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Yeast cell wall supplementation modulates pre-weaning stress in male Nili Ravi buffalo calves

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Abstract

The effects of yeast cell wall (YCW) supplementation on growth performance, cell-mediated immune response, blood metabolites, and health scores in early-weaned male buffalo *(Bubalus bubalis)* calves were evaluated. Forty Nili Ravi buffalo calves were randomly divided into four groups and supplemented with four dietary treatments, namely control (animals without prebiotics), YCW-2 (yeast cell wall fed at 2 g/calf/day), YCW-4 (yeast cell wall fed at 4 g/calf/day), and cMOS-4 (commercial mannan-oligosaccharide fed at 4 g/calf/day). Milk intake, dry matter intake (DMI), and health scores were recorded daily, whereas body weight (body weight) and structural developments (hip height, wither height, heart girth, and body length) were recorded weekly. Feed efficiency and average daily gain (ADG) were calculated at the end of the experiment. Blood samples were collected fortnightly to determine glucose, non-esterified fatty acids, beta-hydroxybutyric acid (βHBA), blood urea nitrogen (BUN), catalase, malondialdehyde, creatinine, hepatic enzymes, cholesterol, triglycerides, and total serum protein profile. The cell-mediated immune response was determined by the application of dinitrochlorobenzene directly to the skin. The results of the study revealed that supplementation of YCW and cMOS increased DMI, body weight, feed efficiency, ADG, and structural growth in buffalo calves, whereas faecal scores were significantly improved in supplemented groups compared to the control. Glucose, BUN, and βHBA improved in supplemented animals more than in the control group, indicating bioactivity that contributed to the improvement of gut health. Supplementation with YCW improved the growth performance, physiological responses, and gut health of early-weaned male buffalo calves.

Keywords: faecal score, growth, male buffalo calf, serum chemistry, structural measurements
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Introduction

In Pakistan, buffalo calf mortality is high during the pre-weaning period (Ahmad *et al*., 2009) because the immunity level is low and the digestive system undergoes drastic changes from birth to three months old as microbes are introduced from the mother's birth canal and the environment (Eckburg *et al*., 2005). During the first month, intestinal microbes are highly unstable (Lucas *et al.*, 2007) and can cause diarrhoea, with a concomitant decrease in the intestinal surface area, digestibility, and absorption of nutrients (Signorini *et al*., 2012). Diarrhoea may be caused by *Escherichia coli*, *Salmonella* species*, Cryptosporidium*, and *Rotavirus* (Dar *et al.*, 2018).

Subtherapeutic doses of antibiotics were used widely in the past to minimize the incidence of intestinal diarrhoea but are now banned because of antibiotic resistance and residue deposition in animal products (Newman, 2002; Spring *et al.*, 2015). The alternatives to subtherapeutic antibiotics are probiotics, prebiotics,

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synbiotics, and organic acids, which are used widely in animal production. Yeast cell wall has prebiotic potential and is obtained from *Saccharomyces cerevisiae* (Quigley III, 2005; Hill *et al.*, 2009; Spring *et al.,* 2015). It binds to certain pathogenic bacteria and prevents microbial colonization in the intestine (Spring *et al.*, 2015; Szandra *et al.,* 2020).

Yeast cell wall contains mannan-oligosaccharide (MOS) and beta-glucan, which support the growth of beneficial bacteria in the intestine including *Bifidobacteria*, *Lactobacilli,* and *Streptococci,* which improve faecal consistency, stimulate the immune system, and promote animal performance (Heinrichs *et al.*, 2003; Baurhoo *et al*., 2007; Ghosh & Mehla, 2012). Yeast cell wall is extracted by the fermentation of waste products from the sugarcane and brewing industries. The fermentation of molasses produces alcohol, which is separated. The remaining waste product is known as distillery yeast sludge and contains an appreciable amount of dead yeast and organic components, which are processed in the laboratory to produce YCW.

There is little information about the use of distillery yeast sludge in calf supplementation. The present study therefore aimed to assess the pre-weaning dietary effects of YCW on growth performance, immune response, and blood metabolites in Nili Ravi buffalo calves.

