranting further examination and validation in fattening bulls.

## 6. CONCLUSIONS

The first achievement of our Trials was to evaluate if rumenocentesis was a technique applicable to beef cattle as it was with dairy cows. The result was positive as long as you have available an effective containment system for animals. None of the studied animals has suffered damage or injury as a result of the procedures for the collection of samples. Having an effective restraint system enabled us to perform the rumenocentesis and subsequent sample collections as well as the ultrasound scans quickly and reducing stress for the animals involved.

Rumenocentesis can be used in beef steer too as well as in dairy cows as a standard routine to detect subacute ruminal acidosis, further studies should to be done in this are to increase our overall understanding on when peak of pH acidity occurs as it was done in dairy cows and see if it is comparable.

Increasing interest of issues regarding antibiotic resistant pathogens are becoming a concern above popular opinion. The European Union has banned supplemental anti-microbial, believing that the daily feeding of various antibiotics to livestock species entering the food supply will lead to a development of antibiotic resistant pathogens. Concern that the United States government will follow the European Union's lead in a ban on antibiotic usage has increased the need for research into alternative methods of improving livestock health and performance. This includes bacterial direct fed microbials, yeast, and yeast culture products such as yeast cell walls.

Numerous research trials have been conducted over the past century on the effectiveness of yeast and yeast cell wall supplementation on cattle performance and health. Unlike antibiotic growth promoters and treatments,

results with yeast supplementation have been highly variable. Under some circumstances, yeast supplementation seems to have beneficial effects on the health and performance of stressed calves. Dietary supplements, such as yeast cell wall, can improve the immune system and assist cattle during high stress periods. There are numerous reports indicating a positive effect on performance of yeast-supplemented cattle during various production phases.

The Trail I and II's results suggest that yeast supplementation do not have beneficial effects with all type of diet condition. Growing group's steer seems to have a better response at yeast live cell and yeast cell wall supplementation; that is really interesting because those are the animals just imported from France and then subjected to great stress due to the travel, new housing and of course new diet too. These positive physiological effects in association with the increased level of performance we observed indicate that supplementation of LY and YCW products could improve the total productivity of cattle during the receiving period. Additional studies should be performed on different breed and diets also considering the microbiological asset and population of the rumen.

Since public opinion is paying increasing attention to animal welfare and the quality of the productions, it should also be regarded as the yeast supplementation affect, on different kind of diets, the meat quality at the slaughter house and how rumen wall is like at the post-mortem visit.

The third trial of this study provides encouraging preliminary data on rumen wall ultrasonography in beef steers, using the right probe it is possible to accurately measure and discriminate the different layers of the rumen wall.

Transabdominal ultrasonography of the rumen wall was easily applicable and has the potential to be a suitable diagnostic tool to identify fattening bulls affected by SARA.

However, as for the rumenocentesis, an effective restraint system is required to perform this exam in a safe way for the operator and even for the animal.

Although the sample of the animals of our test was high for the third trial we had considered just a breed, Charolaise bulls, other breeds should be considered to see if there is a species-specific effect.

Further studies should merge also our trials arguments and demonstrate how yeast live cells and yeast cell wall may affect the rumen wall layers and how they appear at ultrasound scan.

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