https://doi.org/10.17221/70/2021-CJAS

Prebiotics supplementation modulates pre-weaning stress in male cattle calves by improving growth performance, health scores and serum biomarkers

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Citation: Raza M., Yousaf M.S., Ahmad J., Rashid M.A., Majeed K.A., Tahir S.K., Ashraf S., Numan M., Khalid A., Rehman H. (2022): Prebiotics supplementation modulates pre-weaning stress in male cattle calves by improving growth performance, health scores and serum biomarkers. Czech J. Anim. Sci., 67: 102–113.

Abstract: Neonatal calves are prone to gastrointestinal infections and microbial dysbiosis that lead to high morbidity and mortality. Prebiotics can be used to mitigate the adverse effects of gut diseases and microbial dysbiosis. Forty male Holstein-Friesian calves (2 ± 1 day old) were divided into four dietary treatments: control (milk without prebiotics), YCW-2, YCW-4 (milk containing 2 or 4 g/day/calf of yeast cell wall, respectively), and cMOS (milk containing commercial mannan-oligosaccharides 4 g/day/calf). Milk intake, feed intake, and health scores were recorded daily, whereas body weight, dry matter intake (DMI), and body measurements were recorded weekly. Feed efficiency (FE) was determined at the end of the trial (eight weeks). Cell-mediated immunity (CMI) was assessed by the topical application of dinitrochlorobenzene. Blood samples were collected fortnightly to determine glucose, non-esterified fatty acid (NEFA), blood urea nitrogen (BUN), and beta-hydroxybutyric acid (βHBA). Cell wall supplemented calves had significantly higher ($P < 0.05$) final body weights, DMI, and body measurements along with improved ($P < 0.05$) faecal scores. Feed intake was higher ($P < 0.05$) in both the YCW-supplemented calves. Glucose was lower ($P < 0.001$), whereas BUN and βHBA were significantly higher in the YCW-2 animals. No differences were observed in FE, CMI, and NEFA between all the experimental animals. The yeast cell wall (2 g) may have the potential to improve the growth performance and health status of neonatal calves.

Keywords: cattle calf; yeast cell wall; structural development; blood metabolites; immunity

Neonatal calf mortality during the pre-weaning period remains a high-risk challenge for the livestock sector. The calves face greater health disorders and diseases when shifted pre-ruminant to the ruminant phase. Gastrointestinal infections primarily affect the health of the calves during this

Supported by the Punjab Agricultural Research Board, Lahore, Punjab-Pakistan (Project No. 453).

https://doi.org/10.17221/70/2021-CJAS

phase leading to microbial dysbiosis, diarrhoea, dehydration, and ultimately early calf mortality. Electrolyte therapy and antibiotics are commonly used to treat these ailments to substitute fluid loss and combat invading pathogens (Heinrichs et al. 1995; Lorenz et al. 2011). However, dysbiosis during gastrointestinal disturbances can be corrected by supplementing prebiotics.

The most commonly available prebiotics are mannan-oligosaccharides (MOS) and fructooligosaccharides and they are added in milk replacer or calf starter diets. Mannan-oligosaccharides are obtained from the cell wall fragments of *Saccharomyces cerevisiae* (Hill et al. 2009). It can bind to specific mannose-binding proteins present on the cell membrane of some pathogenic bacteria and prevent their colonization in the intestine by interfering with the binding of carbohydrate residues to the epithelial cell surface (Spring et al. 2000). MOS supplementation facilitates the colonization of beneficial microbes in the gut. Moreover, MOS supplementation has immune-boosting (Wismar et al. 2010) and antioxidant properties (Che et al. 2012) and improves the intestinal mucosal integrity against antibiotic growth promoters (Spring et al. 2000). Studies have reported that prebiotics supplementation improved starter intake, reduced faecal score (Terre et al. 2007), increased growth (Ghosh and Mehla 2012), average daily gain, feed efficiency (Xu and Gorden 2003), and decreased enteric disease severity (Quigley et al. 2002).

White sugar is manufactured from sugar cane after chemical processing and molasses is available to produce by-products. Molasses is fermented by *S. cerevisiae* and distillery yeast sludge (DYS) is the end product of molasses fermentation in the sugarcane industry. This DYS is disposed of in a field or drained in water, which causes environmental pollution. This waste product can be used as a prebiotic in animal feed after processing as it contains high protein content and it can replace the commercially available expensive products.

Keeping in view the importance of prebiotics, the present study was conducted to mitigate the adverse effects of weaning stress in neonatal male cattle calves. It is hypothesized that supplementation of DYS-derived yeast cell wall would reduce the weaning stress and improve gastrointestinal health and development that may ultimately lead to improved growth performance, health status, immunity, and blood metabolites.