Material and Methods

The experimental procedure was approved by the Institutional Guidelines of the Ethical Review Committee at the University of Veterinary and Animal Sciences (DR/53) Lahore, Pakistan. The experiment was conducted at the Buffalo Research Institute, Pattoki, Pakistan. Forty Nili Ravi male buffalo calves (3 ± 1 days old) with an average birth weight of 39.45 ± 1.99 kg were selected and separated from their dam within two hours of birth, tagged, and housed in 1.2 m × 2.4 m individual pens bedded with straw. All calves were fed colostrum at 10% of body weight for three days. Blood samples were collected after the second colostrum feeding to determine the total proteins to ascertain the transfer of passive immunity (Refractometer, TS Meter, American Optical, Buffalo, NY, USA).

The value of >5.5 g/dL for total serum proteins was used as a cut-off for the indication of the transfer of adequate passive immunity from the dams to the calves. All calves (10 calves/group) were randomly assigned to four treatments and supplemented with prebiotics in milk as i) control with no prebiotics, ii) laboratory-produced YCW-2 g/calf/day, iii) YCW-4 g/calf/day, and iv) commercial mannan oligosaccharides (cMOS) (Harbin Ao Bi Li Biotechnology, Harbin, China) 4 g/calf/day. All the calves were fed 4 L of milk until the sixth week of the research trial, 2 L of milk during the seventh week, and 1 L of milk during the eighth week. Weaning was started at the beginning of the seventh week of the experiment, which lasted eight weeks. Water and calf starter (Table 1) were offered *ad libitum* to all calves.

The management practices were started on the third week of the experiment with a one-week interval for deworming followed by vaccination against *Clostridia perfringens* types *C* and *D* (Toxipra, Marush, Pakistan), foot and mouth disease (Aftovaxpur, Merial, France), haemorrhagic septicaemia (HS®)(Niab, Pakistan), and bovine respiratory disease (Elite 9, Boehringer Ingelheim Vetmedica, Inc. Germany). A booster dose of vaccine was administered after 21 days. Calves were treated following the veterinary practice at the farm with an oral solution of electrolytes in the event of poor faecal scores.

Crude protein, dry matter, crude fibre, crude fat, and ash contents were determined according to the guidelines of AOAC (1991), whereas acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by the process described by Van Soest *et al.* (1991).

Distillery yeast sludge was collected from Murree Brewery, Rawalpindi, 46000, Punjab, Pakistan, and washed by adding distilled water at a ratio of 1:12 overnight. The supernatant was discarded, and the process was repeated four times to remove the contaminants. After washing, DYS was added to a beaker, which was placed in an ice-filled container to keep the temperature under 40 ℃. Distillery yeast sludge was sonicated (VCX-750, Sonics & Material, Inc., Newtown, CT, USA) at an amplitude of 70 Hz to extract the YCW contents. A pulse of 10 seconds was given with a delay of 10 seconds for 10 minutes. Distilled water was added to DYS after sonication and the mixture was centrifuged. Pellets were collected and dried at a temperature range of 30–40 ℃ and powdered. The final dried product contained 95% dry matter, 1.2% crude fat, 0.0% crude fibre, 29.5% crude protein, 8.05% neutral detergent fibre, and 25% ash.

Dry matter intake (DMI) and milk intake were recorded daily, whereas body weight gain and body measurements, i.e., heart girth, body length, hip height, and wither height, were recorded weekly until the experiment was completed. Feed, faecal score, ocular discharge, general appearance, nasal discharge, and respiration were determined daily (Lesmeister & Heinrichs, 2004).

Table 1 Composition of starter ration for male Nili Ravi buffalo calves

Ingredients	Inclusion (%)
Ground maize	55.00
Soy hulls	12.00
Molasses	5.00
Soybean meal	25.00
Mineral premix	0.80
Salt	0.50
Lime	1.50
Vitamin premix	0.20
Analysed content (%)	
Dry matter	89.00
Crude protein	18.50
Total digestible nutrient	81.25
Crude fibre	7.35
Crude fat	3.01
Ash	3.52
Neutral detergent fibre	17.56
Acid detergent fibre	8.85
Calcium	0.76
Phosphorus	0.50
Metabolizable energy (Mcal, kg)	2.88

Blood samples were collected fortnightly from the jugular vein four hours after morning feeding in plain test tubes (Terumo Co. Ltd., Tokyo, Japan). Serum was separated to determine blood glucose (Cat. No. 11538, Biosystems, Barcelona, Spain), blood urea nitrogen (BUN) (Cat. No. 11537, Biosystems, Barcelona, Spain), non-esterified fatty acid (NEFA) (Cat. No. FA115, Randox, West Virginia, USA), beta-hydroxybutyric acid (βHBA) (Cat. No. H7587-58, Pointe Scientific, Canton, MI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, albumin, globulins (DiaSys, Diagnostic System GmbH, Germany), total cholesterol, triglycerides, creatinine (Human, Gesellschaft für Biochemica und Diagnostica mbH, Germany), MDA (Ohkawa *et al.*, 1979), and catalase levels (Hadwan & Abed, 2016).

