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Article

Growth Performance of Buffalo Calves in Response to Different Diets with and without *Saccharomyces Cerevisiae* Supplementation

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Simple Summary: Buffalo breeding in Italy significantly increased in the new century, because of mozzarella cheese, the main product derived from buffalo milk. Indeed in recent years a growing interest was seen also meat production. The nutritional factors that significantly affect animal performance are: the forage/concentrate ratio, diets' energy and protein content, supplementations (i.e. vitamins, minerals, additives) and the feeding systems. The ban of the use of antibiotics for auxinic purpose (Reg. 1831/2003/EC) increased the interest for feed additives potentially able to modulate rumen metabolism, thus enhancing nutrient utilization and animal performance. Among such additives, yeast cultures of *Saccharomyces cerevisiae* (SC) have found great interest in ruminant nutrition. In such contest, the aim of the present trial was to evaluate the growth performance of buffalo calves fed diets characterized by different forage/concentrate ratio, with or without the *Saccharomyces Cerevisiae* supplementation.

Abstract: The aim of the present trial was to evaluate the growth performance of buffalo calves fed diets characterized by different forage/concentrate ratio with or without the *Saccharomyces Cerevisiae* supplementation (CBS 493.94, Yea-Sacc®). Twenty-four male buffalo calves (mean age 145.1 ± 16.1 days; mean weight 108.0 ± 18.7) were assigned randomly to 4 groups, homogeneous for age, fed four different diets: diet 1 F:C ratio 50:50; diet 2 F:C ratio 30:70; diet 3 F:C ratio 50:50 + Yea-Sacc® and diet 4 F:C ratio 30:70 + Yea-Sacc®. Buffalo calves were individually weighted before the start of the experiment and the data were used as a covariate, monthly until the end of the trial. Dry matter intake (DMI), daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. The differences in diets' composition significantly ($p < 0.01$) affected all these parameters. In particular, the animals fed diet 1 and diet 3 showed higher DWG (0.91 and 0.88 g/d vs. 0.68 and 0.66 for group 2 and 4) and DMI (5.8 and 5.3 kg/d, respectively) compared to the other groups (4.3 and 4.4 kg/d, for group 2 and 4), as well as final body weight (370.5 and 334.1 kg for group 1 and 3 vs. 272.8 and 273.1 kg of group 2 and 4, respectively). Indeed, the supplementation with Yea-Sacc® at the dosage of 1×10^{18} did not affect buffaloes' growth performance.

Keywords: buffalo calves; *Saccharomyces cerevisiae*; growth performance

1. Introduction

In recent years, the interest in buffalo breeding in Italy has increased significantly, thanks to the main product derived from buffalo milk, Mozzarella di Bufala Campana, which received the Protected Designation of Origin (DOP) in 1996. As a consequence, an increase in the buffalo population from around 200,000 heads in 2000 to over 400,000 has been reported, as reported by ANASB in 2020 (1, 2). Furthermore, buffalo meat has gained more and more popularity in recent

years, because of the nutraceutical properties highlighted by some studies (3-5). mainly concerning its low fat and cholesterol content, so much that it has been defined "the healthiest meat among red meats intended for human consumption" (6).

The dietary factors that significantly affect animal performance and meat quality are: energy and protein level, the forage/concentrate ratio, the supplementations (i.e. vitamins, minerals, additives) and the feeding systems. Terramoccia et al. (7) and Iommelli et al. (8) reported a better degradation of crude protein (CP) in buffaloes compared to bovines. Other studies demonstrated higher digestibility of roughage in buffaloes compared to cattle (9-10). Scientific data regarding growth and physiological response of buffalo calves fed different diets during and after weaning is scarce. Also, it's difficult to compare the studies in which the growth and physiological response of buffalo calves to dietary treatments have been evaluated mainly because the term "buffalo calf" is used for animals with a body weight ranging between 40 and 220 kg (11).