MATERIAL AND METHODS

Animal care

The experimental procedure was approved by the Institutional Animal Care and Use Committee at the University of Veterinary and Animal Sciences (DR/53).

Animals, treatments and management

The experiment was conducted at the experimental station, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan after getting formal approval from the animal care committee of the same university. Forty Holstein-Friesian male calves (2 ± 1 days of age) were used in the study. The calves were separated from dams within 2 h after birth, marked with an ear tag, weighed, and housed in 1.2 m × 2.4 m open-sided individual pens with straw bedding. The calves were given four litres of quality colostrum (> 50 mg/ml of immunoglobulin G) within 30 min after birth and another three litres of fresh colostrum at the next feeding time. After the second colostrum feeding, blood was collected to determine the total proteins using a refractometer (TS Meter; American Optical, Buffalo, NY, USA) to ascertain the transfer of sufficient passive immunity. Later, the calves were blocked by birth weight in a complete randomized block design and randomly assigned to four dietary treatment groups with ten calves (n = 10) in each group. The grouping was as follows: control with no prebiotics in milk; laboratory-produced yeast cell wall (YCW) at 2 g/calf/day (YCW-2) in milk; YCW 4 g/calf/day (YCW-4) in milk; and commercial mannan-oligosaccharides (cMOS; Harbin Ao Bi Li Biotechnology, Harbin, China) in milk. The total duration of the research trial was eight weeks and weaning was started at the age of seven weeks.

Calves were fed whole milk twice daily during the morning (07:00) and evening (19:00) using a feeding bottle with a nipple until weaning. The calves received 4 l/day of milk from week 1 to 6 and 2 l/day during week 7 and thereafter 1 l/day till the end of the experiment. Calf starter (Table 1) and water were provided *ad libitum* throughout the research trial. Animals were weaned when calf starter intake reached 800 g for consecutive three days, after which calf starter was given at a daily

https://doi.org/10.17221/70/2021-CJAS

Table 1. Composition of starter ration

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
Analysis	
Dry matter (%)	89
Crude protein (%)	18.5
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

maximum of 2 400 g. Deworming was carried out in week 3. In week 4, the calves were vaccinated against foot and mouth disease (Aftovaxpur; Merial, Lyon, France), followed by *Clostridium perfringens* type C and D (Toxipra; Marush Pharma, Lahore, Pakistan), haemorrhagic septicaemia (HS® Vaccine Niab, Faisalabad, Pakistan), and bovine respiratory diseases (Elite 9; Boehringer Ingelheim Vetmedica, GmbH, Rohrdorf, Germany) with a one-week interval. Booster doses of vaccines were administered after 21 days. In the case of faecal score, the animals were treated with veterinary practice at the farm using an oral electrolyte solution.

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

Yeast cell wall preparation

Collected distillery yeast sludge (DYS) was washed by adding distilled water at a ratio of 1 : 12 and kept overnight. The supernatant was discarded and the procedure was repeated four times to remove the toxic metals. After washing, DYS was added to a beaker which was placed in an ice-filled container to keep the temperature under 40 °C. DYS was sonicated by using a sonicator (VCX 750, Sonics & Material, Inc., Newtown, CT, USA) with an amplitude of 70% to extract the yeast cell wall content. A pulse of 10 s was given with a delay of 10 s for 10 minutes. After sonication, distilled water was added in DYS and centrifuged. Pellet was collected, dried, and powdered. The final product contains 25% crude protein, 1.2% crude fat, 42.3% nitrogen free extract, 0.0% crude fibre, 8.05% neutral detergent fibre, and 25% ash.

Growth, structural measurements and health scoring

The calves were weighed on an electronic digital scale at birth and weekly thereafter until week 8. Fortnightly measurements of body weights (BW) were carried out in the morning before feeding. The change in BW in relation to baseline weight before the start of the experiment was considered as average daily gain (ADG). Feed intake was recorded daily for each calf. The calves were offered a measured amount of diet twice a day. Mean dry matter intake (DMI) of the individual calf was calculated after determining the dry matter content of feed offered and the remainder. These values were used to measure the final and weekly DMI for the calves. The structural growth measurements, including wither height, hip height, heart girth, and body length were recorded at the start of the experiment and thereafter weekly until the completion of the experiment.

