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Use of a commercial feed supplement based on yeast products and microalgae with or without nucleotide addition in calves

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ABSTRACT

The use of feed additives with antioxidant and immune response modulatory activity could be a useful strategy in suckling calves to reduce morbidity and mortality. This strategy is based on several feed additives tested for these purposes. The aim of the paper is the examination of a commercial feed additive for adult cows for use in calves, with and without nucleotide supplementation. Seventy-five Holstein Friesian male calves were divided in 3 groups, with each calf randomly assigned to a group according to birth order. All calves received 2 L of pooled colostrum within 2 h of birth. The commercial feed supplement group was orally administered with 5 g/head of Decosel (dried brewer's yeast lysate (*Saccharomyces cerevisiae*), brewer's yeast walls (*Saccharomyces cerevisiae*), diatoms, spirulina, barley flour, calcium carbonate; Agroteam srl, Torrimpietra, Italy) and the nucleotides + commercial feed supplement group was orally administered with 5 g/head of an additive containing 2.5 g of Decosel and 2.5 g of nucleotides once daily from birth to 25 d. The control group was orally administered 20 mL of fresh water/head once daily. Calves that received the supplement and the nucleotides showed lower rates of protein and metabolizable energy conversion, with longer villi and greater crypt depth in duodenum. Moreover, the commercial feed supplement alone increased antioxidant capacity [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and ferric-reducing antioxidant power] in plasma some activity of antioxidant liver enzymes, and peripheral blood mononuclear cell viability after in vitro concanavalin A and H_2O_2 stimuli. Dietary supplementation with a commercial feed supplement containing yeast products (yeast cell walls and hydrolyzed yeast) and microalgae enhanced the redox balance and gut morphology in calves, allowing calves to improve their immune response, increasing resistance to stress. Moreover, these beneficial effects were strongly potentiated when dietary nucleotides were added to the supplement.
Key words: calves, yeasts, peripheral blood mononuclear cell, redox balance

INTRODUCTION

Production of healthy and efficient calves plays a pivotal role in economic viability, sustainability and long-term success of the dairy industry. The preweaning and weaning periods are often associated with high prevalence of mortality and morbidity; thus, they are considered one of the most challenging periods in calf management (Fischer et al., 2019). This is confirmed by the frequent use of antimicrobials in the first weeks of calf life. Targeted dietary strategies are needed to guarantee profitable performance in intensive and large-scale farming. Accordingly, researchers turned to alternative milk feeding procedures and particularly to how different dietary components can support the growth and wellbeing of animals, prevent adverse events (Alizadeh et al., 2016; Alugongo et al., 2017; Jonova et al., 2021; Samarasinghe et al., 2021a,b), and promote animal health with beneficial effects on farm costs, as well as support a general decline in the use of antimicrobials in animals (Langford et al., 2003).

Among feed additives, yeasts, microalgae, and nucleotides have generated extensive interest by farmers and researchers (Yalçın et al., 2010; Shurson, 2018; Ding et al., 2021). Yeast-based products are notable for their heterogeneous composition, including AA, peptides, saccharides, enzymes, nucleotides, cofactors, and micronutrients (Shurson, 2018). Economic advantages of yeast compounds could be linked with

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high feed conversion, faster growth rates, and reduced mortality (Eicher et al., 2010; Nocek et al., 2011). But, such benefits are highly variable, depending on animal species, growth stage, diet, dose, and yeast features (Wallace, 1994). Several studies reported the beneficial effects of different forms of yeast on the health status of monogastrics and adult ruminants (Jiang et al., 2015; Salinas-Chavira et al., 2018; Shi and Kim, 2019; He et al., 2021), whereas literature describing performance responses of preruminants is still conflicting and variable, and few studies tested their effects under nonstressful conditions (Lesmeister et al., 2004; Pisoni and Relling, 2020). Concerted efforts to employ natural feed additives in the animal diet led to the discovery of nucleotides as effective nutritional supplements for specific physiological conditions including immune challenge, oxidative stress, nutrient digestion and morphological changes (Ding et al., 2021). Dietary nucleotides play a role in setting immune response and morpho-functional features of the gut, which are involved in growth processes during early life (Mohamed et al., 2020). As observed in nonruminant animals, dietary nucleotides may improve the proliferation of peripheral blood neutrophils and affect the maturation and differentiation of intestinal lymphocytes (Manzano et al., 2003; Lee et al., 2007). Moreover, recent studies also reported positive effects of nucleotides in immunomodulation in preruminant calves (Dinardo et al., 2022).

Over the past decade, microalgae gained global popularity as a feed additive due to their nutritional value and antioxidant content (Bleakley and Hayes, 2017). The promising prospects for economic sustainability and cost-effective production make microalgae a valuable feed supplement for the livestock industry. Inclusion of microalgae in animal diets positively affects meat quality and improves the productivity of ruminant and monogastric species by increasing their growth performance (Madeira et al., 2017), thereby increasing the efficiency of gain.

Thus, there is potential for several feed additives to improve health and growth performance of ruminants and monogastric animals, but several aspects still need to be clarified regarding their effectiveness in preruminants. Particularly, whereas yeast effects in adult ruminants are clear, the effect in preruminants is unknown, as is the effect of nucleotide inclusion with yeast. For these reasons, our study aimed to investigate the functional potential of a novel dietary supplement based on yeast hydrolysate, yeast cell wall, and microalgae in the newborn calf diet. Furthermore, the possible synergistic effect due to the combined use of the novel formulation and nucleotides was also examined under normal farm conditions.

MATERIALS AND METHODS

All animal use and procedures for the study were approved by the Ethical Committee for Animal Welfare of Animals employed in scientific research of the Department of Veterinary Medicine of the University of Bari (Approval n. 07/2020).

Animal Housing and Feeding

Seventy-five Holstein Friesian male calves were divided in 3 groups, with each calf randomly assigned to a group according to birth order. All the calves were born and reared on the same commercial dairy farm (Azienda Montecamplo, Laterza, Italy; 40°38′09″N 16°51′22″E). Soon after birth, each calf was moved to an individual 1.2- × 2.4-m stall and housed and managed as described by Dinardo et al. (2022). Before calf recruitment, 196 cows were milked within 4 h after calving on 96 farms in the Apulia region of Italy to collect colostrum that was then pooled. All the collected colostrum was stored at 4°C until reaching the volume of 1,000 L of total collected colostrum. The volume was reached in 36 h, so that the first colostrum collected was stored at refrigeration conditions no more than 36 h. All the colostrum was pooled and stored at −20°C in 2 L plastic bottles, so the colostrum fed to calves had the same composition. After colostrum pooling, Brix degrees were measured using a refractometer (Pal-Colostrum) and a sample was stored at −20°C separately for IgG concentration analysis through radioimmunoassay test (Triple J Farms). The Brix degrees were 26.4, and the IgG concentration was approximatively 90 g/L.