Assessment of cell-mediated immune response was measured on the 42nd day of the experiment by applying dinitrochlorobenzene (DNCB). An area of 15 × 15 cm from the tuber ischiatic, tuber coxae, and sacral vertebrae was hair clipped and 1 × 1 cm in these areas was sensitized with 0.2 ml of dimethylsulfoxide (DMSO), (Cat. No. 67-68-5, Scharlau, Spain). After 15–20 min, 0.2 ml of 5% DNCB (Cat. No. 601097, Merck, Germany) (W/V) in absolute alcohol was applied. On the 44th day of the experiment, sensitization was repeated. Two sites ~10 cm apart from the sensitization site were selected and their thickness was recorded on Day 51 of the experiment with a digital vernier calliper (Lishui Nanguang Measuring & Cutting Co. Ltd, Zhejiang, China)**.** Acetone and olive oil (4:1) were applied at the control site, whereas 0.5% DNCB (0.5 ml) in the same vehicle was applied at the challenging dose site. Skin thickness of the challenging dose site was recorded at 24, 48, and 72 hours with a digital Vernier calliper (Burton *et al.*, 1989).

The data were analysed with Statistical Package for Social Sciences (version 20, SPSS Inc., Chicago, IL, USA). Initial body weight, final body weight, weight gain, total feed intake, ADG, feed efficiency, body measurements, and cell-mediated immune response were analysed using one-way ANOVA, whereas the repeated measures ANOVA technique was applied to weekly body weights, calf starter intake, faecal scoring, and blood metabolites. The analysis included differences in the subject main effect (treatment), within-subject main effect of sampling (week), and interaction between treatment and week. Duncan's multiple range tests were applied to significant data (P <0.05).

Results and Discussion

On the day of recruitment, the total serum proteins of the calves were 6.20 ± 0.19, 6.60 ± 0.37, 6.33 ± 0.15, and 6.63 ± 0.12 g/dL for the control, YCW-2, YCW-4, and cMOS groups, respectively. No mortality was observed during the study period. Starter intake was higher (P <0.001) in the animals supplemented with YCW and cMOS than in the control (Table 2). No variation (P >0.05) was observed in milk intake. DMI was higher (P <0.001) in

YCW-2, YCW-4, and cMOS-4 than in the control. Average daily DMI and feed efficiency were also higher (P <0.001) in the supplemented animals than in the control (Table 2).

Treatment and week interaction for starter intake was greater (P <0.001) in the supplemented calves than in the control during the study (Table 3). Body weight, weight gain, and ADG were greater (P <0.001) in the supplemented animals than in the control (Table 2). Treatment and week interaction for weight gain were greater (P <0.05) during the fifth to eighth weeks in the supplemented animals compared to the control (Table 3). Similar results were recorded by Hill *et al.* (2009), who reported that supplementation of yeast to calves increased the starter intake and DMI, possibly because of the low incidence of diarrhoea. Yeast cell wall supplementation does not allow gram-negative bacteria to colonize the intestine and can work as an alternative to antibiotics (Heinrichs *et al.*, 2003; Lesmeister & Heinrichs, 2004). Sharma *et al.* (2018) reported that a combination of MOS and *Lactobacillus acidophilus* increased the DMI in Murrah buffalo calves and stated that MOS and *L. acidophilus* decreased the bacterial load and improved gut health, which increased DMI.

During the current study, supplementation of YCW and cMOS did not increase (P >0.05) starter intake during the first two weeks because most of the daily energy requirement was fulfilled by milk. However, the starter intake increased (P <0.05) from the third week to the eighth week as energy requirements increased with body weight and the rumen was developing (Table 3). Similarly, Heinrichs *et al.* (2003) reported that supplementation of MOS increased starter intake during the sixth week.