Calf health was monitored daily by assessing faecal score, ocular discharge, general appearance, and nasal discharge and respiration as described previously by Lesmeister & Heinrichs (2004). Nasal scores and ear scores were recorded daily before the morning feeding using a 0–3 (nasal score) or 0–4 (ear score) scale developed by the University of Wisconsin-Madison (McGuirk 2008). For determination of faecal consistency, faeces were scored daily before the morning milk feeding by using a 1–5 scale (1 = normal, 2 = soft, 3 = runny, 4 = watery, and 5 = watery, mucous, bloody). Scores of more than two were considered to indicate diarrhoea.

https://doi.org/10.17221/70/2021-CJAS

Blood collection and analysis

Blood samples were collected fortnightly from the jugular vein 4 h (1 100 h) after milk feeding in plain test tubes (Terumo Co., Ltd, Tokyo, Japan). Later, the blood samples were centrifuged at 1 500 g for 10 min at 4 °C. The supernatant was aspirated and stored at –40 °C until analysis. The serum was used to estimate glucose (Cat#11538; Biosystems, Barcelona, Spain), blood urea nitrogen (BUN, Cat#11537; Biosystems, Barcelona, Spain), non-esterified fatty acid (NEFA, Cat#FA115; Randox, Kearneysville, WV, USA) and beta-hydroxybutyric acid (βHBA, Cat#H7587-58; Pointe Scientific Inc., Canton, MI, USA) by using commercial kits.

Cell-mediated immune response

Cell-mediated immune (CMI) response was measured by applying dinitrochlorobenzene (DNCB) as previously described by Burton et al. (1989). Briefly, on day 42 of the experiment, an area of 15 × 15 cm was hair clipped at *tuber ischiadicum*, *tuber coxae*, and sacral vertebrae. A small area was used for sensitization. For this purpose 0.2 ml of dimethyl sulfoxide (DMSO; Cat#67-68-5; Scharlau, Barcelona, Spain) was applied to the skin and thereafter 0.2 ml of 5% DNCB (Cat#601097-25g; Merck KGaA, Darmstadt, Germany) (w/v) in absolute alcohol was applied. Sensitization was repeated on day 44 in the same manner. On day 51 of the experiment, two sites about 10 cm apart from the sensitization site were selected and their thickness was recorded using a digital vernier calliper (Lishui Nanguang Measuring and Cutting Co., Ltd., Zhejiang, China). To the control site, the vehicle (4 : 1 acetone and olive oil) was applied and to the challenging site 0.5 ml of 0.5% DNCB in the same vehicle was applied. Skin thickness was recorded using a digital vernier calliper at 24 h, 48 h, and 72 h after the application of a challenging dose.

Statistical analysis

Data were analysed using SPSS v20 (SPSS Inc., Chicago, IL, USA). Total feed intake, initial weight, final weight, weight gain, ADG, feed efficiency, CMI, and body measurements were analysed using one-way ANOVA. Health scoring, DMI, weekly body weight gain, and blood metabolites were analysed by the repeated-measures procedure using GLM of SPSS; the analysis included the between-subjects main effect of treatment (supplementation), the within-subjects main effect of the period of sampling (week), and interaction between treatment × week. Tukey's test was applied to significant data ($P < 0.05$).

RESULTS

Serum proteins

Total serum protein concentrations of the calves, measured on the day of recruitment, did not differ significantly between the treatment groups and were 6.23 ± 0.25, 6.04 ± 0.17, 6.14 ± 0.24 and 5.88 ± 0.12 g/dl for control, YCW-2, YCW-4 and cMOS groups, respectively. We used a value of > 5.2 g/dl (Magalhaes et al. 2008) for total serum proteins as a cut-off for the indication of the transfer of adequate passive immunity from the dams to the calves included in the present study.

Growth performance, dry matter intake and feed efficiency

No mortality was observed during the entire study period. Final BW, ADG, and weight gain were higher ($P < 0.001$) in the supplemented calves compared with the non-supplemented calves (Table 2). During the first five weeks of age, the supplementations did not affect body weight. The BW gain was higher ($P < 0.05$) during the sixth to eighth weeks in the supplemented animals than in the control animals (Table 3). Dietary inclusion of prebiotics (YCW/mannan-oligosaccharides) resulted in the higher consumption ($P < 0.01$) of starter compared with the non-supplemented calves (Table 3). The treatment × time interaction was also significant ($P < 0.01$) for the dry matter intake in the calves (Table 3). During the first two weeks, starter intake remained the same in all the groups. In weeks 4, 5, and 6, starter intake was higher ($P < 0.01$) in the YCW-2 group compared with the other groups (Table 3). Total DMI and daily DMI were also higher ($P < 0.01$) in the supplemented calves compared with the non-

https://doi.org/10.17221/70/2021-CJAS

Table 2. Effects of dietary supplementation of prebiotics on body weight, dry matter intake (DMI), average daily gain (ADG), feed efficiency, and health scoring in Holstein-Friesian male calves