All calves received 2 L of pooled colostrum within 2 h of birth. Thereafter, calves received 2 L of the pooled colostrum for the next 5 feeding sessions, every 12 h, at 0700 and 1900 h. Colostrum was thawed in a 50°C water bath until a temperature of 38°C was reached. If calves did not suckle colostrum, they were fed through a plastic probe placed in the abomasum. After 3 d, 2 L of milk replacer was fed, through scaled buckets, provided with valves and teats, every 12 h. Milk replacer (DM 95.5%, CP 23.5%, fat 19.1%, lactose 49.9%; Elvor Starter 60) was diluted in water at 13% (130 g of milk powder in water brought up to 1 L) and administered within 1 h from preparation at the temperature of 39°C. During the trial, all the calves consumed the provided amount of milk replacer without refusals. Fresh calf starter (DM 87.40%, CP 17.95%, NDF 14.1%; UniCalf19, Plantamura Carlo Alimenti Zootecnici) was offered ad libitum beginning at d 1 in a manger, and intake was recorded daily, weighing administered calf starter and its individual daily refusals. Fresh, clean water was offered free choice. The commercial feed supplement

Table 1. Additive chemical composition and nucleotide aminoacidic composition

Item	Percentage
Gross nucleotide chemical composition	
Moisture	7.70
Protein	55.63
Free nucleotides sodium salt ($7H_2O$)	55.87
Total nucleic acids	74.12
Nucleotide composition (total nucleotides = 100%)	
Cytidine monophosphate	21.50
Uridine monophosphate	25.71
Adenosine monophosphate	24.21
Guanosine monophosphate	28.57

group (**SG**) was orally administered with 5 g/head of Decosel [dried brewer′s yeast lysate (*Saccharomyces cerevisiae*), brewer′s yeast walls (*Saccharomyces cerevisiae*), diatoms, spirulina, barley flour, calcium carbonate; Agroteam srl] and the nucleotides + commercial feed supplement group (**NSG**) was orally administered with 5 g/head of an additive containing 2.5 g of Decosel and 2.5 g of nucleotides (Table 1) once daily from birth to 25 d. Additives were mixed with water to obtain a slurry, which was then administered directly in the mouth using a large syringe, as described by Maggiolino et al. (2019). The dose used in the present trial was indicated by the manufacturer, according to previous empirical trials. The control group (**CG**) was orally administered 20 mL of fresh water/head once daily. All the calves were clinically monitored by a veterinarian. During the trial, 18 calves (6 SG, 5 NSG, and 7 CG calves) showed diarrhea. Fifteen were excluded from the trial (5 from SG, 5 from NSG, and 5 from CG) as they needed antimicrobial therapy, whereas the other 3 had a total recovery from clinical signs after hydration therapy lasting no more than 3 d and continued to be included in the trial. The trial lasted 4 mo, from March to June 2021.

Blood and Tissue Sampling

Blood samples were collected from each calf at T0 (within 2 h after birth and before first colostrum and additive administration), T3 (d 3 of life and immediately before the sixth colostrum feeding), T7 and T15 (d 7 and 15 of life, respectively), and T25 (slaughter). Blood was drawn from the jugular vein through sterile Vacutainer tubes (Becton Dickinson and Co.). At all blood sampling time points described above, 9 mL of lithium-heparin (plasma) and 9 mL of clot activator (serum) tubes were collected. All the tubes were centrifuged at the farm (1,500 × *g* for 10 min at room temperature), and both serum and plasma were stored immediately at −20°C until analysis. Four 9-mL EDTA tubes for peripheral blood mononuclear cell (**PBMC**) analysis were collected at T25 and transported at room temperature to the laboratory no more than 2 h after sampling. After blood sampling at T25, calves were transported 67 km to an abattoir and slaughtered. Soon after evisceration, a 5-cm-long duodenum sample was taken at 20 cm from the pylorus from each calf and fixed in 10% buffered formalin. Moreover, a sample of liver (100 g) was taken and stored at −20°C until analysis.

Growth Performance Parameters

All calves were weighed at birth (before first colostrum) and at 25 d of life (slaughter). In addition, the total amount of milk and starter fed to each calf from birth to 25 d of life was recorded daily. As the quantity of milk replacer and calf starter intake in each experimental group was not different, all subsequent parameters were calculated from the combined intake of both feeds. Gross composition of both milk and starter were used to calculate DM, ME (Rauba et al., 2019), and CP intake of each calf. To determine DM conversion rate, ME conversion rate, and CP conversion rate, the amounts of DM, ME, and CP necessary for a weight gain of 1 kg were estimated, respectively.

Tissue Collection and Histological Measurements

For intestinal morphological evaluation, samples of the duodenum approximately 20 cm from the pylorus were taken from all the animals at the slaughterhouse and fixed by immersion in 10% buffered formalin for 24 h. After fixation, fragments were washed in distilled water, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin. Serial sections (5 μm) were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, were stained with haematoxylin and eosin (Zitnan et al., 2008). The height of villi and the depth of crypts were randomly detected with a 10× and 20× magnification using an HD camera (DS-Fi2 high-definition color camera, Nikon Corporation) connected to an optical microscope (Nikon Eclipse Ni-U). An image analysis system (NIS Elements BR, Nikon Corporation) was used to carry out morphometric analyses. Villi height was calculated as the average of at least 10 villi with intact lamina propria and measured from the tip of the villus to the villus-crypt junction. Five fields per slide were randomly selected to measure crypt depth, defined as the depth of the invagination between 2 adjacent villi.

Determination of Serum IgG Concentration

The concentration of IgG in serum samples was determined with commercially available radial immunodiffusion test (Triple J Farm). Briefly, 5 μL of each serum sample were transferred to a radial immunodiffusion plate containing antibovine IgG antibody within agarose gel. Three standard solutions of bovine serum (18.0, 147.2, and 280.3 mg of IgG/L) were provided in the radial immunodiffusion kit and included in each test plate. After a 20-h incubation at room temperature, precipitin ring diameters were measured and plotted on a concentration–diameter best fit line, established using standard sera. Samples were tested in duplicate.

Total Antioxidant Capacity and Antioxidant Enzyme Activity

Liver samples were homogenized (1:10, wt/vol) in 100 m*M* phosphate buffer, pH 7.4 with an electrically driven Teflon pestle. The homogenate was centrifuged at 4°C for 20 min at 7,000 × *g* and then the supernatants were collected for antioxidant parameter analysis. Separate plasma samples were analyzed without dilution. Total antioxidant capacity (ferric-reducing antioxidant power; **FRAP**) assay was used to measure total antioxidant potential as described by Benzie and Strain (1996) with a slight modification. Three milliliters of freshly prepared FRAP reagent (1 mL of a 10 m*M* tripyridil-s-triazine solution in 40 m*M* HCl plus 1 mL of 20 m*M* $FeCl_3$ in 10 mL of H_2O solution and 10 mL of 300 m*M* acetate buffer, pH 3.6) was incubated at 37°C for 40 min after mixing with 100 μL of plasma sample or supernatant. The absorbance of the reaction mixture was recorded at 593 nm and the antioxidant power was expressed as micromole Trolox equivalents per milliliter. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (**ABTS**) radical scavenging activity was assayed by the methods of Re et al. (1999) with some modification. Briefly, ABTS radical cation was produced by mixing 7 m*M* ABTS stock solution with 2.45 m*M* potassium persulfate and keeping the mixture in the dark at room temperature for 12 to 16 h before use. For the evaluation of antioxidant capacity, the solution was then diluted in PBS to obtain the absorbance value of 0.70 ± 0.02 at 734 nm. Plasma or supernatant samples (10 μL) were added to 990 μL of diluted ABTS radical cation solution and incubated at 30°C for 5 min. The reagent blank was prepared by adding 10 μL of PBS instead of the sample. Then, the scavenging of the ABTS radical cation was determined spectrophotometrically at 734 nm. Antioxidant activity was expressed as a percentage inhibition (I) of ABTS radical cation and calculated by the equation

$$\% \text{ I} = 100 \times (\text{Absorbance734 Control} - \text{Absorbance734 Sample})/\text{Absorbance734 Control}.$$

(Absorbance734 Sample and Absorbance734 Control stand for absorbance values respective to the control and the sample.) Each sample analysis was performed in triplicate.

