



Growth performance and hemobiochemical parameters in South African dusky kob (*Argyrosomus japonicus*, Sciaenidae) offered brewer's yeast (*Saccharomyces cerevisiae*) as a feed additive

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Abstract

There is some evidence that single-cell proteins such as yeast have the potential to improve feed utilization in aquaculture fish, but this has not been investigated in the economically important dusky kob, *Argyrosomus japonicus*. This study was, therefore, designed to determine the effect of graded levels of dietary inactivated brewer's yeast, *Saccharomyces cerevisiae*, on the growth performance and hemobiochemical parameters of dusky kob in a 6-week feeding period. Five isonitrogenous and isoenergetic diets—consisting of three brewer's yeast-containing diets at rates of 50,150, and 300 g/kg dry matter (BY5P0, BY15P0, and BY30P0, respectively); a commercial dusky kob diet containing 10% probiotic mix but no brewer's yeast (BYOP1, positive control); and a commercial dusky kob diet with neither the probiotic mix nor the yeast (BYOP0, negative control)—were formulated. A total of 65 fish, weighing an average of 7.02 ± 0.10 g, were randomly distributed to each of 20 replicate tanks. Each dietary treatment was randomly allocated to four tanks and offered to fish at a rate of 2.8% fish body weight per day. A total of 10 fish from each tank were randomly sampled once a week for length and weight measurements. Blood was drawn from five fish per tank (20 fish per treatment) for hematology and serum

biochemical analyses at the end of the 6 weeks. Fish on the BY0P0 diet achieved the highest weight gain of 18.53 ± 0.69 g after 6 weeks. Growth rate was significantly reduced in the groups fed BY15P0 and BY30P0 diets compared to the BY0P0, BY0P1, and BY5P0 groups. Fish fed the BY0P0 diet recorded the highest average feed conversion efficiency (FCE) of 0.22, while the BY30P0-fed group recorded the lowest FCE of 0.15. Hematocrit and alanine transaminase levels declined with increasing levels of yeast. It can be concluded that the maximum inclusion level of brewer's yeast that does not impair growth performance and health of dusky kob is 50 g/kg.

KEYWORDS

blood parameters, dusky kob, feed conversion efficiency, growth, probiotic mix, *Saccharomyces cerevisiae*

1 | INTRODUCTION

Brewer's yeast has rarely been incorporated as an additive in fish diets but rather as a fishmeal replacement or as a protein source (Nayar, Hegde, Rao, & Sudha, 1998; Oliva-Teles & Gonçalves, 2001). However, its ability to stimulate intestinal brush border enzymes (Buts, Bernasconi, Van Craynest, Maldague, & De Meyer, 1986) and to facilitate nutrient digestion and utilization means it should be evaluated as an additive in diets for dusky kob. As yeast has the ability to out-compete pathogenic bacteria in the gut flora (Ofek, Mirelman, & Sharon, 1977), the inclusion of brewer's yeast in the diets of the dusky kob, *Argyrosomus japonicus*, should result in improved growth performance. A growing number of studies have reported the ability of probiotics to increase the growth rate and welfare of farmed fish (Carnevali et al., 2004; Lara-Flores, Olvera-Novoa, Guzman-Mendez, & Lopez-Madrid, 2003; Macey & Coyne, 2005; Wang & Xu, 2006). Brewer's yeast, *Saccharomyces cerevisiae*, has been recognized as having the potential to be a substitute for live food in the production of certain fish (Nayar et al., 1998) or as a potential replacement for fishmeal (Oliva-Teles & Gonçalves, 2001). Composition of brewer's yeast has been reported to be: 44.35% crude protein, 5.90% ether extract, 4.80% crude fiber, 3.30% lysine, and 0.90% methionine (Sacakli, Koksall, Ergun, & Ozsoy, 2013).

The use of yeast in fish feed is justified because of its ability to stimulate the intestinal brush border disaccharidases, an antiadhesive effect against pathogens, and the stimulation of nonspecific immunity (Buts et al., 1986). The cell wall of *S. cerevisiae* contains glucans, mannoproteins, and chitins, which are important, naturally occurring immunostimulants with some growth-promoting effects in fish (Estaban, Rodriguez, & Mesguer, 2004). Yeast competes with pathogens to occupy the intestinal cells, a process that is crucial to the expression of the cytopathogenic effect (Li, Shi, Guan, Zhang, & Tian, 1998). Mannan oligosaccharides derived from the cell wall of yeast are known to improve digestion and gut health by specifically binding to and blocking glycoprotein receptors on the gut-invading pathogens, subsequently improving animal growth (Newman, 2001).