Body weights during the study did not differ from week 1 to week 4 (Table 3). However, a difference (P <0.05) was observed from week 5 to week 8. Possibly there was an increase in starter intake with the supplementation of YCW and MOS, which improved gut health, reduced the incidence of diarrhoea, and hence increased body weight (Hill *et al.,* 2009). Similarly, Shimkus (2004) found that supplementation of MOS or MOS + probiotics in combination increased ADG, whereas Heinrichs *et al.* (2003) and Morrison *et al.* (2010) reported no differences in body weight and ADG in calves supplemented with MOS. Feed efficiency was greater (P <0.05) in the supplemented calves than in the control in the current study, which may be because of an increase in DMI and weight gain. Similarly, Sharma *et al.* (2018) reported that supplementation of prebiotics and probiotics in combination increased feed efficiency in Murrah buffalo calves. Lesmeister *et al.* (2004) stated that supplementation of yeast cultures did not improve feed efficiency in calves.

The faecal score was lower (P <0.05) in the supplemented calves than in the control (Table 2). Treatment and week interaction for the faecal score was lower (I<0.001) during the weeks 1–8 in the supplemented animals than in the control animals (Table 3), whereas many researchers (Heinrichs *et al.*, 2003; Galvão *et al.,* 2005; Hill *et al.,* 2009) found that supplementation of MOS and yeast culture improved the faecal score in calves and decreased the number of days of scours. No variation (P >0.05) was seen in ocular discharge, general appearance, nasal discharge, and respiration in supplemented calves and the control (Table 2). Similar results were reported by Lesmeister & Heinrichs (2004).

Structural developments, including wither height, hip height, heart girth, and body length, were greater (P <0.05) in the supplemented animals than in the control (Table 4). Changes in structural development (cm/day) were greater (P <0.05) in the supplemented animals than in the control (Table 4). Similar findings were observed by other researchers (Lesmeister et al., 2004; Lesmeister & Heinrichs, 2004; Sharma et al., 2018) in which increases in the heart girth, wither height, and hip height were noticed with supplementation of MOS and probiotics (P <0.05). However, Hill et al. (2009) and da Silva et al. (2012) found that supplementation of MOS had no effect (P >0.05) on structural measurements in calves.

The cell-mediated immune response is determined using a delayed-type hypersensitivity skin test by applying dinitrochlorobenzene. There was no difference (P>0.05) in cell-mediated immune response in the supplemented and control calves (Table 4). Similarly, Masucci et al. (2011) and Roodposhti & Dabiri (2012) observed no difference (P >0.05) in delayed-type hypersensitivity when calves were treated with probiotics, prebiotics, and synbiotics.

Mean blood glucose (Table 5) was lower (P <0.001) in the YCW-supplemented animals followed by the cMOS and control animals. No difference (P >0.05) was observed in blood glucose during the second and third weeks, whereas in the sixth and eighth weeks, it was lowest (P <0.05) in the YCW-4 group, compared with the other treatments and control groups.

Table 2 Effects of dietary supplementation of prebiotics on body weight, dry matter intake (DMI), average daily gain (ADG), feed efficiency, and health score in the Nili Ravi male buffalo calves

	Treatments					
Variables	**Control**	**YCW-2**	**YCW-4**	**cMOS**	**SEM**	***P* value**
Body weight (kg)						
Initial	37.38	39.15	39.7	41.55	1.99	0.679
Final	64.12[b]	75.92[a]	76.47[a]	77.63[a]	2.29	0.002
Gain	26.25[b]	36.76[a]	36.76[a]	36.08[a]	0.69	0.001
*ADG (kg/day)	0.47[b]	0.66[a]	0.66[a]	0.64[a]	0.006	0.001
Total starter intake (kg)	13.15[b]	23.57[a]	22.65[a]	22.13[a]	0.70	0.001
**Total DMI (kg)	40.05[b]	49.33[a]	48.52[a]	48.05[a]	0.62	0.001
Daily DMI (kg/day)	0.72[b]	0.88[a]	0.86[a]	0.86[a]	0.01	0.001
***Feed efficiency	0.65[b]	0.74[a]	0.76[a]	0.75[a]	0.02	0.001
Health scoring						
Faecal score	2.36[a]	1.17[b]	1.18[b]	1.19[b]	0.027	0.001
Ocular discharge	1.00	1.00	1.00	1.00	-----	-----
General appearance	1.00	1.00	1.00	1.00	-----	-----
Nasal discharge and respiration	1.04	1.04	1.04	1.06	0.004	0.128
Average number of days scoured	6.33[a]	1.83[b]	2.67[b]	2.00[b]	0.32	0.001

YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4: yeast cell wall content fed at 4 g/d/calf; cMOS: commercial mannan-oligosaccharide fed at 4 g/d/calf; *ad libitum* calf starter and water were offered throughout the trial; SEM: standard error of mean

*ADG: average daily gain

**Total DMI: dry matter of milk + dry matter of calf starter

***Feed efficiency: weight gain (kg)/ DMI (kg)

[a-b]Values in a row without a common superscript differ significantly (P <0.05)

Table 3 Effect of prebiotic treatment on body weight, starter intake, and faecal score in Nili Ravi male buffalo calves

Variables	Treat-ment	Mean	Age (Week)									SEM	*P* value		
			0	1	2	3	4	5	6	7	8		Trt	Week	Trt × Week
Body weight (kg)	Control	48.06^{b}	37.87	39.63	41.62	43.82	46.13^{b}	47.50^{b}	53.48^{b}	58.40^{b}	64.12^{b}	1.143	0.034	0.001	0.001
	DYS-2	54.56^{ab}	39.15	42.08	44.95	48.30	52.37^{ab}	57.13^{a}	62.60^{a}	68.57^{a}	75.92^{a}				
	DYS-4	54.89^{ab}	39.70	42.53	45.73	48.48	52.6^{ab}	57.13^{a}	62.58^{a}	68.78^{a}	76.47^{a}				
	MOS-4	56.33^{a}	41.55	44.05	46.65	50.12	53.87^{a}	58.48^{a}	64.35^{a}	70.27^{a}	77.63^{a}				
Starter intake (kg)	Control	1.65^{b}		0.07^{b}	0.20^{b}	0.35^{b}	0.78^{b}	1.20^{b}	2.15^{b}	3.35^{b}	5.07^{b}	0.05	0.001	0.001	0.001
	DYS-2	2.95^{a}		0.14^{a}	0.39^{a}	0.87^{a}	1.61^{a}	2.69^{a}	3.75^{a}	5.81^{a}	8.35^{a}				
	DYS-4	2.83^{a}		0.13^{a}	0.41^{a}	0.82^{a}	1.43^{a}	2.52^{a}	3.77^{a}	5.68^{a}	7.92^{a}				
	MOS-4	2.77^{a}		0.13^{a}	0.43^{a}	0.80^{a}	1.63^{a}	2.72^{a}	3.73^{a}	5.17^{a}	7.55^{a}				
Faecal score	Control	2.36^{a}		3.21^{a}	2.71^{a}	2.84^{a}	2.40^{a}	2.12^{a}	1.98^{a}	1.91^{a}	1.69^{a}	0.027	0.001	0.001	0.001
	DYS-2	1.17^{b}		1.78^{b}	1.29^{b}	1.10^{b}	1.14^{b}	1.00^{b}	1.02^{b}	1.05^{b}	1.00^{b}				
	DYS-4	1.18^{b}		1.93^{b}	1.24^{b}	1.17^{b}	1.09^{b}	1.00^{b}	1.00^{b}	1.02^{b}	1.00^{b}				
	MOS-4	1.19^{b}		1.83^{b}	1.31^{b}	1.24^{b}	1.17^{b}	1.02^{b}	1.00^{b}	1.00^{b}	1.00^{b}				

YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4: yeast cell wall content fed at 4 g/d/calf; cMOS: commercial mannan-oligosaccharide fed at 4 g/d/calf; SEM: standard error of mean

$^{a-b}$Values in a column without a common superscript differ significantly (P <0.05)

Table 4 Effects of prebiotic treatment on body measurements and cell-mediated immune response in Nili Ravi male buffalo calves