All enzyme activities were measured as described by Maggiolino et al. (2020). The activity of superoxide dismutase (**SOD**; EC1.15.1.1) was measured using the method described by Misra (1985). The enzymatic activity was based on the 50% inhibition rate of epinephrine auto-oxidation at 480 nm. Activity of SOD was expressed as U/mL (in plasma) and U/mg protein (in liver tissue). Catalase (**CAT**; EC 1.11.1.6) activity was evaluated by following the decrease in absorbance of H_2O_2 at 240 nm (Clairborne, 1985). One unit of enzyme activity was defined as the amount of enzyme required to degrade 1 μmol of H_2O_2 in one minute. Activity of CAT was expressed as U/mL (in plasma) and U/mg protein (in liver tissue). Glutathione peroxidase (**GSH-Px**; EC1.11.1.9.) activity was determined as described by Gunzler (1986). The reaction measured the rate of glutathione (**GSH**) oxidation by tert-butyl hydroperoxide, catalyzed by GSH-Px. A constant concentration of GSH was maintained by the addition of exogenous glutathione reductase and NADPH, which converted the GSSG to GSH. The rate of GSSG formation was then measured by the change in the absorbance of NADPH at 340 nm. The GSH-Px activity was expressed as nanomole of NADPH oxidized per minute per milliliter (in plasma) and nanomole of NADPH oxidized per minute per milligram protein (in liver tissue). Protein concentration was determined by Bradford's method using BSA as standard (Bradford, 1976).

Peripheral Blood Mononuclear Cell and Monocyte Isolation

Peripheral blood was used to isolate PBMC using a Ficoll–Hypaque gradient as reported in Latronico et al. (2007). Briefly, 20 mL of whole blood, diluted 1:1 with cold 0.9% (wt/vol) sodium chloride solution (0.9%), was layered on 10 mL of Histopaque-1077 solution and centrifuged at 400 *g* × 35 min at 20°C. The white cell rings were recovered, washed twice with PBS, then pellets were suspended in Iscove's modified Dulbecco's medium (**IMDM**), without calcium and magnesium (Sigma Aldrich). Cells were counted in a Burker cell-counting chamber, and the viability of cells were obtained by Trypan blue dye exclusion (Sigma Aldrich). For the isolation of monocytes, PBMC suspension in IMDM supplemented with 10% fetal calf serum was

plated in 96-well flat-bottom microplates (100 μL, 2 × 10^6 cells/well). After incubation for 1 h at 37°C in 5% CO_2, nonadherent lymphocytes were collected and adherent cells, represented by monocytes (about 2 × 10^5 cells/well) were washed several times. Samples of PBMC suspension (100 μL, 2 × 10^5 cells/well) were plated in 96-well U-bottom microplates in serum-free medium and treated with concanavalin A (**ConA**, at final concentration of 5 μg/mL), LPS (at final concentration of 10 μg/mL), or H_2O_2 (at a final concentration of 1 m*M*) and cultured at 37°C in 5% CO_2. Nontreated PBMC represented the negative sample. After incubation for 20 h, the plates were centrifuged at 400 × *g*, 10 min, 20°C in a microplate centrifuge, then the culture medium was collected, centrifuged at 10,000 × *g* and supernatants were stored at −80°C until analysis, whereas cells were subjected to the viability assay. Successively, microcultures were generated with the resulting purified lymphocytes (6 × 10^4 cells in 200 μL; in flat-bottom 96-well plates) and employed for carrying out the lymphocyte proliferation test for phytohemagglutinin-M (from *Phaseolus vulgaris*; 2.25 μg/mL) and poleweed mitogen (**PKW**) lectin (from *Phytolacca americana*; pokeweed; 2.25 μg/mL), respectively, under culture conditions for 5 d.

Assessment of PBMC Viability and Proliferation

Cell viability or cytotoxicity of PBMC from the different groups of animals was detected using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (**MTT**) assay as reported by Di Bari et al. (2014). This assay is based on the reduction of MTT by the mitochondrial succinate dehydrogenase in viable cells, to a blue formazan product, which can be spectrophotometrically measured by a microplate reader. This assay was specifically identified to detect cell viability and proliferation because it allows to distinguish healthy cells from cells that, though still alive, have already lost their vital functions. Briefly, after removal of culture medium, cells were rinsed with PBS and incubated at 37°C, 5% CO_2 for 2 h with 0.5 mg/mL MTT. After centrifugation of the microplates, the reaction was stopped by removing the medium and the formazan crystals in the cells were solubilized with absolute ethanol. The absorbance values at 560 and 690 nm were recorded by means of a VersaMax Microplate Reader (Molecular Devices). The difference between the absorbance of each sample at 560 and 690 nm was calculated. Results are expressed as optical density.

For the PKW and phytohemagglutinin (**PHA**) tests, triplicate microcultures were incubated either with each of the 2 mitogens, respectively, dissolved in PBS, or with PBS alone (controls). Following 5-d incubation with the mitogens, lymphocyte proliferation was assessed upon inclusion of the nonradioactive thymidine analog 5-bromo-2'-deoxyuridine (**BrdU**; 100 μ*M*), in the microcultures, for 2 h, at 37°C, in the dark. Upon centrifugation of the microwell plates (250 × *g*; 20 min at room temperature) and removal of the supernatant, the cellular pellets were dried under a gentle stream of warm air (35°C) for 15 min, and kit denaturating solution (200 μL) was dispensed in the wells and left to react, at room temperature for 30 min. Incorporation of the nucleotide in proliferating cells was evaluated by an anti-BrdU monoclonal antibody, conjugated with peroxidase (7.5 U/mL; from Roche Diagnostics GmbH; Heil and Reifferscheid, 1992). Incubation with the monoclonal antibody was extended for 90 min. Upon removal of the reaction buffer and triple washing (1× kit washing buffer), 100 μL of ready to use substrate (tetramethylbenzidine) were added to the microwells, for a 30-min incubation, at room temperature, in the dark. The reaction was stopped by inclusion of 5 M sulfuric acid and the reaction was assessed by a Multiskan FC microwell plate reader (Thermo Scientific), at 450 nm wavelength. Results were expressed in terms of absolute absorbance (optical density).

Determination of Reactive Oxygen Species in PBMC and Monocytes

Intracellular free oxygen radicals were detected loading PBMC (2 × 10^5 cells/well in 96-well plates) or monocytes (2 × 10^5 cells/well in 96-well plates) with 10 μ*M* 2′,7′-dichlorofluorescein diacetate (**DCFH-DA**) in phenol red-free IMDM at 37°C for 30 min (Latronico et al., 2021). After incubation, the culture medium was removed, and cells were rinsed twice with PBS. Cells were resuspended in phenol red-free IMDM and the spectrofluorometric analysis was performed at 485 nm excitation/525 nm emission. Negative sample was represented by cells treated in the same experimental conditions only with DCFH-DA. Reactive oxygen species (**ROS**) production was expressed as arbitrary units of photoluminescence intensity.

Statistical Analysis

Different data sets were considered and subjected to statistical analysis. The growth performance data were subjected to analysis with mixed model according to the following model:

$$Y_{ijk} = \mu + \alpha_i + T_j + BW_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} represents the growth performance dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the fixed effect of the jth dietary treatment (j = 1, 2, 3), BW_{ij} is the birth weight as covariate, and ε_{ijk} is the error term.

Villus length and crypt depth data were subjected to analysis with mixed model according to the following model:

$$Y_{ijk} = \mu + \alpha_i + T_j + SW_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} represents the dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the effect of the jth dietary treatment (j = 1, 2, 3), SW_{ij} is the weight at slaughter as covariate, and ε_{ijk} is the error term.