Ozório, Portz, Borghesi, and Cyrino (2012) reported that dried dietary yeast was palatable to tilapia, *Oreochromis niloticus*, juveniles and that a 15% inclusion level promoted growth and efficient diet utilization. However, the daily growth coefficient (% body weight/day) decreased with increasing levels of dietary yeast. Most beneficial effects of

yeast or yeast products as feed additives have been reported in fish species that can tolerate water temperatures lower than 20°C, such as rainbow trout (Rumsey, Hughes, Smith, Kinsella, & Shetty, 1991), common carp (Eleraky, Yahya, & Rahsa, 2014), sea bass (Oliva-Teles & Gonçalves, 2001), and sea bream (Salnur, Gultepe, & Hossu, 2009). The nutrient quality of the feed and environmental conditions, such as water temperature and salinity, may determine the outcome of feed utilization efficiency in fish (Handeland, Imsland, & Stefansson, 2008). Dusky kob juveniles feed optimally between 25 and 26.4°C (Bernatzeder & Britz, 2007). There is currently no literature detailing the role of fish gut structure in metabolizing inactivated yeast, but there is evidence which shows that there is a relationship between feeding habits and the anatomy of the digestive system in fish (Bond, 1979). The above-mentioned fish (sea bass, sea bream, and rainbow trout), as well as dusky kob, are carnivorous species with relatively short digestive tracts, whereas tilapia and carp are herbivorous species with relatively long digestive tracts. There is a great possibility that the disparity in the anatomy of their digestive systems may have a bearing on how they digest and metabolize yeast or any single-cell proteins (SCP) in their diets.

The dusky kob has numerous attributes, including being a recreational fish with high market value, exhibiting fast growth rates and high tolerance to fluctuating salinity (Whitfield, 1998), poor water quality, and low oxygen levels (Fitzgibbon, Strawbridge, & Seymour, 2007). Consequently, formulating an affordable feed could promote dusky kob aquaculture in South Africa. Therefore, the objective of the current study was to investigate the effects of including dietary brewer's yeast in a commercial feed at four levels (0, 50, 150, and 300 g/kg) on the growth performance, hematological, and serum biochemical parameters of the dusky kob.

2 | MATERIALS AND METHODS

2.1 | Experimental site

This study was carried out at the Marine Research Aquarium of the Department of Agriculture, Forestry and Fisheries in Sea Point, Cape Town, South Africa. The experimental system was a recirculating aquaculture system consisting of 20 black, high-density polyethylene grow-out tanks (465 L capacity, 67 cm deep, and 94 cm in diameter) with flattened conical floors coated with white fiberglass resin to allow for better fish visibility. The sea water temperature was maintained at 25°C via a heat pump and with dissolved oxygen at 5.5–6.0 mg/L via air lines. The filtration system included a protein skimmer or foam fractionator, a sand filter, and biological filtration media. Ultraviolet lights (55 W) were fitted on the water route between the filtration system and the fish holding tanks.

2.2 | Experimental fish

The handling of live fish was conducted in compliance with the South African Animals Protection Act, 1962 (Act 71 of 1962). Ethical clearance was obtained from the North-West University's Animal Research Ethics Committee (NWU-00691-17-S9). Dusky kob fingerlings were sourced from a commercial fish farm based in Mtunzini, Kwa-Zulu Natal, off the South African east coast. They were transported in 950 L of sea water, with a salinity of 28 ppt and a temperature of 27°C. Pure oxygen was injected into the water at a rate of around 11 mg/L per minute. Upon arrival, 65 fish were randomly distributed to each of the 20 tanks and left to acclimatize for a period of 6 weeks prior to trial commencement. Commercial fishmeal diet (Marifeed Pty Ltd) was given to the fish during the acclimatization period. At the start of the experiment, the fish weighed, on average, 7.02 ± 0.10 g.

2.3 | Experimental diets

The ingredients and chemical composition of formulated experimental diets are shown in Table 1. A commercial brewer's yeast product (inactivated *S. cerevisiae*) was sourced from a health food distributor, Nature's Choice, based in Meyerton, South Africa. An animal feed probiotic, used in one of the experimental diets, was developed by the

TABLE 1 Ingredients and chemical composition of the experimental diets (g/kg dry matter)