Variables	Treatments				SEM	*P*-value
	control	YCW-2	YCW-4	cMOS		
Body weight (kg)						
Initial	32.79	33.97	34.42	33.48	0.720	0.679
Final	62.01^{b}	75.31^{a}	71.94^{a}	69.91^{a}	1.620	0.001
Gain	29.22^{b}	41.34^{a}	37.52^{a}	36.43^{a}	1.340	0.001
ADG (kg/day)	0.52^{b}	0.73^{a}	0.67^{a}	0.65^{a}	0.024	0.001
Total starter intake (kg)	26.19^{b}	44.14^{a}	41.20^{a}	38.73^{a}	1.980	0.001
Total DMI (kg)[1]	47.88^{b}	63.86^{a}	61.24^{a}	59.04^{a}	1.760	0.001
Daily DMI (kg/day)	0.85^{b}	1.14^{a}	1.09^{a}	1.05^{a}	0.030	0.001
Feed efficiency[2]	0.61	0.65	0.61	0.6	0.030	0.766
Weaning age (days)	53^{a}	38^{b}	43^{b}	42^{b}	1.010	0.001
Health scoring						
Faecal score	2.13^{a}	1.74^{b}	1.76^{b}	1.82^{b}	0.030	0.001
Ocular discharge	1.00	1.02	1.00	1.00	0.018	0.247
General appearance	1.04	1.05	1.05	1.03	0.010	0.822
Nasal discharge and respiration	1.00	1.02	1.01	1.00	0.007	0.445
Average number of days scored	5.72^{a}	2.30^{b}	3.00^{b}	3.80^{ab}	0.484	0.001

cMOS = commercial mannan-oligosaccharides fed at 4 g/day/calfYCW-2 = yeast cell wall content fed at 2 g/day/calf; YCW-4 = yeast cell wall content fed at 4 g/day/calf

[1]Dry matter of milk + dry matter of calf starter; [2]weight gain (kg)/DMI (kg)

[a,b]Values in a row without common small letters differ significantly ($P < 0.05$)

Calf starter and water were offered *ad libitum* throughout the research trial

supplemented calves (Table 2). However, the supplementation did not affect feed efficiency (FE) in experimental animals (Table 2).

Body measurements and cell-mediated immune response

Body structures in terms of hip height, wither height, heart girth, and body length were more developed ($P < 0.05$) in the animals supplemented with YCW and cMOS than in the control animals (Table 4). Higher ($P < 0.05$) body structural developments were observed in the calves supplemented with 2 g of YCW than in the other calves. No difference was observed in the cell-mediated immune response between animals from all the treatments (Table 4).

General health and faecal scores

Generally, the health of the calves was good. Respiratory and general appearance scores averaged approximately one throughout the experimental period and were not affected by the treatments as no differences were observed in ocular discharge, general appearance, nasal discharge, and respiration (Table 2). Faecal scores were the same for all the supplemented calves and showed a typical pattern with a peak in week 2 followed by a decline and stabilization at a score of < 2 by five weeks of age. In week 6, there was an outbreak of coccidiosis resulting in higher faecal scores in some of the experimental calves (Figure 1). The treatment × week interaction was significant ($P < 0.05$) for faecal scores reflecting lower values of the score in the supplemented calves than in the control in weeks 2, 3, 4, 5 and 8 (Figure 1).

Every animal from each group was treated against scours once or more than once. For faecal scores, the animals were treated with oral electrolyte solution. The animals in the control group were treated for a minimum of three and a maximum of eleven days. The animals in the YCW-2 group were treated for a minimum of one and a maximum of five days. The animals in the YCW-4 group were treated for

Czech Journal of Animal Science, 67, 2022 (3): 102–113

https://doi.org/10.17221/70/2021-CJAS

Table 3. Effects of dietary supplementation of prebiotics on weekly body weight, average daily gain (ADG), starter intake, and dry matter intake (DMI) in Holstein-Friesian male calves