Liver parameters were subjected to ANOVA according to the general linear model (**GLM**) procedure as reported in the following model:

$$Y_{ijk} = \mu + \alpha_i + T_j + \varepsilon_{ijk},$$

where Y_{ijk} represents the liver parameters as dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the effect of the jth dietary treatment (j = 1, 2, 3), and ε_{ijk} is the error term.

The ROS level parameters data were subjected to ANOVA according to the GLM procedure (SAS Institute Inc., 2018) as reported in the following model:

$$Y_{ijkl} = \mu + \alpha_i + T_j + H_k + (T \times H)_{jk} + \varepsilon_{ijkl},$$

where Y_{ijk} represents the ROS levels as dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the effect of the jth dietary treatment (j = 1, 2, 3), H_k is the effect of the kth H_2O_2 in vitro stimulation (k = 1, 2), $(T \times H)_{jk}$ is the binary interaction the jth dietary treatment and the kth H_2O_2 in vitro stimulation (jk = 1,...6) and ε_{ijk} is the error term. Pairwise comparison was performed by Bonferroni test.

Cell viability parameter data were subjected to ANOVA as reported in the following model:

$$Y_{ijkl} = \mu + \alpha_i + T_j + V_k + (T \times V)_{jk} + \varepsilon_{ijkl},$$

where Y_{ijk} represents the cell viability as dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the effect of the jth dietary treatment (j = 1, 2, 3), V_k is the effect of the kth in vitro stimulation (k = 1,...4), $(T \times K)_{jk}$ is the binary interaction of the jth dietary treatment and the kth in vitro stimulation (jk = 1,...12), and ε_{ijk} is the error term. Pairwise comparison was performed by Bonferroni test.

Plasma immunoglobulin, oxidative parameters and antioxidant enzymes were subjected to ANOVA according to the GLM procedure (SAS Institute Inc., 2018) as reported in the following model:

$$Y_{ijkl} = \mu + \alpha_i + T_j + D_k + (T \times D)_{jk} + \varepsilon_{ijkl},$$

where Y_{ijk} represents the dependent variables, μ is the overall mean; α_i is the ith calf random effect (i = 1,...60), T_j is the effect of the jth dietary treatment (j = 1, 2, 3), D_k is the effect of the kth days of life (k = 1,...5), $(T \times D)_{jk}$ is the binary interaction of the jth dietary treatment and the kth day of life (jk = 1,...15), and ε_{ijk} is the error term. A Tukey test was applied to evaluate the differences among means when the effect of time or the binary interaction of treatment × time was significant.

Significance was set at $P < 0.05$, and the results are expressed as least squares means and standard error of the means. All the analyses were performed using SAS software (SAS Institute Inc., 2018).

RESULTS

Growth Performance, Intestinal Morphology, and Serum IgG Concentration

Growth performance patterns of calves are reported in Table 2. The calves in the 3 experimental groups had similar DM conversion ratios. In contrast, ME and CP conversion ratios were higher in the NSG group than the CG group ($P = 0.0227$; $P = 0.0345$). The SG group had intermediate values between CG and NSG groups both for ME and CP conversion ratio. Figure 1 shows results of duodenal villus height and crypt depth. A significant increase ($P < 0.0001$) of villi height was found in SG and in NSG compared with CG, with higher values in NSG compared with SG ($P = 0.0188$). Moreover, CG showed lower crypt depth compared with SG ($P = 0.0212$) and NSG ($P < 0.0001$). Serum IgG concentrations are reported in Figure 2. Levels of IgG changed significantly during the experimental period, whereas no differences were found according to feeding treatments. Overall, the IgG concentration increased up to T3 ($P < 0.0001$) and then remained stable.

Total Antioxidant Capacity and Antioxidant Enzymes in Plasma and Liver Tissue

The total antioxidant capacity of plasma, expressed through FRAP assay, showed an increase of total anti-

Table 2. Growth performance parameters in control (CG) or supplemented (SG and NSG) calves for the first 25 d of life

Growth performance[1]	Treatment group[2]			SEM	*P*-value
	CG	SG	NSG		
Live weight at calving (kg)	42.70	42.97	43.01	1.54	0.2963
Live weight at slaughter (kg at 25 d)	56.89	57.44	59.37	1.21	0.4122
Combined DMCR (kg of feed/kg of LWG)	1.45	1.40	1.36	0.31	0.2822
Combined CPCR (kg of CP/kg of LWG)	0.33[a]	0.30[ab]	0.25[b]	0.02	0.0227
Combined MECR (Mcal/kg of LWG)	18.27[a]	15.73[ab]	12.91[b]	1.54	0.0345

[a,b]Different letters indicate significant differences (a, b: $P < 0.05$) among groups for each parameter.

[1]DMCR = DM conversion rate; CPCR = CP conversion rate; MECR = ME conversion rate. LWG = live weight gain.

[2]CG = control group; SG = yeast-microalgae group; NSG = yeast-microalgae and nucleotides group. Data are reported as LSM ± SEM.

oxidant capacity up to T7 in both SG and NSG groups ($P < 0.0001$). Further, the total plasma antioxidant capacity measured with FRAP assay was higher in SG and NSG than in CG ($P < 0.01$) from T3, and we observed higher values in NSG compared with SG from T7 to T15 ($P < 0.01$). In contrast, the ABTS assay recorded an increase in plasma antioxidant capacity at T3 ($P < 0.0001$) and decreased at T7 ($P < 0.0001$) both in SG and NSG groups, which were higher than the CG group, from T3 to T25 ($P < 0.0001$). The CG group, on the other hand, did not show any significant variation from birth to T25 in calves.

Plasma antioxidant enzyme activities and total antioxidant capacity are presented in Table 3. During the trial, SOD activity increased constantly both in SG ($P < 0.0001$) and NSG ($P < 0.0001$), with higher values compared with CG, from T3 to T25 ($P < 0.0001$). The trend in CG did not show any variation over time. Overall, NSG calves showed higher SOD levels than SG at T7 and T15 ($P < 0.0001$). Activity of GSH-Px increased ($P < 0.0001$) at T3, and then remained stable up to T25 in CG and SG. Conversely, the NSG group recorded an increasing trend throughout the whole experimental trial ($P < 0.0001$). Compared with CG, higher GSH-Px levels were recorded in both NSG and SG from T3 to T25 ($P < 0.0001$), as well as higher antioxidant activity in NSG compared with SG at T15 and T25 ($P < 0.0001$).

Figure 3 shows the antioxidant enzymes activities (SOD, GSH-Px, CAT) and total antioxidant capacity (FRAP and ABTS) in liver tissue. Relative to SG and CG, the NSG group had the highest GSH-Px ($P < 0.0001$), SOD ($P = 0.0220$), and CAT activities ($P < 0.0001$), as well as the highest maximum antioxidant capacity, evaluated with both FRAP and ABTS assays ($P < 0.0001$). Moreover, SG showed higher GSH-Px activity and FRAP values than CG ($P < 0.01$).

Peripheral Blood Mononuclear Cell Viability

Cell viability was assessed in isolated PBMC, and after treatment with LPS, ConA, H_2O_2 (Figure 4), PHA, and PKW (Figure 5). The challenge with ConA induced higher proliferative response in PBMC collected from NSG ($P = 0.0308$) compared with CG and SG. Moreover, after H_2O_2 stimulus, NSG showed higher cell viability relative to CG ($P = 0.0178$). The PKW stimulation showed a greater reaction for SG and NSG compared with CG ($P < 0.0001$)

Reactive Oxygen Species in PBMC and Monocytes Cytoplasm

Results of levels of intracellular ROS in isolated PBMC and after H_2O_2 stimulus are reported in Figure 6. Untreated PBMC from CG showed higher ($P = 0.0191$) intracellular ROS levels than those collected from SG and NSG. After H_2O_2 challenge, intracellular ROS levels increased significantly only in PBMC from CG ($P = 0.0208$).