Ingredients	Diets				
	BY0P0	BY0P1	BY5P0	BY15P0	BY30P0
Fishmeal	700	694.0	687.0	681.0	574.0
Brewer's yeast	0	0	50	150	300
Bulk agent (cellulose)	200	190	170	93	65
10% probiotic mix	0	20	0	0	0
Premix (vitamins and minerals)	7	7	7	7	7
Fish oil (mL)	93	89	86	69	54
Proximate composition					
Dry matter	922	981	961	938	952
Ash	101.6	110.5	114.4	137.1	155.0
Moisture	78	19	39	62	48
Crude protein	486.9	488.5	494.9	486.6	456.8
Crude fiber	12.2	16.6	15.4	10.8	18.7
Crude lipid	270.0	281.6	215.5	260.0	314.3
Nitrogen-free extract	43.4	76.8	113.9	50.2	12.3

Note: Diets: BY0P0 = Negative control: commercial kob feed diet with neither brewer's yeast nor probiotics; BY0P1 = Positive control: commercial kob diet with 10% animal probiotic mix but no brewer's yeast; BY5P0 = commercial kob diet containing 50 g brewer's yeast/kg without probiotics; BY15P0 = commercial kob diet containing 150 g brewer's yeast/kg without probiotics; BY30P0 = commercial kob diet containing 300 g brewer's yeast/kg without probiotics.

Council for Scientific and Industrial Research and sourced from Biocentric Technologies, Johannesburg, South Africa. The probiotic product contained three *Bacillus cereus* isolates (*B. cereus* B006, *B. cereus* CFF001, and *B. cereus* TS101) with a total cell concentration of 1×10^9 CFU/g.

Five isonitrogenous and isoenergetic diets, consisting of three brewer's yeast-containing commercial dusky kob diets at rates of 50, 150, and 300 g/kg DM (BY5P0, BY15P0, and BY30P0, respectively); a commercial dusky kob diet containing 10% probiotic mix without brewer's yeast (BY0P1, positive control); and a commercial dusky kob diet with neither the probiotic mix nor the yeast (BY0P0, negative control), were formulated. The dietary treatments were hand-prepared at the Marine Research Aquarium as follows: The commercial dusky kob pellets (8 mm) were granulated (<0.8 mm) with a Krups Burr Grinder model GVX 2. The powdered pellets were proportionally mixed with preweighed quantities of brewer's yeast and multivitamins/minerals. Water was added to the final dry mixture. The mixture was kneaded to produce dough, which was then rolled out to a thin layer using a kitchen dough roller. The thin layer was dried to constant weight using a household floor fan. The flaked pieces of dried food were then graded to different sizes ranging from 1 to 4 mm using a corn kernel hand grinder with an adjustable pressure disc for flake sizing.

2.4 | Feeding strategy and sampling

Each dietary treatment was allocated to four replicate tanks, and fish were consistently hand-fed twice a day at a rate of 2.8% of the fish body weight. Because the dusky kob has photophobia and tends to be cannibalistic, the fish were not exposed to any artificial lighting. Ten fish from each of the 20 tanks were randomly sampled once a week to measure standard length and body mass (Mettler Toledo electronic balance model: Viper SW 15). The 10 sampled fish were returned to the same tank from which they were sampled. A 1-mL dose of 2-phenoxyethanol per 5 L of water was used to anesthetize fish during the measurements. Feed conversion efficiency (FCE) was calculated based

on the collective feed mass offered for the experimental period. Feed offered was adjusted on a weekly basis after body mass measurements (rate of feeding: 2.8% of fish population body mass per day). Tanks were monitored daily before and during feeding for any mortality and for behavioral changes in the fish.

2.5 | Hematology and serum biochemical analyses

At the end of the 6-week feeding trial, a random sample of five fish per tank (20 fish per treatment) was anesthetized using 2-phenoxyethanol before blood was collected. The caudal fin was cut using a dissecting kit scissor to let the fish bleed into collecting tubes. The samples were collected in bottles containing ethylenediaminetetraacetic acid as an anticoagulant at a concentration of 5 mg/mL of blood sampled. Some blood samples were stored in a slanting wooden rack at room temperature to allow the blood to clot. The clotted blood was centrifuged for 15 min at 3,500 rpm. The serum was pipetted out into a clean and sterilized bottle for serum biochemical analysis.

Manual blood count (light microscope; [Olympus, Tokyo, Japan] under oil immersion at $\times 100$ magnification) was performed for hematocrit, thrombocytes, lymphocytes, monocytes, neutrophils, basophils, and eosinophils. Alkaline phosphatase (ALP) activity was measured using the modified method of Wright, Leathwood, and Plummer (1972), while aspartate aminotransferase (AST) and alanine transaminase (ALT) activities were determined according to the methods described by Reitman and Frankel (1957). Total protein (TP) was analyzed according to the Biuret method standardized for the RA-1000 (Technicon method no. SM4-0147K82, 1982). Albumin and creatinine were determined according to the Technicon methods numbers SM4-0131K82 (1982) and SM4-0141K82 (1982), respectively. Technicon reagents were used for TP, albumin, and creatinine assays. Total globulin fraction was determined by subtracting the albumin from the TP. A standard RA-1000 enzymatic method was applied for the analysis of triglycerides using the Boehringer Mannheim GPO-PAP kit. The moncholesterol (CHOD-PAP) method was used to determine cholesterol content. Urea content was estimated by following the Crest Biosystems Modified Berthelot method (Fawcett & Scott, 1960).