	Treatments					
Variables	**Control**	**YCW-2**	**YCW-4**	**cMOS-4**	**SEM**	***P* value**
Withers height (cm)						
Initial	72.33	74.89	74.46	74.86	0.71	0.13
Final	83.65[b]	92.10[a]	92.00[a]	92.52[a]	0.71	0.001
Total gain	11.31[b]	17.21[a]	17.53[a]	17.66[a]	0.63	0.001
Change, cm/d	0.20[b]	0.30[a]	0.31[a]	0.31[a]	0.01	0.001
Hip height (cm)						
Initial	75.42	77.19	75.69	75.22	0.82	0.37
Final	86.99[b]	94.32[a]	93.16[a]	92.41[a]	1.09	0.001
Total gain	11.57[b]	17.12[a]	17.48[a]	17.19[a]	0.90	0.001
Change, cm/d	0.20[b]	0.30[a]	0.31[a]	0.30[a]	0.01	0.001
Heart girth (cm)						
Initial	78.82	80.93	78.78	81.66	1.15	0.214
Final	92.70[b]	99.35[a]	97.91[a]	99.45[a]	1.54	0.033
Total gain	13.88[b]	18.41[a]	19.13[a]	17.78[a]	0.84	0.005
Change, cm/d	0.25[b]	0.33[a]	0.34[a]	0.31[a]	0.01	0.005
Body length (cm)						
Initial	76.23	81.33	77.58	79.33	1.67	0.223
Final	87.77[b]	98.56[a]	93.95[a]	94.91[a]	1.73	0.005
Total gain	11.53[b]	17.23[a]	16.37[a]	15.58[a]	0.73	0.001
Change, cm/d	0.20[b]	0.31[a]	0.29[a]	0.27[a]	0.01	0.001
Cell-mediated immune response						
24-hour	2.61	2.86	2.45	1.93	0.45	0.559
48-hour	1.69	1.54	1.63	1.23	0.37	0.839
72-hour	0.36	0.30	0.37	0.46	0.12	0.86

YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4: yeast cell wall content fed at 4 g/d/calf; cMOS: commercial mannan-oligosaccharide fed at 4 g/d/calf; SEM: standard error of mean

[a-b]Values in a row without a common superscript differ significantly ($P < 0.05$)

The lowest values of glucose were observed in week 8 of the experiment in the supplemented animals (Table 5). Similarly, Quigley *et al*. (1991) reported that glucose concentration decreased as calves aged and early weaning was achieved, whereas some researchers reported no effects of prebiotics and probiotics on glucose concentrations (Ballou, 2011; da Silva *et al*., 2012; Dar *et al*., 2019). The calf's stomach resembles that of monogastric animals during the pre-weaning stage and energy requirements are fulfilled by milk. Therefore, during this period, the glucose level is high. The primary source of energy shifts from glucose to volatile fatty acids with age and rumen development, which decreases the blood glucose concentration.

Increases in BUN and βHBA concentrations with age indicate the fermentative changes in the developing rumen. The concentration of BUN and βHBA can be used as a tool to determine the efficiency of dietary nitrogen utilization and rumen epithelial metabolic activity by the calves. The rumen of the newborn calf is not functioning metabolically, so at this stage levels of BUN and βHBA are low.

With the increase in DMI and the development of rumen microbiota, the production of BUN and βHBA increases, which indicates the degradation of dietary proteins from calf starter and rumen papillae growth. Mean BUN and βHBA (Table 5) concentrations were higher (P <0.001) in the YCW-4 animals compared with the other supplemented and control animals.

No differences (P >0.05) were observed in BUN and βHBA in the second and fourth week, whereas they increased (P <0.05) in the sixth and eighth weeks in the supplemented animals compared to the control. Similarly, Quigley *et al*. (1991) and da Silva *et al*. (2012) reported that supplementation of MOS, inulin, and yeast increased BUN and βHBA levels, whereas Dar *et al*. (2018) reported that supplementation of prebiotics and probiotics did not affect BUN and βHBA levels.

Table 5 Effects of prebiotic treatment on blood metabolites in Nili Ravi male buffalo calves