Figure 7 shows results of intracellular ROS levels in isolated monocytes, before and after H_2O_2 challenge. In both CG and SG, intracellular ROS rose ($P = 0.0384$) after treatment of monocytes with H_2O_2, whereas no increase ($P = 0.0243$) was observed in H_2O_2-treated cells isolated from NSG.

DISCUSSION

Emerging evidence suggests that the use of some bioactive dietary supplements in calves' diets could be a suitable strategy to counteract the high prevalence of pathological events and, consequently, of high mortality and culling rates (Alugongo et al., 2017; Samarasinghe et al., 2021a,b). Some studies suggest that yeast prod-

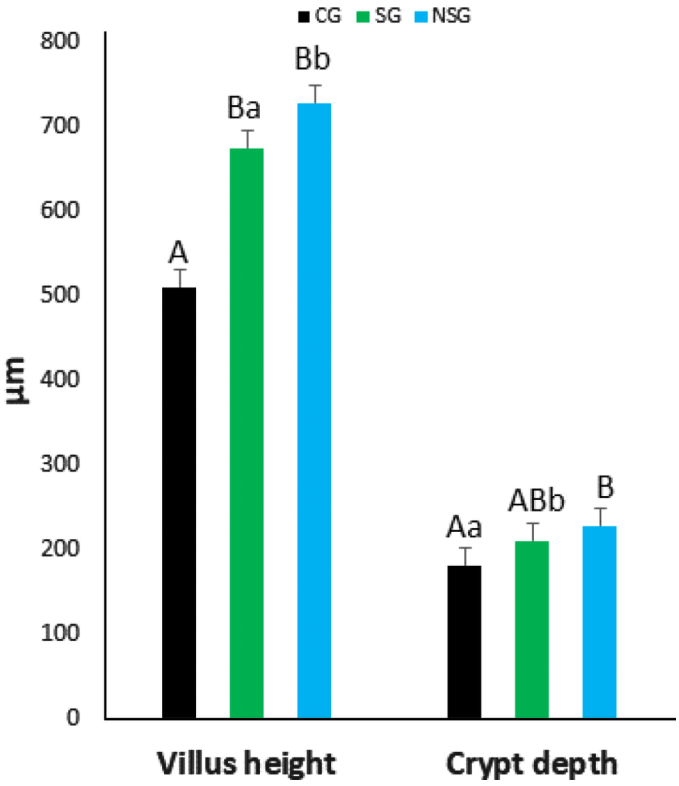

Figure 1. Effects of feeding treatments on gut mucosa morphology in control group (CG), yeast-microalgae group (SG), or yeast-microalgae and nucleotides group (NSG) of calves at 25 d of age. Data are reported as LSM ± SEM. Different letters indicate significant differences (A, B: $P < 0.01$; a, b: $P < 0.05$) among groups.

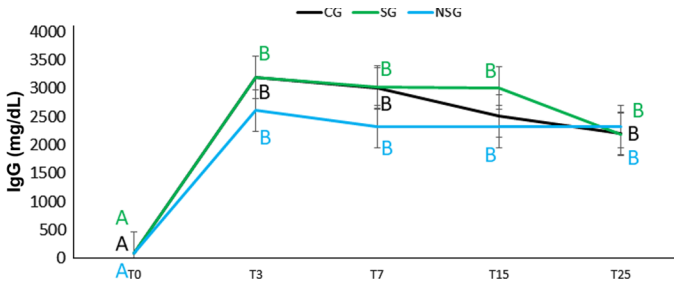

Figure 2. Serum IgG concentration (mg/dL) in control (CG) or supplemented (SG and NSG) calves for the first 25 d of life. Blood samples were collected at T0 (within 2 h after birth and before first colostrum and additive administration), T3 (d 3 of life and immediately before the sixth colostrum feeding), T7 (7 d of life), T15 (15 d of life), and T25 (slaughter at d 25). CG = control group; SG = yeast-microalgae group; NSG = yeast-microalgae and nucleotides group. Data are reported as LSM ± SEM. Different letters of the same color indicate significant differences (A, B: $P < 0.01$) among sampling days within each group.

ucts, as well as monocellular algae and spirulina are effective in improving immune response and antioxidant status in calves (Alugongo et al., 2017). Moreover, (Dinardo et al., 2022) reported that nucleotides positively affect immune function and oxidative profile in dairy calves. So, aiming to evaluate more than the effect of each single ingredient, but the potential synergistic effect, in the present research we hypothesized that a commercial product, composed of several bioactive ingredients useful for immune response and antioxidant

Table 3. Plasma levels of ferric-reducing antioxidant power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) in control (CG) or supplemented (SG and NSG) calves for the first 25 d of life

		Sampling day[2]						ANOVA		
Item	Treatment[1]	T0	T3	T7	T15	T25	SEM	Day	Treatment	Day × treatment
FRAP (µmol TE/mL)[3]	CG	55.45	56.83^{X}	56.57^{X}	55.47^{X}	55.62^{X}	0.45	<0.001	<0.001	<0.001
	SG	54.20^{A}	$70.61^{B,Y}$	$62.11^{C,Y}$	$62.83^{C,Y}$	$64.31^{C,Y}$				
	NSG	55.53^{A}	$61.14^{B,Z}$	$65.57^{C,Z}$	$65.38^{C,Z}$	$65.49^{C,Y}$				
ABTS (% I)[4]	CG	22.92	23.15^{X}	22.61^{X}	22.84^{X}	22.66^{X}	0.39	<0.001	<0.001	<0.001
	SG	23.21^{A}	$48.01^{B,Y}$	$33.77^{C,Y}$	$32.29^{C,Y}$	$33.35^{C,Y}$				
	NSG	22.63^{A}	$49.98^{B,Y}$	$33.76^{C,Y}$	$32.89^{C,Y}$	$33.79^{C,Y}$				
SOD (U/mL)	CG	8.40	8.34^{X}	8.26^{X}	8.04^{X}	8.46^{X}	0.14	<0.001	<0.001	<0.001
	SG	8.50^{A}	$9.67^{B,Y}$	$9.74^{B,Y}$	$13.33^{C,Y}$	$15.59^{D,Y}$				
	NSG	8.42^{A}	$9.25^{B,Y}$	$11.50^{C,Z}$	$14.67^{C,Z}$	$15.40^{D,Y}$				
GSH-Px (nmol of NADPH oxidized/mL)	CG	19.96^{A}	$25.48^{B,X,x}$	$25.02^{B,X}$	$25.38^{B,X}$	$25.43^{B,X}$	0.39	<0.001	<0.001	<0.001
	SG	20.40^{A}	$28.45^{B,Y}$	$30.09^{B,Y}$	$28.22^{B,Y}$	$29.43^{B,Y}$				
	NSG	19.77^{A}	$27.48^{B,XY,y}$	$30.87^{C,Y}$	$31.76^{C,Z}$	$35.30^{D,Z}$				

[A–D]Different letters indicate significant differences ($P < 0.01$) among sampling days within each group.

[x,y;X–Z]Different letters indicate significant differences (X–Z: $P < 0.01$; x, y: $P < 0.05$) among groups within each sampling day.

[1]CG = control group; SG = yeast-microalgae group; NSG = yeast-microalgae and nucleotides group.