2.6 | Statistical analysis

Measurements from multiple fish per tank were averaged before analysis, such that each replicate tank had one value. All the data from the reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Weekly growth performance data were analyzed using repeated-measures analysis using the Proc Mixed procedures of Statistical Analysis System (2010) according to the following model:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

where Y_{ijk} = dependent variable; μ = population mean; D_i = effect of diets; W_j = effect of week; $D \times W_{ij}$ = effect of interaction between diets and week; and E_{ijk} = random error associated with observation ijk , which was normally and independently distributed.

The general linear model procedures of the Statistical Analysis System (Statistical Analysis System, 2010) were used to analyze hematological and serum biochemical data according to the following statistical linear model:

$$Y_{ij} = \mu + D_i + E_{ij},$$

where Y_{ij} = dependent variable; μ = population mean; D_i = effect of diet; and E_{ij} = random error associated with observation ij , which was normally and independently distributed. For all statistical tests, significance was declared at

$p \leq .05$. Least-squares means were compared using the probability of difference (pdiff) option in the lsmeans statement of Statistical Analysis System.

3 | RESULTS

3.1 | Feed intake, growth performance, and FCE

The white resin coating on the tank floor made it possible to visually confirm the presence or absence of uneaten feed. Observations during feeding and immediately after feeding indicated that, for all the dietary treatments, all the feed offered was consumed within 2 min of being in contact with water. Syphoning of the tanks after 10 min of feeding confirmed this observation. Statistical analysis demonstrated a significant interactive effect between dietary treatments and time (weeks) ($p < .05$) on fish weight and standard length. Fish weight at the start of the feeding trial and also after the first week of feeding were similar ($p > .05$) across the five treatment groups. The effect of the experimental diets on fish weight and standard length per week is presented in Table 2. The effect of the dietary treatments did not differ regarding the FCE in the fish ($p > .05$). At the end of the 6-week feeding period, the negative control group recorded an FCE value of 0.22, BYOP1-fed group recorded 0.21, BY5PO recorded 0.20, BY15PO recorded 0.19, and the BY30PO-fed group recorded an FCE value of 0.15.

3.2 | Hematology and serum biochemical analyses

Experimental diets significantly influenced lymphocyte, thrombocyte, monocyte, and hematocrit counts but not the eosinophil, basophil, and neutrophil counts (Table 3). There was a notable decrease in hematocrit levels with incremental levels of brewer's yeast, with BY5PO-fed fish recording 42.06%, BY15PO recording 38.89%, and BY30PO recording 36.96%. The inclusion of a probiotic mix in the dusky kob diet lowered the levels of some of the hematological parameters compared to the negative control (BYOP0): eosinophils (1.25% in BYOP0 vs. 1% in BYOP1),

TABLE 2 Weekly weight (g) and standard length (cm) of dusky kob in response to dietary treatments