Variables	Treatment	Age (week)										SEM	*P*-value		
		means	0	1	2	3	4	5	6	7	8		treatment (TR)	week	TR × week
Body weight (kg)	control	45.24^{b}	32.92	34.6	37.06	40.26	43.99	48.07^{b}	51.74^{b}	56.56^{b}	62.01^{b}	1.13	0.017	0.001	0.001
	YCW-2	50.02^{a}	33.97	35.91	38.39	42.35	46.97	52.82^{a}	58.92^{a}	65.58^{a}	75.31^{a}				
	YCW-4	49.76^{a}	34.42	36.6	39.33	43.74	47.39	51.58^{ab}	57.89^{a}	64.98^{a}	71.94^{a}				
	cMOS-4	48.2^{ab}	33.68	35.47	38.42	42.25	46.09	50.03^{ab}	55.87^{ab}	62.16^{ab}	69.91^{a}				
ADG (kg)	control	0.52^{b}		0.26	0.35	0.46	0.53	0.58^{b}	0.53^{b}	0.69^{b}	0.78^{c}	0.013	0.001	0.001	0.001
	YCW-2	0.73^{a}		0.32	0.31	0.57	0.66	0.84^{a}	0.87^{a}	0.95^{ab}	1.39^{a}				
	YCW-4	0.67^{a}		0.33	0.41	0.60	0.52	0.60^{b}	0.90^{a}	1.01^{a}	0.99^{bc}				
	cMOS-4	0.65^{a}		0.29	0.42	0.55	0.55	0.56^{b}	0.84^{a}	0.90^{ab}	1.11^{ab}				
Starter intake (kg)	control	3.27^{b}		0.27	0.72	1.04^{b}	1.74^{c}	2.73^{c}	4.45^{b}	5.55^{b}	9.67^{b}	0.262	0.001	0.001	0.001
	YCW-2	5.51^{a}		0.29	0.9	2.00^{a}	3.50^{a}	5.26^{a}	7.35^{a}	10.45^{a}	14.38^{a}				
	YCW-4	5.15^{a}		0.35	1.09	1.85^{a}	2.55^{bc}	4.02^{b}	6.39^{ab}	9.86^{a}	15.07^{a}				
	cMOS-4	4.8^{a}		0.29	0.75	1.89^{a}	2.65^{ab}	4.00^{b}	6.10^{ab}	9.27^{a}	13.68^{a}				
DMI (kg)	control	5.98^{b}		3.88	4.29	4.56^{b}	5.19^{c}	6.07^{c}	7.61^{b}	6.77^{b}	9.52^{b}	0.117	0.001	0.001	0.001
	YCW-2	7.98^{a}		3.90	4.44	5.42^{a}	6.76^{a}	8.32^{a}	10.18^{a}	11.12^{a}	13.71^{a}				
	YCW-4	7.65^{a}		3.96	4.61	5.28^{a}	5.91^{bc}	7.23^{b}	9.33^{ab}	10.60^{a}	14.32^{a}				
	cMOS-4	7.38^{a}		3.90	4.31	5.32^{a}	6.01^{ab}	7.21^{b}	9.13^{ab}	10.07^{a}	13.09^{a}				

cMOS = commercial mannan-oligosaccharides fed at 4 g/day/calf; YCW-2 = yeast cell wall content fed at 2 g/day/calf; YCW-4 = yeast cell wall content fed at 4 g/day/calf

$^{a–c}$Values in a column without common small letters differ significantly ($P < 0.05$)

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Table 4. Effects of dietary supplementation of prebiotics on structural growth and cell-mediated immune response in Holstein-Friesian male calves

Variables	Treatments				SEM	*P*-value
	control	YCW-2	YCW-4	cMOS		
Wither height (cm)						
Initial	75.06	75.76	75.54	75.2	0.82	0.936
Final	85.62^{b}	90.93^{a}	87.35^{b}	87.7^{b}	0.61	0.001
Total gain	10.55^{b}	15.16^{a}	11.81^{b}	12.49^{ab}	0.76	0.002
Hip height (cm)						
Initial	79.22	79.14	79.7	78.07	0.81	0.586
Final	89.15^{b}	93.44^{a}	91.18^{ab}	91.56^{ab}	0.68	0.001
Total gain	9.92^{b}	12.3^{a}	11.48^{ab}	13.48^{a}	0.73	0.001
Heart girth (cm)						
Initial	74.97	75.74	75.05	74.44	0.8	0.745
Final	89.86^{b}	94.69^{a}	93.59^{a}	92.68^{ab}	0.94	0.006
Total gain	14.89^{b}	18.94^{a}	18.54^{a}	18.23^{a}	0.78	0.003
Body length (cm)						
Initial	75.13	74.82	74.24	73.27	1.07	0.636
Final	89.87^{b}	94.08^{a}	91.77^{ab}	91.89^{ab}	0.69	0.003
Total gain	14.73^{b}	19.25^{a}	17.52^{ab}	18.61^{a}	0.83	0.003
Cell-mediated immune response (mm)						
24 hour	2.58	2.86	2.90	2.65	0.35	0.901
48 hour	1.97	1.97	2.12	1.69	0.30	0.801
72 hour	0.87	0.53	0.74	0.65	0.19	0.650

cMOS = commercial mannan-oligosaccharides fed at 4 g/day/calf; YCW-2 = yeast cell wall content fed at 2 g/day/calf; YCW-4 = yeast cell wall content fed at 4 g/day/calf

^{a,b}Values in a row without common small letters differ significantly ($P < 0.05$)