The ban of the use of antibiotics for auxinic purpose (12) has led to an increasing interest for those feed additives that being able to modulate rumen metabolism, may enhance nutrient utilization and animal performance (Newbold et al., 1996). Among these, yeast cultures of *Saccharomyces cerevisiae* (SC) have found great interest in ruminant nutrition (13). SC is able to grow rapidly in the rumen and to facilitate fiber digestion. The micro-nutrients in SC also stimulate cellulolytic bacteria growth. In addition, *Saccharomyces cerevisiae* protects ruminal fermentation from lactic acid accumulation (14). According to the theory proposed by Newbold et al. (13), SC in the rumen environment can utilize the remaining dissolved oxygen, saving anaerobic microorganisms from the toxic effect of oxygen, finally resulting in a higher digestion rate and a better growth performance (15). Indeed, reports on performance responses of ruminants fed on yeast culture are controversial. Growth performance were similar or reduced according Mutsvangwa et al. (16) and Kamra et al. (17), whereas other author reported an increase of weight gain, feed intake and feed conversion rate after yeast supplementation (18-19).

Weaning represents a critical period for calves, due to a multifactorial stress as it incorporates nutritional, physical and psychological factors, which cause stress and, consequently, several negative effects on performance, including an increase in the mortality rate of calves.

In such contest, the aim of the present trial was to evaluate the growth performance of buffalo calves fed diets, characterized by different forage/concentrate ratio with or without the *Saccharomyces Cerevisiae* supplementation.

2. Materials and Methods

2.1. Study Site

The experiment was conducted at the Regional Experimental buffalo farm "Improsta" located in Eboli (Salerno province) characterized by Mediterranean sub-continental climate, with annual mean temperature of 15.2° C and average annual rainfall of 842 mm.

The trial was performed from March to October 2019 according to the Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and approved by the local Bioethics Committee (protocol number: 2019/0013729 of February 2019).

2.2. Experimental Diets

Two experimental diets were formulated and administered to the buffalo calves with or without the supplementation of a commercial product Yea-Sacc® (Alltech Inc., Dunboyne, Co. Meath, Ireland), a yeast culture of *Saccharomyces cerevisiae* CBS 493.94, added to the diets in ratio of 1×10^{10} . The diets were formulated as follow:

- 1) F:C ratio 50:50
- 2) F:C ratio 30:70
- 3) F:C ratio 50:50 + Yea-Sacc®
- 4) F:C ratio 30:70 + Yea-Sacc®

In Table 1 the diets characteristics are reported. *Saccharomyces cerevisiae* was daily added to 500 g of concentrate.

Table 1. Ingredients and chemical composition of the experimental diets.

Diet	Unit	1	2	3	4
Supplementation		.	.	Yea-Sacc®	Yea-Sacc®
F:C ratio		50/50	30/70	50/50	30/70
Ingredients					
Wheat bran	%	30.7	61.2	30.7	61.2
Corn meal	%	18.4	9.2	18.4	9.2
Aalfalfa hay	%	49.2	.	49.2	.
Mixed hay*	%	.	27.6	.	27.6
VMS1**	%	1.7	.	1.7	.
VMS2***	%	.	2.0	.	2.0
Yea-Sacc®	%	.	.	0.23	0.23

Diet 1: F:C ratio 50:50; Diet 2: F:C ratio 30:70; Diet 3: F:C ratio 50:50 + Yea-Sacc®; Diet 4: F:C ratio 30:70 + Yea-Sacc®. **Phleum Pratense L., Lolium italicum L., Trifolium pratense L.* ** VMS1: vitamin-mineral supplementation (0.46% Vitamin mix ADE: Vit. A: 8.000.000 UI, Vit D: 200.000 UI, Vit E: 3.000 mg; 0.46% buffer mix: calcium: 25%, phosphorus: 1%; 0.77% Phosphorus mix: calcium 5%, phosphorus: 15%). *** VMS2: vitamin-mineral supplementation (0.46% Vitamin mix ADE: Vit. A: 8.000.000 UI, Vit D: 200.000 UI, Vit E: 3.000 mg; 1.53% buffer mix: calcium: 25%, phosphorus: 1%).

2.3. Chemical Composition

Samples (1 kg) of each diet were monthly collected before feeding and analysed according to AOAC (20) procedures. In particular, diets were milled to pass a grid of 1.1 mm and analysed for dry matter (DM), crude protein (CP) and ether extract (EE). Moreover, structural carbohydrates fractions, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), were analysed according to Van Soest et al. (21). The starch content was determined through polarimetric detection (Polax L, Atago, Tokyo, Japan) as suggested by the official procedure (22). The physically effective NDF (peNDF), useful to guarantee adequate ruminal activity and the maximum effectiveness of rumen function, was measured with the support of Penn State Particle Separator (PSPS), consists of 3 meshes of 19 mm, 8 mm and 4 mm. The peNDF concentration was evaluated considering the percentage of particle fraction retained (greater than 4 mm) multiplying by the percentage of NDF in TMR sample (23). UFL (unité fourragère du lait) was calculated according to INRA (24) equation.