Diets	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
BYOP0	^W 7.28 ± 0.21 ^a	8.25 ± 0.53 ^a	11.93 ± 0.28 ^b	15.05 ± 0.41 ^c	16.70 ± 0.84 ^b	20.75 ± 1.57 ^b	25.80 ± 0.51 ^c
	^L 7.40 ± 0.06 ^a	7.60 ± 0.11 ^a	8.38 ± 0.09 ^a	9.28 ± 9.09 ^c	9.68 ± 0.17 ^b	10.40 ± 0.23 ^a	10.98 ± 0.32 ^c
BYOP1	^W 6.75 ± 0.06 ^a	7.95 ± 0.25 ^a	9.58 ± 0.25 ^a	13.37 ± 0.28 ^{bc}	14.88 ± 0.43 ^{ab}	17.37 ± 0.58 ^a	21.80 ± 1.09 ^b
	^L 7.23 ± 0.03 ^a	7.53 ± 0.13 ^a	7.95 ± 0.05 ^a	9.03 ± 0.03 ^{bc}	9.25 ± 0.06 ^a	9.75 ± 0.10 ^a	10.58 ± 0.15 ^{bc}
BY5PO	^W 6.80 ± 0.27 ^a	8.25 ± 0.31 ^a	10.43 ± 0.75 ^{ab}	12.45 ± 0.67 ^{ab}	14.60 ± 0.61 ^{ab}	17.57 ± 0.37 ^a	21.35 ± 0.49 ^b
	^L 7.23 ± 0.11 ^a	7.50 ± 0.08 ^a	8.18 ± 0.19 ^a	8.78 ± 0.11 ^{ab}	9.22 ± 0.10 ^a	9.75 ± 0.09 ^a	10.40 ± 0.10 ^{abc}
BY15PO	^W 6.90 ± 0.15 ^a	7.52 ± 0.16 ^a	9.55 ± 0.46 ^a	11.52 ± 0.63 ^{ab}	13.78 ± 0.32 ^a	16.23 ± 0.59 ^a	18.67 ± 1.27 ^{ab}
	^L 7.32 ± 0.08 ^a	7.40 ± 0.08 ^a	8.00 ± 0.11 ^a	8.50 ± 0.13 ^a	9.07 ± 0.08 ^a	9.55 ± 0.10 ^a	9.98 ± 0.21 ^{ab}
BY30PO	^W 7.37 ± 0.26 ^a	7.52 ± 0.15 ^a	9.17 ± 0.36 ^a	11.10 ± 0.54 ^a	12.60 ± 0.39 ^a	15.35 ± 0.41 ^a	16.95 ± 0.63 ^a
	^L 7.45 ± 0.09 ^a	7.42 ± 0.08 ^a	7.92 ± 0.06 ^a	8.37 ± 0.17 ^a	8.83 ± 0.09 ^a	9.87 ± 0.58 ^a	9.62 ± 0.09 ^a
	^W NS, ^L NS	^W NS, ^L NS	^W *, ^L NS	^W *, ^L *	^W *, ^L NS	^W *, ^L NS	**

Note: Diets: BYOP0 = Negative control: commercial kob feed diet with neither brewer's yeast nor probiotics; BYOP1 = Positive control: commercial kob diet with 10% animal probiotic mix but no brewer's yeast; BY5PO = commercial kob diet containing 50 g brewer's yeast/kg without probiotics; BY15PO = commercial kob diet containing 150 g brewer's yeast/kg without probiotics; BY30PO = commercial kob diet containing 300 g brewer's yeast/kg without probiotics. ^Wweight; ^Llength. Means along the same column with different superscripts denote significant differences ($p < .05$). NS: $p > .05$; * $p < .005$; ** $p < .001$.

TABLE 3 Hematological parameters of dusky kob in response to dietary treatments

	Diets				
	BYOPO	BYOP1	BY5PO	BY15PO	BY30PO
Hematocrit (%)	40.15 ± 2.1 ^a	38.61 ± 0.53 ^{ab}	42.06 ± 1.41 ^b	38.89 ± 1.80 ^{ab}	34.94 ± 0.94 ^a
Lymphocytes (%)	89.5 ± 2.33 ^{ab}	86.25 ± 1.55 ^{ab}	90 ± 1.00 ^{ab}	93.25 ± 1.70 ^b	87.00 ± 2.92 ^{ab}
Monocytes (%)	7.00 ± 1.22 ^{ab}	9.75 ± 1.89 ^b	8.25 ± 1.60 ^{ab}	3.75 ± 0.75 ^a	10.00 ± 2.91 ^b
Eosinophils (%)	1.25 ± 0.96 ^a	1.00 ± 0.56 ^a	1.75 ± 1.18 ^a	1.75 ± 0.63 ^a	1.75 ± 0.75 ^a
Basophils (%)	0.75 ± 0.75 ^a	0.00 ^a	0.00 ^a	0.50 ± 0.50 ^a	0.50 ± 0.29 ^a
Thrombocytes (6.2/hpf)	3.45 ± 2.08 ^a	3.25 ± 1.32 ^a	2.575 ± 0.50 ^a	2.225 ± 0.60 ^a	4.3 ± 1.76 ^a
Neutrophils (%)	1.50 ± 0.87 ^{ab}	3.00 ± 0.91 ^b	0.00 ^a	0.75 ± 0.75 ^{ab}	0.75 ± 0.75 ^{ab}

Note: Diets: BYOPO = Negative control: commercial kob feed diet with neither brewer's yeast nor probiotics; BYOP1 = Positive control: commercial kob diet with 10% animal probiotic mix but no brewer's yeast; BY5PO = commercial kob diet containing 50 g brewer's yeast/kg without probiotics; BY15PO = commercial kob diet containing 150 g brewer's yeast/kg without probiotics; BY30PO = commercial kob diet containing 300 g brewer's yeast/kg without probiotics. In a row, means with common superscripts do not differ ($p > .05$).

basophils (0.75% in BYOPO vs. 0% in BYOP1), lymphocytes (89.50% in BYOPO vs. 86.25% in BYOP1), and hematocrit (40.15% in BYOPO vs. 38.61% in BYOP1).