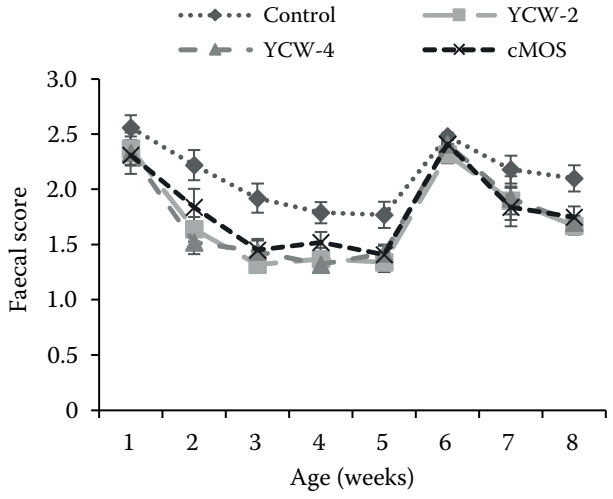

Figure 1. Effects of dietary supplementations of prebiotics on faecal scores in Holstein-Friesian male calves

Week: $P < 0.001$; treatment: $P < 0.001$; treatment × week: $P < 0.05$

a minimum of one day and a maximum of seven days. cMOS supplemented animals were treated for a minimum of three days and a maximum of six days. Furthermore, seven animals from the control group and two animals, one from YCW-2 group and the other from YCW-4 group were given sulfonamides (Disulf; ICI Pakistan Limited, Lahore, Pakistan) during the coccidiosis outbreak, whereas no animal from the cMOS group suffered from coccidiosis.

Blood metabolites

The mean glucose level was lower ($P < 0.001$) in the YCW-2 supplemented animals compared with the other groups. Prebiotics addition did not influence the glucose during week 2 and 4. However, in week 6 and 8, glucose concentration

https://doi.org/10.17221/70/2021-CJAS

was lower ($P < 0.05$) in the YCW-2 group than in the other groups. On the other hand, as the calves aged, βHBA and BUN levels were increased for all supplemented groups (Table 5). There were no differences between groups except in weeks 6 and 8 after birth when βHBA and BUN concentrations in the YCW-2 group were higher ($P < 0.01$) than in the other groups. On a temporal scale, both metabolites were non-significant among treatments in weeks 2 and 4. No effect of dietary treatments was observed for NEFA in the calves (Table 5).

DISCUSSION

The early period of neonatal calf life is very crucial as the calves can easily be infected with intestinal and respiratory pathogens. During this pre-weaning period, poor growth performance and veterinary intervention increase the cost of the weaned calf. To combat these problems, prebiotics have been used to improve the health and performance of calves during the pre-weaning period (Gosh and Mehla 2012). Presently, we sought to investigate the use of yeast cell wall contents as a dietary management tool to improve the health and growth performance of neonatal calves.

Growth performance, dry matter intake and feed efficiency

Results revealed that the supplementation of YCW to the calves significantly increased body weight, body weight gain (BWG), ADG, starter intake, and total DMI. Various studies have demonstrated that the supplementation of yeast cell culture to pre-weaned calves increased BWG, starter intake, total DMI, and ADG (Hill et al. 2009; Roodposhti and Dabiri 2012).

In calves, DMI, BWG, and health were improved when *Saccharomyces cerevisiae* fermentation product was included in the calf starter (Lesmeister et al. 2004). Recently, it has been observed that feeding of MOS resulted in higher ADG and BW compared to the control animals (He et al. 2017). In the present study, starter intake was very low during the first

Table 5. Effects of dietary supplementation of prebiotics on selected blood metabolites in Holstein-Friesian male calves

Variables	Treatment	Age (weeks)					SEM	*P*-value		
		means	2	4	6	8		treatment (TR)	week	TR × week
Glucose (mmol/l)	control	4.19^{a}	4.45	4.06	4.39^{a}	3.88^{a}	0.051	0.001	0.001	0.014
	YCW-2	3.59^{b}	4.32	4.18	3.31^{c}	2.58^{c}				
	YCW-4	3.89^{ab}	4.53	4.27	3.70^{bc}	3.07^{b}				
	cMOS	4.06^{a}	4.62	4.36	3.98^{ab}	3.31^{b}				
BUN (mmol/l)	control	1.55^{c}	1.21	1.41	1.29^{c}	2.26^{c}	0.023	0.001	0.001	0.001
	YCW-2	2.13^{a}	1.26	1.39	2.64^{a}	3.22^{a}				
	YCW-4	2.06^{ab}	1.49	1.61	2.34^{b}	2.79^{b}				
	cMOS	1.93^{b}	1.19	1.54	2.13^{b}	2.86^{b}				
NEFA (mmol/l)	control	0.35	0.36	0.34	0.34	0.35	0.004	0.053	0.070	0.226
	YCW-2	0.32	0.32	0.32	0.33	0.32				
	YCW-4	0.32	0.33	0.35	0.31	0.3				
	cMOS	0.32	0.31	0.33	0.36	0.3				
βHBA (mmol/l)	control	0.42^{c}	0.32	0.37	0.40^{c}	0.60^{c}	0.005	0.001	0.001	0.001
	YCW-2	0.55^{a}	0.36	0.37	0.64^{a}	0.81^{a}				
	YCW-4	0.49^{b}	0.37	0.38	0.52^{b}	0.70^{b}				
	cMOS	0.47^{b}	0.33	0.39	0.51^{b}	0.68^{b}				