			Age (Weeks)					*P* value		
Variables	**Trt**	**Means**	**2**	**4**	**6**	**8**	**SEM**	**Treat-ment**	**Week**	**Trt × Week**
Glucose mg/dL	Control	77.68[a]	82.55	83.5	80.06[a]	64.63[a]	0.609	0.001	0.001	0.001
	YCW-2	62.96[b]	78.69	82.33	51.75[b]	39.08[b]				
	YCW-4	62.01[b]	79.03	78.63	51.91[b]	38.47[b]				
	cMOS-4	63.98[b]	82.93	82.47	50.69[b]	39.81[b]				
*BUN mg/dL	Control	4.95[b]	4.04	4.80	5.19[b]	5.77[c]	0.052	0.001	0.001	0.001
	YCW-2	5.68[a]	3.98	5.30	6.36[a]	7.05[b]				
	YCW-4	6.01[a]	4.16	5.03	6.51[a]	8.33[a]				
	cMOS-4	5.80[a]	3.83	5.14	6.52[a]	7.71[ab]				
Creatinine mg/dL	Control	1.37	1.42	1.33	1.28	1.45	0.040	0.920	0.028	0.266
	YCW-2	1.43	1.41	1.47	1.39	1.44				
	YCW-4	1.37	1.39	1.40	1.31	1.37				
	cMOS-4	1.41	1.45	1.39	1.41	1.39				
**βHBA mmol/L	Control	0.41[b]	0.35	0.37	0.36[b]	0.54[b]	0.007	0.001	0.001	0.001
	YCW-2	0.50[a]	0.34	0.41	0.56[a]	0.71[a]				
	YCW-4	0.51[a]	0.33	0.39	0.55[a]	0.76[a]				
	cMOS-4	0.51[a]	0.36	0.38	0.55[a]	0.74[a]				
***NEFA mmol/L	Control	0.51	0.49	0.47	0.57	0.51	0.011	0.746	0.001	0.955
	YCW-2	0.49	0.44	0.45	0.55	0.55				
	YCW-4	0.47	0.46	0.43	0.55	0.48				
	cMOS-4	0.49	0.46	0.45	0.57	0.50				

Trt: treatment; YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4: yeast cell wall content fed at 4 g/d/calf; cMOS: commercial mannan-oligosaccharide fed at 4 g/d/calf; *BUN: blood urea nitrogen; SEM: standard error of mean

**βHBA: beta hydroxybutyric acid

***NEFA: non esterified fatty acids

[a-c]Values in a column without a common superscript differ significantly (P <0.05)

No differences (*P* <0.05) were observed in serum catalase and MDA levels in the supplemented animals (Table 7). Similar results were found by Zhang *et al.* (2012), in which supplementation of MOS did not affect serum MDA levels in piglets. In the current study, no differences (*P* >0.05) were observed in total proteins, albumin, globulins, and albumin to globulin ratio in the control animals (Table 6). Similar results were found by Nargeskhani *et al.* (2010). Differences in total cholesterol and triglyceride concentration in the animals supplemented with YCW

and cMOS were insignificant (Table 6). Similarly, Szandra *et al.* (2020) found that supplementation of MOS and inulin did not affect total cholesterol and triglyceride levels in supplemented animals.

During the current study, no differences (P >0.05) were observed in serum ALT and AST levels (Table 7). Similarly, Rekiel *et al.* (2007) and Dar *et al.* (2019) reported that supplementation of probiotics, prebiotics, and synbiotics did not affect the serum ALT and AST levels in crossbred calves. However, serum triglyceride levels dropped as calf age progressed, which was opposite to the current findings, in which serum triglycerides were not affected by YCW and cMOS supplementation. Serum creatinine level was not affected by the supplementation of YCW and cMOS (Table 5).

Similar results were found by Kehoe & Carlson (2015), in which supplementation of non-medicated feed additives did not influence the serum creatinine level in calves. In another study, Abdalla *et al.* (2013) found that supplementation of *S. cerevisiae* did not affect the serum creatinine level.

Table 6 Effects of prebiotics on serum cholesterol, triglycerides, and total protein profile in Nili Ravi male buffalo calves