For serum biochemistry, dietary treatments only influenced ($p < .05$) ALT and AST, while the remaining serum metabolites were not significantly affected (Table 4). The inclusion of the probiotic mix in the diet (BYOP1) significantly lowered the levels of ALT, AST, creatinine, and cholesterol when compared to the negative control (BYOPO).

TABLE 4 Effect of dietary treatments on concentration of selected blood serum metabolites in dusky kob

Metabolites	Diets				
	BYOPO	BYOP1	BY5PO	BY15PO	BY30PO
Urea (mmol/L)	3.05 ± 1.12 ^a	3.03 ± 0.12 ^a	2.93 ± 0.10 ^a	3.18 ± 0.10 ^a	3.28 ± 0.18 ^a
CREA (umol/L)	27.50 ± 6.20 ^{ab}	21.50 ± 1.84 ^a	20.25 ± 0.85 ^a	35.00 ± 5.33 ^b	25.75 ± 1.70 ^{ab}
BUN/CREA	25.50 ± 9.39 ^a	36.75 ± 4.80 ^a	41.25 ± 1.25 ^a	14.25 ± 8.25 ^a	35.00 ± 5.14 ^a
TP (g/L)	38.75 ± 1.80 ^a	38.50 ± 0.64 ^a	40.00 ± 1.47 ^a	38.00 ± 1.08 ^a	39.00 ± 0.91 ^a
ALB (g/L)	14.25 ± 0.48 ^a	14.75 ± 0.25 ^{ab}	15.50 ± 0.29 ^b	14.25 ± 0.25 ^a	14.50 ± 0.29 ^{ab}
GLOB (g/L)	24.50 ± 1.32 ^a	23.75 ± 0.85 ^a	24.50 ± 1.19 ^a	23.75 ± 0.85 ^a	24.50 ± 0.65 ^a
ALB/GLOB	0.60 ± 0.00 ^a	0.63 ± 0.05 ^a	0.65 ± 0.29 ^a	0.60 ± 0.00 ^a	0.60 ± 0.00 ^a
AST (U/L)	142.75 ± 14.49 ^b	122.00 ± 7.14 ^{ab}	93.50 ± 3.92 ^a	109.50 ± 6.33 ^a	103.00 ± 8.97 ^a
ALT (U/L)	61.25 ± 5.25 ^b	40.50 ± 5.68 ^{ab}	42.00 ± 4.53 ^{ab}	36.50 ± 6.34 ^a	31.75 ± 4.05 ^a
ALKP (U/L)	45.25 ± 4.48 ^a	46.75 ± 4.70 ^a	45.25 ± 5.12 ^a	64.00 ± 12.76 ^a	53.75 ± 2.29 ^a
CHOL (mmol/L)	2.27 ± 0.21 ^a	2.16 ± 0.19 ^a	2.16 ± 0.24 ^a	2.18 ± 0.09 ^a	2.09 ± 0.10 ^a
TRIG (mmol/L)	3.54 ± 1.05 ^a	4.06 ± 1.26 ^a	3.55 ± 0.53 ^a	3.80 ± 0.72 ^a	3.04 ± 0.43 ^a

Note: Diets: BYOPO = Negative control: commercial kob feed diet with neither brewer's yeast nor probiotics; BYOP1 = Positive control: commercial kob diet with 10% animal probiotic mix but no brewer's yeast; BY5PO = commercial kob diet containing 50 g brewer's yeast/kg without probiotics; BY15PO = commercial kob diet containing 150 g brewer's yeast/kg without probiotics; BY30PO = commercial kob diet containing 300 g brewer's yeast/kg without probiotics. In a row, means with common superscripts do not differ ($p > .05$).

Abbreviations: ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BUN/CREA, blood urea nitrogen/creatinine; CHOL, cholesterol; CREA, creatinine; GLOB, globulin; TP, total protein; TRIG, triglycerides.