βHBA= beta-hydroxybutyric acid; BUN= blood urea nitrogen; cMOS = commercial mannan-oligosaccharides fed at 4 g/day/calf; NEFA = non-esterified fatty acids; YCW-2 = yeast cell wall content fed at 2 g/day/calf; YCW-4 = yeast cell wall content fed at 4 g/day/calf

$^{a–c}$Values in a column without common small letters differ significantly ($P < 0.05$)

https://doi.org/10.17221/70/2021-CJAS

two weeks and became significant afterward, being higher in the supplemented calves. The inclusion of the yeast cell wall did not influence BWG during the first four weeks. It may be due to the fact that all animals were provided with a fixed quantity of milk (4 l/day) and starter intake was practically very low during this period. It appears that the growth of the calves depends on milk during this early period of life and the quantity of calf starter is not enough to induce its impact on the growth. Thereafter, the supplemented calves consumed more dry matter compared with the control calves that might have stimulated the development of the rumen and therefore improve growth performance. Though the amount of calf starter consumed by the calves supplemented with yeast cell wall became significant in week 3, its impact in terms of BWG and ADG appeared during week 5. The calves consumed more starter when YCW was supplemented at the rate of 2 g daily. Yeast supplementation has been shown to stimulate the growth of ciliated protozoa and cellulolytic bacteria that stabilize the rumen pH (Cangiano et al. 2020) and promote early rumen development as well as nutrient digestibility, hence increasing feed intake and ADG. Ghosh and Mehla (2012) observed that 4 g/day MOS supplementation improved ADG, average daily feed intake, and feed efficiency of the calves.

Consistent with the growth performance, yeast cell wall supplementation was also translated into the structural growth of the pre-weaned calves. In our study, body structural measurements were higher ($P < 0.05$) in the supplemented animals especially in the YCW-2 group than in the control animals. The results are also similar to Sharma et al. (2018), who noted increased heart girth, hip height, and body height in the MOS-fed buffalo calves. Lesmeister et al. (2004) observed that hip height and hip width were increased in the calves receiving starter supplemented with yeast fermentable products, while others failed to observe the effects with the same product (Alugongo et al. 2017).

Cell-mediated immune response

The delayed-type hypersensitivity skin test has been employed to measure the proliferative response of T-lymphocytes following dermal application of dinitrochlorobenzene. In the present study, we were unable to find the influence of the YCW or MOS supplementation on cell-mediated immunity in neonatal calves. Similar results were also observed by Roodposhti and Dabiri (2012), where supplementations of probiotics, prebiotics, and synbiotics did not affect delayed-type hypersensitivity in calves.

General health and faecal scores

The neonatal period of growing calves is the most crucial period of life due to an attenuated immune response that may result in intestinal and respiratory distresses leading to high mortality and morbidity. The inclusion of YCW has been suggested to ameliorate faecal score and, hence, improve the health and growth of the calves (Brewer et al. 2014). Our data suggested that the inclusion of YCW in whole milk improved general health conditions during the neonatal period. The calves receiving supplementation have on average lower ($P < 0.05$) faecal scores than the control animals. The lowest values of faecal scores were observed in animals when supplemented with 2 g yeast cell wall per calf per day. Similarly, Hill et al. (2009) found that the supplementation of MOS and yeast culture products improved the faecal scores in the calves. Our results are also consistent with other researchers where calves were fed YCW products (Magalhaes et al. 2008; Ghosh and Mehla 2012). However, the ameliorating effects of YCW were diminished during weeks 6 and 7 due to an outbreak of coccidiosis in some of our experimental animals. During this infection, more calves that had faecal scores ≥ 3 were in the control group than among the YCW fed calves. The greater occurrence of coccidiosis in the control group might have adversely affected the health and therefore lead to compromised growth. Interestingly, we observed that faecal scores were improved again in the supplemented groups in week 8 suggesting greater effects of YCW on improving the intestinal health. Though we are not sure how the yeast cell wall improved the faecal scores and health of neonatal calves, it is presumed that the yeast cell wall contains various components like mannoproteins, β-glucans, and oligosaccharides that may have decreased the attachment of pathogenic bacteria to the intestinal epithelial cells by directing them to bind with YCW components (White et al. 2002). It has been known that MOS has the ability to agglutinate gram-nega-

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tive bacteria by interacting with mannose-sensitive lectins located on the bacterial surface. Therefore, competitive inhibition of pathogenic gram-negative bacteria like *Escherichia coli* by the YCW components might be the cause of improved faecal scores and lower incidence of calf diarrhoea in our study.