2.4. Animals

Twenty-four male buffalo calves (mean age 145.10 ± 16.12 days; mean weight 108.00 ± 18.72) were recruited. The animals were divided into 4 groups homogeneous for age and randomly assigned to a different dietary treatment. Feeding of the animals was carried out by TMR once a day at 9:00 AM. All animals were housed in well-ventilated sheds provided with individual feeding and watering arrangements and dewormed and vaccinated according to farm protocol before the start of the experiment. The trial lasted 240 days and the dry matter intake (DMI) was registered daily by the difference between feed offered and refusals. Buffalo calves were individually weighted before the start of the experiment and the data were used as a covariate, and successively each month until the end of the trial. In addition, daily weight gain (DWG) and feed conversion ratio (FCR) were calculated.

2.5. Statistical Analysis

Data were analysed using a one-way ANOVA with group (1, 2, 3 and 4) as factors. Initial body weight of buffalo was used as covariate factor. The differences were considered significant at $p < 0.05$.

All the statistical procedures were performed using JMP software (version 14; SAS Institute, Cary, NC, USA).

3. Results

In Table 2, the chemical composition of the diets is reported. The differences in diets' composition affected their energy content (UFL 0.84 for diet 1 and 3 vs. 0.86 for diet 2 and 4, respectively). The peNDF content in all the tested diets was appropriate for growing buffaloes (25).

Table 2. Chemical composition of the experimental diets.

Diet	Unit	1	2	3	4
Supplementation		.	.	Yea-Sacc®	Yea-Sacc®
Chemical composition					
CP	% of DM	16.0	15.6	16.0	15.6
NDF	% of DM	46.4	42.4	46.4	42.4
ADF	% of DM	33.6	30.2	33.6	30.2
ADL	% of DM	11.3	8.4	11.3	8.4
EE	% of DM	3.6	3.2	3.6	3.2
peNDF	% of DM	57.8	50.6	57.8	50.6
Starch	% of DM	20.8	22.4	20.8	22.4
UFL	% of DM	0.84	0.86	0.84	0.86

Diet 1: F:C ratio 50:50; Diet 2: F:C ratio 30:70; Diet 3: F:C ratio 50:50 + Yea-Sacc®; Diet 4: F:C ratio 30:70 + Yea-Sacc®. CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: lignin detergent fibre; EE: ether extract; peNDF: physically effective NDF; NFE: nitrogen free extract; UFL: feed units for lactation.

The dietary treatment significantly affected DMI, final body weight and DWG of buffaloes (Table 3). In particular, animals fed diets 1 and 3 showed the highest values compared to the other groups ($p < 0.01$). No differences were detected for FCR. The supplementation with Yea-Sacc® at the dosage of 1×10^{10} did not affect the buffalo's growth performance.

Table 3. Growth performance of buffalo calves fed the experimental diets.

Group	DWG, g/d	Initial body weight, kg	Final body weight, kg	DMI, kg/d	FCR
1	0.91A	137.8	378.5A	5.8A	6.37
2	0.68B	94.0	272.8B	4.3B	6.32
3	0.88A	101.4	334.1AB	5.3A	6.02
4	0.66B	98.8	274.1B	4.4B	6.67
RMSE	0.21	6.10	27.79	0.313	0.561

Group 1: F:C ratio 50:50; Group 2: F:C ratio 30:70; Group 3: F:C ratio 50:50 + Yea-Sacc®; Group 4: F:C ratio 30:70 + Yea-Sacc®. DWG: daily weight gain; DMI: dry matter intake; FCR: feed conversion ratio. A, B, C: values on the same row with different superscripts differ ($p < 0.01$). RMSE: root mean square error.