4 | DISCUSSION

4.1 | Growth performance and FCE

The nutrient quality of the feed and environmental conditions such as temperature may determine the outcome of feed utilization efficiency of any fish species (Handeland et al., 2008). Dusky kob juveniles feed optimally between 25 and 26.4°C (Bernatzeder & Britz, 2007). However, Hidalgo and Alliot (1988) observed optimum feed utilization and growth of sea bass juveniles between 15 and 20°C. These differing physiological aspects between dusky kob and sea bass may explain the contrasting outcomes when using brewer's yeast as a feed supplement. The protein content of BY30P0 diet (456.8 g/kg) fell within the optimum dusky kob protein requirement as reported by Daniel (2004). Thus, suboptimal protein content was likely not the cause of the low feed utilization observed in the BY30P0 group. Poor FCE (0.09–0.12) was also observed in juvenile dusky kob when fed seaweed *Ulva*-based diets in a 9-week feeding trial (Madibana, Mlambo, Lewis, & Fouche, 2017). Meagre, *Argyrosomus regius*, a close relative of the dusky kob that was used in the current study, was able to attain FCE values between 0.51 and 0.74 after 3 months of feeding (Abdelhamid, Fedekar, Abou, & Radwan, 2013), a much longer feeding period compared to the 6 weeks used in the current study.

In their studies on sea bass, Metailler and Huelvan (1993) also tested the inclusion of 100, 200, and 300 g/kg of lactic yeast, baker's yeast, and brewer's yeast in isoproteic fishmeal-based diets. The authors reported no differences in growth and feed utilization among the groups, with the exception of those fish that were fed brewer's yeast-containing diets. These previous results are in contrast to our findings where fish fed on diets containing more than 150 g yeast/kg (BY30P0) had lower growth performance compared to those fed the control and BY5P0 diets. Moreno, Sanchez-Dmontero, Ballesteros, and Sinisterra (1991) discovered that most SCPs have high concentrations of nucleic acids, with brewer's yeast nucleic acid nitrogen making up to 20–25% of the nitrogen (Rumsey, Hughes, et al., 1991). Excess dietary nucleic acid supply has been reported to be toxic in most monogastric animals and humans as the capacity to excrete uric acid is limited, leading to suppressed growth and metabolism disorders (Tusé, 1984). However, because of their active liver uricase, fish have been reported to cope with an excess of dietary nucleic acid supply (De la Huiguera, Sanchez-Muniz, Mataix, & Varela, 1981). However, some authors suggest that high levels of nucleic acids content may have harmful or growth-suppressing effects in fish (Davies & Wareham, 1988), a possible explanation for the results we observed in the current study.

Rumsey, Hughes, and Kinsella (1990) suggested that reduced growth in fish offered more than 50 g brewer's yeast/kg may be because of the unavailability of intracellular nutrients from intact yeast cells. Indeed, Rumsey, Kinsella, Shetty, and Hughes (1991) found that the digestibility of intact brewer's yeast cells in rainbow trout was lower than that of disrupted cells and resulted in reduced growth performance. In the current study, results suggest that the inclusion rate of brewer's yeast should not exceed 5% in dusky kob diets as weight gain decreased at higher yeast inclusion levels.

Although there was no dietary treatment-induced reductions in feed intake in the current study, Rumsey, Hughes, et al. (1991) observed a depression in feed intake in rainbow trout when offered diets containing 250 g brewer's yeast/kg diet. Rumsey, Winfree, and Hughes (1992) implicated purine bases as being responsible for the depressing feed intake in farm animals. Also in contrast to the findings of the current study, Solomon, Ataguba, and Itodo (2017) observed a reduction in feed intake in African catfish, *Clarias gariepinus*, fed a brewer's yeast diet compared to those fed a soybean diet.

Inclusion of brewer's yeast in experimental diets for Nile tilapia, *O. niloticus*, resulted in improved growth rates (Asadi Rad, Zakeri, Yavari, & Mousavi, 2012). Welker and Lim (2011) suggested that probiotics like brewer's yeast may improve the digestion of farmed fish by stimulating the production of digestive enzymes or through other alterations in the gut environment, which could result in improved growth performance. Contrary to the findings of this study, supplementation with active brewer's yeast, *S. cerevisiae*, in Nile tilapia diets (10⁴ CFU/g diet) significantly improved the final body weight, specific growth rate, and feed conversion ratio (Essa et al., 2010). The authors

suggested that the positive effects of the yeast may be because of unidentified growth factors that elicit a response at low concentrations. The *S. cerevisiae* and related yeast species are known to secrete polyamines such as putrescine, spermidine, and spermine (Tabor & Tabor, 1985), which are thought to be essential growth factors. According to Peulen, Deloyer, and Dandrifosse (2002), those polyamines play a fundamental role in proliferation, rapid growth, and regeneration of tissues.

When provided in certain concentrations in fish diets, probiotics have been reported to beneficially modify the intestinal microbiota by secreting antibacterial substances (bacteriocins and organic acids) (Meyers, 2007). They also compete with pathogens for nutrients and space, thereby preventing the adhesion of pathogens to the intestinal walls, thus rendering them inactive (Weiss, 2013). However, the probiotic mix used in this study did not show any growth-promoting effects, and it may not be beneficial to dusky kob diets.