[2]Blood samples were collected at T0 (within 2 h after birth and before first colostrum and additive administration), T3 (d 3 of life and immediately before the sixth colostrum feeding), T7 (7 d of life); T15 (15 d of life), and T25 (slaughter at d 25). Data are reported as LSM ± SEM.

[3]TE = Trolox equivalents.

[4]% I = percentage inhibition.

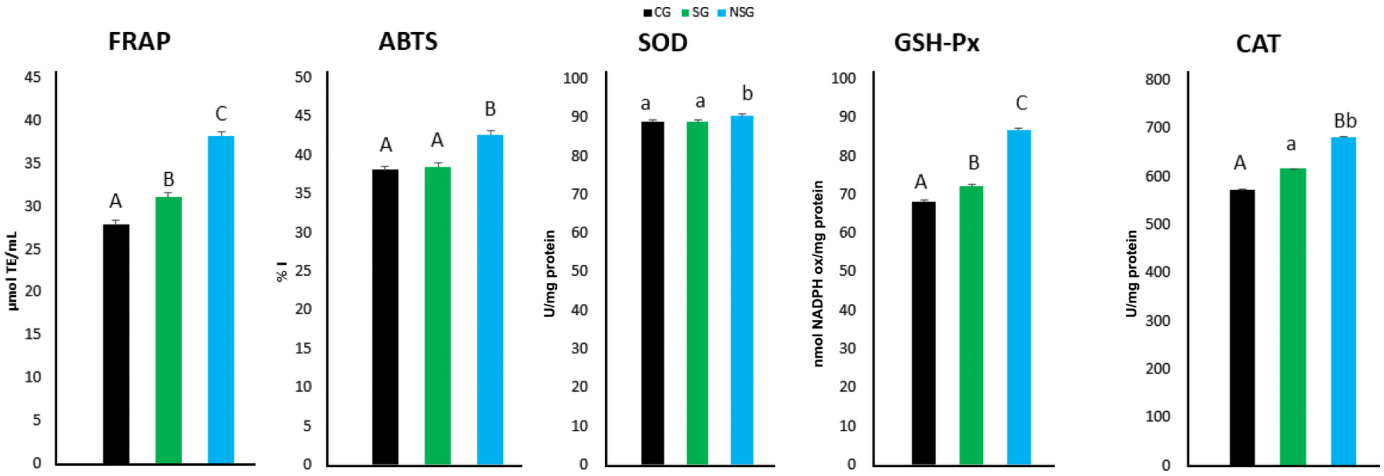

Figure 3. Liver levels of ferric-reducing antioxidant power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in control group (CG), yeast-microalgae group (SG), or yeast-microalgae and nucleotides group (NSG) of calves after the first 25 d of life. Data are reported as LSM ± SEM. Different letters indicate significant differences (A-C: $P < 0.01$; a, b: $P < 0.05$) among groups. TE = Trolox equivalents; % I = percentage inhibition; NADPH ox = NADPH oxidized.

activity in calves, could be improved in its efficacy with the addition of nucleotides.

Serum IgG concentration was monitored to gain a perspective on humoral immunity development. Levels of serum IgG reflected the typical developmental pattern in calves receiving colostrum, as shown by the peaks after 3 d. Colostrum confers passive immune protection to the newborn by the transfer of maternal immunoglobulins. Calves are capable of immunoglobulin absorption during a brief period up to 24 h after birth (Fischer et al., 2019), and becuase all animals received the same amount of colostrum, no changes in IgG levels were observed among experimental groups. Serum IgG levels were not improved by dietary supplementation, although administration of experimental additives started soon after calving and before first colostrum suckling bout, as reported by other authors in preweaning calves supplemented with dried seaweed (Samarasinghe et al., 2021b). We hypothesize that yeast, microalgae, and nucleotides have an indirect role in immune response regulation, primarily through the remodeling of intestinal mucosa after gut closure (Be-

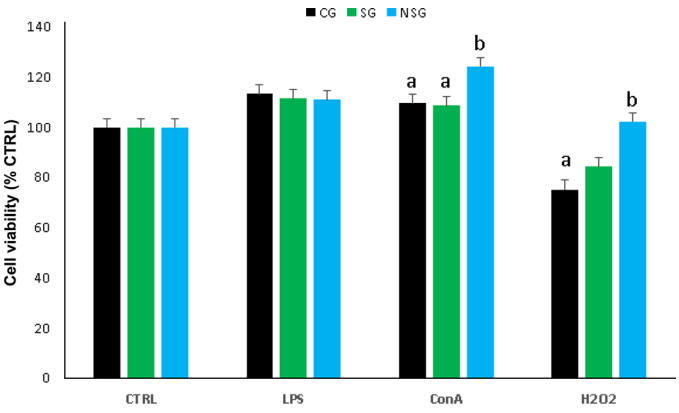

Figure 4. Effect of feeding supplementation and in vitro treatment on cell viability and proliferation of peripheral blood mononuclear cells (PBMC). Cell viability, expressed as a percentage of cell survival in comparison with control (CTRL), was detected by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay in PBMC from the different groups of animals untreated (CTRL) or treated for 20 h at 37°C, 5% CO_2 with LPS, concanavalin A (ConA), or H_2O_2. Samples were tested in triplicate. Values are mean ± SD (one-way ANOVA followed by Dunnett's post hoc test; a, b = $P < 0.05$).

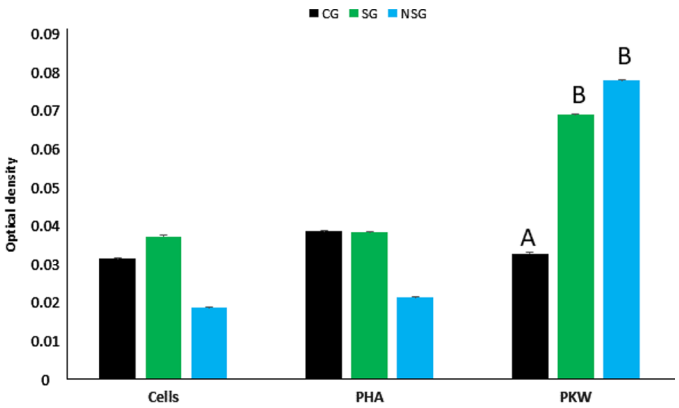

Figure 5. Effect of feeding supplementation and in vitro treatment on cell viability and proliferation of peripheral blood mononuclear cells (PBMC). Cell viability, expressed as optical density, was determined without stimulation (cells) after treating with phytohemagglutinin (PHA) and pokeweed (PKW). Samples were tested in triplicate. Values are mean ± SD (one-way ANOVA followed by Dunnett's post hoc test; A, B = $P < 0.01$).