Blood metabolites

Blood metabolites including blood glucose and blood urea nitrogen (BUN) are indicators of fermentative changes taking place in the developing rumen. In the present study, glucose ($P < 0.01$) concentration was decreased with the growth of calves as well as with prebiotics supplementation ($P < 0.01$). Young calves during the pre-ruminant phase resemble monogastric animals and glucose is being used as a major source of energy. The energy is primarily derived from milk, therefore, the glucose concentration is higher during this phase. However, glucose decreases as the age of calves progresses and the rumen is developed. Therefore, the level of glucose is considered an indicator of rumen development. Like ADG and BWG, glucose levels did not differ between treatments during weeks 2 and 4. Thereafter, glucose started decreasing in the supplemented animals only and the lowermost value of glucose was observed in YCW-2 animals in week 8. This reduction of glucose concentration is believed to be associated with higher starter intake. Quigley et al. (1991) reported low blood glucose concentration in the calves weaned on day 28 compared to those weaned later (day 56) that indicated changes in the energy metabolism of calves in relation to weaning age. Contrary to it, Dar et al. (2019) reported no effect on plasma glucose concentration when probiotics and prebiotics were included in the rations of the calves. Ballou (2011) also found that there was no difference in serum glucose and BUN concentrations in the calves fed a blend of prebiotics and probiotics when compared to the non-fed group for three weeks suggesting that the prebiotics and probiotics did not influence the systemic physiology of the calves during the first 21 days of life.

We observed significant effects of age, supplementation, and age × supplementation interaction on BUN (Table 5). In the young calves, potential changes occur in microbial protein synthesis in the rumen. The concentration of BUN can be used as a tool to determine the efficiency of dietary nitrogen utilization by the calves. Higher levels of BUN have also been associated with the efficient functioning of the rumen (Hadorn et al. 1997). As observed in the present study, we found, like previously Rashid et al. (2013), a similar pattern of an increase in BUN concentration with age in buffalo calves. Likewise, Quigley et al. (2006) also reported increased BUN with age in the cattle calves. We found that the calves supplemented with 2 g of YCW had higher BUN levels compared with the other groups in week 7 and 8. Elevated levels of BUN in the calves fed the yeast cell wall might be attributed to higher consumption of starter that resulted in higher degradation of dietary proteins or deamination of amino acids to form ammonia (Hadorn et al. 1997). Although we did not measure the effects of supplementation on the rumen development, we speculate that higher intake of starter in the supplemented groups might have resulted in better ruminal development as the increased levels of BUN were also linked with the efficient functioning of the rumen (Hadorn et al. 1997).

Like BUN, beta-hydroxybutyric acid was also increased with calf age ($P < 0.01$; Table 5), which may also indicate the rumen development. Blood βHBA, a measure of rumen epithelial metabolic activity, is primarily synthesised by the ruminal epithelium by converting butyrate that is formed during ruminal fermentation (Lane et al. 2000). As the rumen of a newborn calf is not functional metabolically, therefore, the level of βHBA is very low at this phase. As soon as the calves started taking solid food, the ruminal microbiota is developed and established. Subsequently, the physical and metabolic development of the rumen occurs and the ruminal walls become the primary source of βHBA production. Similar to the findings of others (Quigley et al. 1991) blood βHBA increased with the age of calves. Our study showed that blood βHBA was higher in the YCW-2 calves compared with the other calves during the last weeks of feeding. Beta-HBA is considered an indicator of rumen development, therefore it may also reflect increased starter intake (Quigley et al. 1991). We also found a similar result as DMI and starter intake were higher in the calves fed prebiotics compared with the non-supplemented calves reflecting the better rumen growth, a desirable attribute in the calf rearing. Similar results were found by Quigley et al. (1991), where supplementation of MOS, in-

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ulin, and yeast increased BUN and βHBA levels in the neonatal calves. On the other hand, Dar et al. (2019) found that supplementation of prebiotics and probiotics did not affect the βHBA level in the calves. This discrepancy in the results may be due to various factors like a dose of YCW, source of YCW, variation in the composition of YCW, nature of the starter, and also the hygienic measures during the experiment.

CONCLUSION

In the present study, supplementations of prebiotics, especially 2 g of YCW, to the neonatal male cattle calves had a positive impact on growth performance, general health, body measurements, faecal scores, and blood metabolites that might be due to improvement in digestibility of dietary fibre and better rumen development suggesting a potential antibiotic growth promoter replacer. However, further studies are needed to investigate the potential role of YCW in the development and fermentation profile of rumen at various phases of weaning.

Conflict of interest

The authors declare no conflict of interest.

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Received: May 4, 2021
Accepted: February 16, 2022
Published online: March 4, 2022