			Age (Weeks)					*P*-value		
Variables	**Trt**	**Means**	**2**	**4**	**6**	**8**	**SEM**	**Treat-ment**	**Week**	**Trt × Week**
Cholesterol mg/dL	Control	84.22	71.91	77.22	89.79	97.97	3.08	0.175	0.001	0.802
	YCW-2	97.53	77.64	89.31	111.67	111.53				
	YCW-4	96.73	78.62	90.99	108.04	109.29				
	cMOS-4	96.19	77.50	87.35	102.73	117.19				
Triglyceride mg/dL	Control	71.69	73.92	67.60	72.98	72.28	2.41	0.499	0.038	0.677
	YCW-2	77.95	74.15	73.80	85.73	78.13				
	YCW-4	74.73	68.19	77.89	81.99	70.87				
	cMOS-4	72.13	68.42	69.82	77.19	73.10				
Total proteins g/dL	Control	6.55	6.60	6.85	6.37	6.35	0.354	0.997	0.324	0.602
	YCW-2	6.58	6.61	6.50	6.64	6.56				
	YCW-4	6.57	6.69	6.58	6.65	6.37				
	cMOS-4	6.56	6.73	6.47	6.45	6.59				
Albumin g/dL	Control	3.95	3.81	3.83	4.08	4.11	0.168	0.752	0.001	0.203
	YCW-2	3.96	3.85	3.96	4.03	4.01				
	YCW-4	3.99	4.02	3.69	4.12	4.12				
	cMOS-4	4.08	4.07	3.81	4.17	4.23				
Globulins g/dL	Control	2.59	2.80	3.02	2.28	2.24	0.24	0.826	0.002	0.59
	YCW-2	2.62	2.76	2.54	2.60	2.55				
	YCW-4	2.69	2.67	2.89	2.53	2.25				
	cMOS-4	2.49	2.67	2.66	2.28	2.36				
*A/G	Control	1.64	1.48	1.32	1.87	1.87	0.166	0.732	0.001	0.677
	YCW-2	1.56	1.41	1.61	1.61	1.62				
	YCW-4	1.61	1.55	1.29	1.74	1.89				
	cMOS-4	1.69	1.56	1.47	1.87	1.86				

Trt: treatment; YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4: yeast cell wall content fed at 4 g/d/calf; cMOS: commercial mannan-oligosaccharide fed at 4 g/d/calf; SEM: standard error of mean

*A/G: albumin to globulin ratio

Table 7 Effect of prebiotic treatment on redox status and hepatic enzymes in Nili Ravi male buffalo calves

Variables	Trt	Means	Age (Weeks)				SEM	*P*-value		
			2	4	6	8		Treat-ment	Week	Trt × Week
Catalase ku/L	Control	1560.94	1785.53	1465.55	1443.34	1549.33	8.84	0.26	0.001	0.619
	YCW-2	1573.49	1760.16	1543.48	1486.84	1503.48				
	YCW-4	1539.74	1737.27	1465.92	1405.25	1550.50				
	cMOS-4	1590.17	1743.86	1552.26	1536.75	1527.83				
MDA µmol/L	Control	29.55	29.92	32.80	32.74	22.75	0.528	0.223	0.001	0.752
	YCW-2	31.57	33.14	34.78	34.52	23.84				
	YCW-4	28.96	30.67	32.63	33.00	19.53				
	cMOS-4	28.08	27.11	30.59	33.00	21.63				
*ALT u/L	Control	18.69	17.06	22.97	14.83	19.87	1.79	0.398	0.316	0.672
	YCW-2	14.49	12.11	16.68	14.44	14.73				
	YCW-4	12.78	13.57	13.77	10.08	13.67				
	cMOS-4	17.89	14.93	16.29	15.99	24.33				
**AST u/L	Control	38.07	33.45	38.49	40.62	39.74	1.41	0.287	0.003	0.535
	YCW-2	38.78	32.58	38.00	45.27	39.26				
	YCW-4	33.42	29.47	34.22	34.02	35.96				
	cMOS-4	38.58	36.94	39.07	41.88	36.45				

YCW-2: yeast cell wall content fed at 2 g/d/calf; YCW-4 - : yeast cell wall content fed at 4 g/d/calf; cMOS-4: commercial mannan-oligosaccharide fed at 4 g/d/calf; SEM: standard error of mean
*ALT: alanine transaminase
**AST: aspartate aminotransferase

Conclusion

The authors conclude that supplementation with YCW and cMOS has a positive impact on growth performance, structural measurements, faecal score, energy metabolites, and rumen development in buffalo calves. Therefore, it could be used for early rumen development, which helps to achieve early weaning and better growth in Nili Ravi male buffalo calves.

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Authors' contributions

Conceptualization, MR, HUR,MAR; methodology, MSY; investigation and formal analysis, MR, BA, AM, RM, SA, MN; writing-original draft preparation, HR, MSY, MR, AK; supervision, MSY, MAR; funding acquisition, HR; resources, HR, MAR, MSY. All the authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declare there are no conflicts of interest.

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