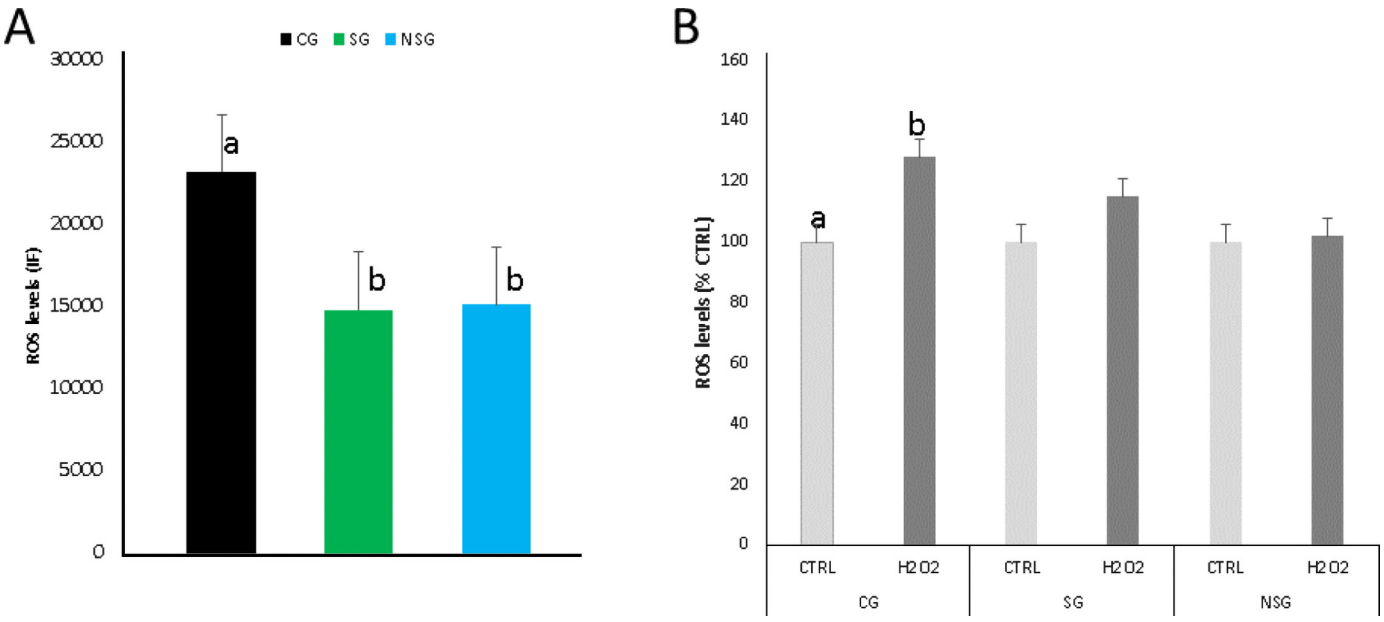

Figure 6. Production of reactive oxygen species (ROS) in peripheral blood mononuclear cells (PBMC). Histograms represent the ROS production in untreated (CTRL; A) and H_2O_2-treated (B) PBMC from the different groups of animals. Results in A are expressed as fluorescence intensity (IF). Results in B are expressed as a relative percentage of IF in comparison with the CTRL, which was set at 100%. Samples were assayed in triplicate. Data are mean values ± SD (one-way ANOVA followed by Dunnett's post hoc test; a, b = $P < 0.05$).

dirli et al., 2009; Valini et al., 2021). It is well known that intestinal tissues and immune response are closely related, not only for the immune origin of intestinal cells but also for the capability to produce immunomodulatory molecules (Dahan et al., 2007).

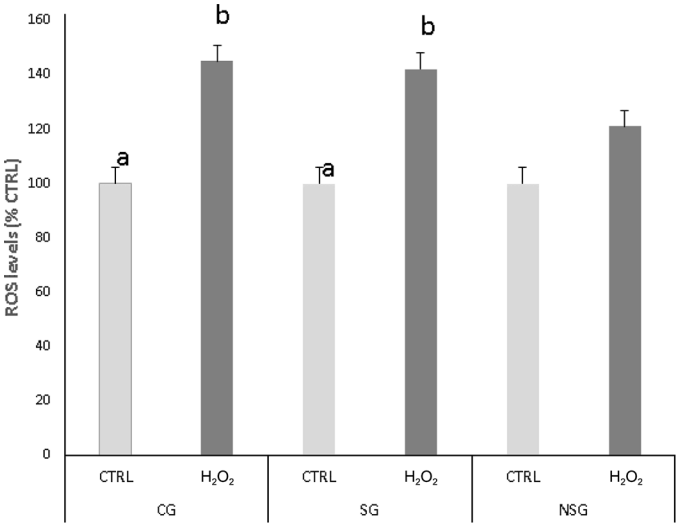

Figure 7. Production of reactive oxygen species (ROS) in monocytes. Histograms represent the ROS production in untreated (CTRL) and H_2O_2-treated monocytes from the different groups of animals. The ROS levels are expressed as a relative percentage of fluorescence intensity in comparison with CTRL, which was set at 100%. Samples were assayed in triplicate. Data are mean values ± SD (one-way ANOVA followed by Dunnett's post hoc test; a, b = $P < 0.05$).

Although no differences were observed in calf growth performance among experimental groups in the first 25 d of life, we recorded a significant improvement of protein and metabolic energy digestive efficiency in calves supplemented with the commercial supplement added with nucleotides. Several studies have been conducted on the use of yeasts in calves, reporting conflicting results. Some authors reported that yeasts could stimulate preweaning DM intake (Galvão et al., 2005; Pinos-Rodríguez et al., 2008), whereas others reported similar results after weaning (Lesmeister et al., 2004). In contrast, others report none of the above-mentioned results (Quigley et al., 1992). These differences could be due to factors such as the strain used, the nature of the diet or the physiological status of the animal as well as the dose and the feeding strategy (Lesmeister et al., 2004; Chaucheyras-Durand et al., 2008; Magalhães et al., 2008). Differently, some authors hypothesize their effect on energy intake and protein due to a significant increase of nutrient digestibility (Zhou et al., 2009). Some in vitro studies reported that yeast culture metabolites are characterized by the ability to inhibit pathogenic microorganisms and support the commensal bacteria (Jensen et al., 2008a). This ability could be a result of gut clearance of pathogenic bacteria and supporting the commensal fraction, favoring all processes for carbohydrate fermentation that result in greater VFA production (Brewer et al., 2014). Our results showed a lack of effectiveness of the commercial di-

etary supplement alone to improve digestive efficiency in calves, whereas the addition of nucleotides significantly improved digestive efficiency. These results are consistent with the higher absorption capacity found in jejunal mucosa, with higher villi and deeper crypts in calves supplemented with the Decosel added with nucleotides. The effectiveness of nucleotides in affecting intestinal mucosa, moreover, is consistent with results obtained by others in species such as chickens (Daneshmand et al., 2017) and pigs (Hernandez et al., 2000), as well as in calves (Dinardo et al., 2022), although other authors observed no differences (Samarasinghe et al., 2021a).

Villi play a key role in nutrient absorption and their anatomy is critical to calf growth efficiency (Daneshmand et al., 2017). Accordingly, yeast-derived saccharides affect gut development and function in chickens by regulating the expression of genes involved in mucosa integrity, uptake processes, and mucin secretion (Yang et al., 2007; Cheled-Shoval et al., 2011). In pigs, He et al. (2021) associated dietary yeast to polyamine-enhanced enterocyte proliferation. The greater development in villi structure observed in NSG could be related to the availability of nucleotides as DNA and RNA precursors in epithelial cell turnover (Alizadeh et al., 2016), combined with a synergistic effect with the biological effects of the yeast products contained in the Decosel. Dietary nucleotides may also preserve gut morphological integrity by alleviating inflammatory processes, as shown in piglets (Jang and Kim, 2019). Consequently, in animals fed with a nucleotide-supplemented diet, the absorbed nutrients may be fully employed for growth rather than for recovery from intestinal injury (Buccigrossi et al., 2010; Wang et al., 2018). In addition to the direct role of nucleotides in preserving or improving gut morpho-functional features, we should also consider the energy savings due to the unnecessary de novo synthesis of endogenous nucleotides that is very energy expensive (Grimble, 1996). In our study, the addition of yeast, microalgae and nucleotides to diets increased feed efficiency (ME and protein conversion rate), but without significant consequences for BW.