4.2 | Hematology and serum biochemical analyses

Hematological characteristics can be used as an effective index to monitor any physiological and pathological changes in fish. There are no existing records of normal ranges of blood parameters for dusky kob, but different studies have established normal ranges for other species (Rambhaskar & Srinivasa Rao, 1987; Xiaoyun, Mingyun, Khalid, & Weinmin, 2009). All the hematological parameters for dusky kob recorded in this study fell within normal ranges for tarpon, *Megalops cyprinoides*; gray mullet, *Mugil cephalus*; Borneo mullet, *Liza macrolepis*; black-tailed trevally, *Caranx carangus*; rosy snapper, *Lutjanus lutjanus*; ten-pounder, *Elops saurus*; and dojo loach, *Misgurnus anguillicadatus*, fish as reported by Rambhaskar and Srinivasa Rao (1987) and Xiaoyun et al. (2009). The effect of dietary treatments was not significant for eosinophil, basophil, and neutrophil counts but influenced thrombocyte, lymphocyte, monocyte, basophil, and hematocrit levels. Although hematocrit levels decreased with an increase in yeast dilution, the levels of all parameters were within normal ranges for fish as reported by Rambhaskar and Srinivasa Rao (1987) and Xiaoyun et al. (2009).

Isolating the notable hematocrit results, the levels recorded from the current study ranged between 34.94% (BY30P0) and 42.06% (BY0P0). These results are in line with normal hematocrit values determined for other marine carnivorous species such as the Asian sea bass, *Lates calcarifer*; the flathead gray mullet, *M. cephalus* (omnivores); and the milkfish, *Chanoschanos* (herbivores) (Satheeshkumar, Ananthan, Senthil Kumar, & Jagadeesan, 2011). As is the case in this study, Allam (2007) reported no negative effect of baker's yeast (10 and 20% of the diet) on hematological parameters of the Nile tilapia. From these two studies, it shows that brewer's yeast does not compromise the hematological status of fish.

Serum biochemistry parameters vary from species to species and can be influenced by many biotic and abiotic factors, such as water temperature, seasonal patterns, feed, age, and the gender of the fish (Jawad, Al-Mukhtar, & Ahmed, 2004). Patriche, Patriche, and Tenci (2009) observed that total serum protein varied with the diet consumed. However, in the current study, the total serum protein recorded for fish fed the two control diets was similar to that of groups fed yeast-supplemented diets. This suggests that yeast supplementation had no impact on the concentration of serum protein in the experimental fish. The same observation was made for concentrations of both globulin and albumin. The levels of globulin and TP indirectly reflect the condition of specific humoral immunity (Maqsood, Samoon, & Singh, 2009). On the other hand, albumin is an important serum protein for the transportation of steroid hormones (Shahsavani, Kazerani, Kaveh, & Gholipour-Kanani, 2010). In addition, the importance of albumin has been described with respect to fish pathology, while the albumin:globulin ratio is widely used as an index of the fish's physiological state (Nakagawa, 1978). The fact that the inclusion of brewer's yeast did not affect either globulin or TP suggests that the humoral immunity of the fish was not negatively affected. Fillet or muscle protein content was not analyzed in this study; however, Javed and Usmani (2015) reported an elevation of both TP and globulin in spotted snakehead's, *Channa punctata*, serum, liver, and muscles. This may suggest that unaffected serum TP when feeding the current experimental diets may well indicate that muscle protein was not affected.

In a 12-week trial to test the effect of replacing dietary fishmeal with yeast (10 and 20%) on blood parameters in Gilthead sea bream, *Sparus aurata*, Salnur et al. (2009) reported a nonsignificant effect on parameters such as ALP, blood urea nitrogen (BUN), serum protein, cholesterol, and triglycerides. Our findings on the effect of yeast on serum protein, cholesterol, triglycerides, and other serum metabolites were in contrast to the study by Salnur et al. (2009). Diet influenced the level of serum metabolites, except for the BUN/creatinine ratio. It is important to note that Salnur et al. (2009) used different yeast inclusion levels, different basal diets, and different fish species compared to the current study, which may explain the discordant results.

In conclusion, the current study demonstrated that supplementation with brewer's yeast at levels above 50 g/kg could be detrimental to the growth of the South African dusky kob. The use of a probiotic mix did not yield any positive influence on fish growth; hence, it may not be beneficial in dusky kob diets. For uncompromised growth performance, future dusky kob diets may include up to 50 g brewer's yeast/kg kob feed.

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