4. Discussion

In this trial, the effects of two different diets, characterized by different forage/concentrate ratio with or without the supplementation of *Saccharomyces cerevisiae* strain CBS 493.94 were investigated. Results showed that the diets with the higher energy and protein level (diets 1 and 3) were able to increase dry matter intake, final body weight and daily weight gain. Comparative studies on the digestive physiology and nutritional needs of buffalo highlighted a greater capacity of fiber utilization compared with cattle and sheep, thus resulting in a better utilization of diets characterized by high complex structural carbohydrates (26). Moreover, in vitro studies demonstrated a higher organic matter utilization by the rumen microorganism in buffalo than in bovine (27-30). Despite that,

in this trial an improvement of buffaloes' growth performance was observed in the groups fed the diets characterized by a high energy value and a lower forage/concentrate ratio. This result is in agreement with those of Abdel Raheem et al. (31) who compared four different diets, characterized by F:C ratio of 80:20, 75:25, 60:40 and 55:45. These authors found an increase of dry matter intake, daily weight gain, final body weight by increasing the concentrate percentage in the diet. They hypothesized that the increase of DMI could be ascribed to the higher palatability of the concentrate compared to the roughage. Also, DMI was strongly influenced by dietary NDF. It is known that forages must constitute at least 40% of the ruminant diet to maintain adequate functionality and physiology of the rumen (32). The high fiber content is the main nutritional difference between forages and concentrates, resulting in a lower energy value of forages. Due to the high forage content in ruminant feed, optimizing forage particle size is a significant feeding strategy to improve forage utilization for ruminants (33). Indeed, it has been well documented that increasing peNDF content in the diet leads to more time spent for ruminating and chewing (34-35) with a positive effect on rumen pH, reducing the risk of sub-acute and acute ruminal acidosis (33). Llonch et al. (36) reported that a percentage of pe-NDF between 6.4% and 15.4% in the diet of beef calves lead to a linear increase in daily rumination time. In our trial, the peNDF content of all the diets was appropriate for growing buffaloes (24).

The inclusion of *Saccharomyces cerevisiae* in the diets has been reported to improve feed intake starting from weaning, by stabilizing ruminal pH and improving fiber digestion, and to stimulate the growth (directly or indirectly) of ruminal cellulolytic bacteria (37). In our trial, SC did not significantly affected growth performance in buffaloes. Contrasting results are reported in the literature. In studies carried out on lactating buffaloes, some authors highlighted differences in the production and composition of milk (3, 4) whereas other author did not found any difference due to the inclusion in the diet with SC (39).

Gamal et al. (40) found an increase in final body weight, daily weight gain and feed conversion ratio (FCR), but no differences in DMI in buffalo calves fed diet supplemented with SC (in ratio of 1%/kg) compared to the control one. The higher growth rate found in animals fed the yeast supplemented diets may be ascribed to an increased flow of microbial protein leaving the rumen and to a higher supply of amino acids in the small intestine as suggested by NagamalleswaraRao et al. (41). Such results are in agreement with those of Saha et al. (42) and Kumar & Ramana (19) who showed significant improvements in groups fed yeast culture added diet. Moreover, Kumar & Ramana (19) reported higher DMI ($p > 0.05$) in calves fed SC culture (CNCM I-1077 strain in ratio of 0.25 g/head/day) supplemented diets compared with the control group. Mutsvangwa et al. (16) found significantly greater dry-matter intake in bulls after the supplementation of Yea-Sacc® compared to control. Despite a similar average daily gain between groups, those authors reported that the FRC efficiency was not significantly improved by the supplementation ($P > 0.05$), in accordance with our results. On the contrary, Kamra et al. (16) found no difference in the body weight gain, feed intake, feed conversion efficiency in calves fed diets supplemented with yeast cell suspension (10ml containing 5×10^9 cells/mL) of *Saccharomyces cerevisiae* (strain ITCCF 2094). It is likely that the differences in the kind of yeast, as well as the dosage, the experimental conditions and the physiological periods of the animals may be responsible for the differences found in the literature.

5. Conclusions

This trial showed that the dietary forage/concentrate ratio affects buffaloes' growth performance in buffalo calves. On the contrary, no differences were found supplementing the diet with a commercial product based on *Saccharomyces cerevisiae* strain CBS 493.94 in ratio of 1×10^8 . Further studies are needed to better define the optimal amount and time of supplementation to achieve best results.

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