A complex interplay occurs between gut mucosal integrity, antioxidant defense, and immune response, which is meaningful for maintaining homeostasis in calves. Gut tissue provides the first line of defense against pathogens and remains the main anatomical location where yeast can play a significant role in immunomodulation (Alugongo et al., 2017). Several polysaccharides contained in yeast cell wall, including mannan-oligosaccharides and β-glucans, have been identified as modulators of immunity, and in vitro studies showed their antioxidant, anti-inflammatory, and immunomodulatory activities (Jensen et al., 2008a,b). Indeed, it has been suggested that yeast cell wall cannot penetrate the endothelium barrier, and only β-glucans are able to move from the lumen and interact with the immune cells stimulating the immune system (Wójcik, 2014). However, although several in vivo studies have been conducted in calves (Lynch and Martin, 2002), lambs (Wojcik, 2010), and piglets (Shen et al., 2009; Weedman et al., 2011), the mechanisms by which yeast products and, particularly, mannan-oligosaccharides and β-glucans stimulate immune response remain unclear, although it is widely recognized their modulation effects on innate immune functions through increasing phagocytosis, and bactericidal killing oxidative burst of immune cells (Rodríguez et al., 2003; Lowry et al., 2005).

At birth, the initiation of respiration elicits an increase in ROS production, and the imbalance between radical species and antioxidant enzymes also leads to inflammatory events (Hussain et al., 2016; Zhu et al., 2017). After birth, calves are exposed to an oxygen-rich environment for the first time once they start to breathe and this results in an increase in the production of ROS (Saugstad, 2003; Abuelo et al., 2014). If the production of ROS overwhelms the antioxidant capacity of the neonatal calf, oxidative stress can occur. This is known to play a key role in the initiation and maintenance of conditions such as diarrhea or pneumonia (Lykkesfeldt and Svendsen, 2007; Sordillo and Aitken, 2009). Furthermore, the overgeneration of ROS may result in poor growth performance and weakened immune response, if not countered by effective antioxidant defenses (Križková et al., 2006), considering that immunological and antioxidant systems are mutually complementary (Alugongo et al., 2017). However, colostrum is also a source of ROS, as it is rich in macromolecules, such as lipids and proteins that are easily oxidized, and has a significant population of immune cells, including macrophages, that use ROS generating systems to kill bacteria (Przybylska et al., 2007).

In the present study, dietary supplementation of yeast helped to maintain the redox balance through increased activity of antioxidant enzymes (SOD, CAT, and GSH-Px) and of total antioxidant capacity, particularly when nucleotides were added to the dietary supplement with yeast products and microalgae. The metabolites from yeast products are able to modulate the in vitro immune response, particularly inhibiting and reducing the oxidative stress and the inflammatory response (Jensen et al., 2007). Dietary yeast compounds consistently increase the activity of serum antioxidant enzymes in pigs and broilers (Li et al., 2016; Yang et al., 2016; Boontiam et al., 2020), and improve total antioxidant capacity in lactating ewes during the peripartum period, as well as in weaning piglets (Sauerwein

et al., 2007; Mavrommatis et al., 2020). The wide spectrum of biological responses orchestrated by dietary nucleotides might explain their heightened effects on the redox state of NSG. It is worth noting that dietary nucleotides exert their cytoprotective role both by neutralizing the hydroxyl radicals and by activating detoxifying enzymes and antioxidant proteins (Gudkov et al., 2006; Kaspar et al., 2009; Ma, 2013). In vivo studies in monogastric animals report the ability of exogenous nucleotides to preserve DNA against genotoxic effect induced by high oxidative loads (Frankič et al., 2006; Weaver and Kim, 2014; Hu et al., 2018), and other in vivo trials report their capacity to prevent some negative effects on oxidative status, including liver injury and intrauterine growth retardation through high SOD levels (Cai et al., 2016; Hu et al., 2018). Even if minor, yeast products and microalgae had a positive impact on calves' redox state. Some authors attribute the ability of yeast-derived glucans to prevent oxidative DNA damage to scavenging both hydroxyl radicals and singlet oxygen in a concentration-dependent manner (Khan et al., 2016; Tang et al., 2017). Microalgae extracts counteracted generation of ROS and superoxide anion in rat macrophages (Kong et al. (2019), and Tomaluski et al. (2021) found increased levels of serum SOD in preweaning calves. Furthermore, algae-derived polysaccharides were suggested to protect chicken intestinal mucosa from oxidative damage by enhancing SOD and GSH-Px enzyme activities (Liu et al., 2020). Based on the current study and considering that nucleotides alone are able to improve calf oxidative status (Dinardo et al., 2022), we hypothesize a synergistic effect among nucleotides, yeasts, and microalgae in NSG calves.

Supplementation with yeast and microalgae did not exert a proliferative effect on untreated and stimulated PBMC, except when treated with PKW. In contrast, the association with nucleotides showed a proliferative effect after H_2O_2, ConA, and PKW stimulation. Available information related to yeast product effects are conflicting (Hester et al., 2012; Zhen et al., 2020), with some studies attributing an antiproliferative effect on PBMC to microalgae extract (Ciaglia et al., 2017; Ciliberti et al., 2019). The effect of dietary yeast on cell proliferation depends on many factors including dose, mode, treatment duration or the structural and chemical properties of yeast components (Eicher et al., 2010). Considering the present results, proliferative effects may be attributed to nucleotides or to a synergistic mechanism involving other components of the supplement (Safaei and Hassanabadi, 2020). Indeed, the biological effect of nucleotides has been investigated and these substances play an immunomodulatory role in calves, promoting PBMC proliferation, which led to the hypothesis that they may improve immune responses in newborn calves (Mashiko et al., 2009; Dinardo et al., 2022). Discrepancies in proliferative responses to different stimulation and mitogen activity, as PHA and PKW, might be associated with interleukin expression of different cell types in isolated PBMC, considering that some of them stimulated B cells (i.e., LPS), whereas others stimulate T cells. Lymphocyte and monocyte stimuli may in turn cause preferential stimulation of cell subsets after binding to the specific receptors, activating different signal pathways that promote the cytokine production (Stanilova et al., 2006).

It is interesting to note that the ROS levels in PBMC and monocytes collected from SG and especially from NSG were lower compared with CG. Similarly, Jensen et al. (2008a) observed that yeast can significantly reduce ROS formation in polymorphonuclear cells, enhancing a positive response in animals that experience inflammation, and Dinardo et al. (2022) reported nucleotides can reduce ROS production in PMBC when orally administered in calves. Counteracting the oxidative stress, dietary supplementation of yeast in combination with nucleotides likely had positive effects on inflammatory signaling cascades. There is not a strict proliferative effect on PBMC calls but rather an increase in their efficiency, both innate and acquired, helping them to reduce ROS concentrations in case of activation. Indeed, oxidative stress and inflammation processes are deeply linked. Moreover, the activation of the immune response requires an effective antioxidant system, because the higher the immune activation, the higher the production of ROS that need to be metabolized by antioxidant systems (Hussain et al., 2016). Obviously, these aspects could be considered of crucial importance for those diseases in which the inflammatory response exacerbates the deleterious effects of the illness.

CONCLUSIONS

Our results suggest that dietary supplementation with yeast products, microalgae, and nucleotides enhanced the redox balance and gut morphology in calves, helping calves overcome dietary challenges by facilitating innate immunity, increasing resistance to stress and ultimately to pathogenic infection. On the other hand, these beneficial effects are strongly potentiated when dietary nucleotides were combined with yeasts. Nevertheless, our results do not fully explain the underlying mechanism of the reported positive effects. Although this study contributes toward the understanding of the immune regulatory mechanisms of these yeast, microalgae, and nucleotide dietary supplements in calves, more in-depth studies are required for a complete mechanistic understanding of such supplementation.

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