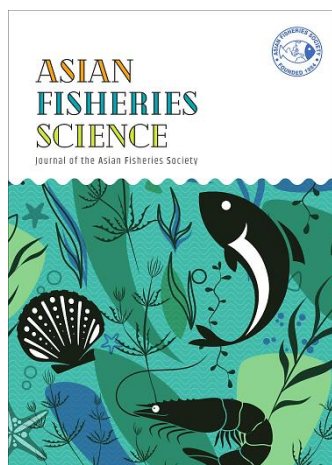




Effect of Graded Levels of Aquapro® Herbal Stimulant on Growth and Intestinal Morphology in Dusky Kob, *Argyrosomus japonicus* (Temminck & Schlegel, 1843)

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Abstract

This study was designed to test the effect of a herbal product, Aquapro®, on the growth performance and gut morphology of dusky kob, *Argyrosomus japonicus* (Temminck & Schlegel, 1843) in a 49-day feeding trial. Four diets, containing Aquapro® at 0 (Aqua0), 50 (Aqua50), 100 (Aqua100), and 150 (Aqua150) g.kg⁻¹ dry matter (DM) were formulated. Forty-five fish (14.44 ± 0.27 g) were randomly distributed into each of 12 experimental tanks. Each dietary treatment was randomly allocated to three tanks and offered to fish at 2.8 % body weight. Ten fish from each tank were randomly sampled weekly for length and weight measurements. At termination, three fish from each tank were sampled for distal intestinal tissues for histology preparation. A non-significant ($P > 0.05$) interaction between the diets and the fish age (weeks) on both the weight and caudal length of the fish was observed. The fish weight decreased significantly with an increase in Aquapro® inclusion in the diets beyond 100 g.kg⁻¹. The Aqua150 diet produced the least weight gain of 24.57 ± 2.44 g. Aquapro® inclusion in the diets did not cause any gut morphological alterations in the fish. In conclusion, Aquapro® product, up to 100 g.kg⁻¹ kob diet does not negatively affect juvenile dusky kob growth and all the tested inclusion levels did not cause gut inflammation, thereby suggesting uninterrupted nutrient absorption.

Keywords: dusky kob, Aquapro® herbal stimulant, growth performance, gut histology

Introduction

Feed additives are used to provide essential nutrients, improve growth performance and feed intake and thus optimise feed utilisation (Wenk, 2003). However, the use of synthetic feed additives in farmed animals is increasingly being frowned upon by consumers who worry about the possible health implications of the synthetic feed supplements. The feed industry is interested in valuable additives, which could be acceptable to consumers. The search for suitable additives in aquaculture feeds is also driven by restrictions or bans in the use of dietary antimicrobial agents, prompting a search for novel nutritional strategies to improve productivity and enhance the health of farm animals. The goal of improving and protecting animal health could be achieved by the best possible combination of so-called pronutrients, which are designed to improve the intrinsic value of the

nutrient mix in animal diets (Rosen 1996). Herbs or their extracts represent one of the types of pronutrients that can be used as feed additives (Bye and Linares, 1999).

Among the benefits of herbs or their extracts in farm animals is the fact that they activate feed intake and digestive enzymes as well as stimulate the immune system (Wenk, 2003). It is suggested that herbs flavour could influence the animal's eating pattern, the secretion of digestive fluids and the feed intake (Barreto et al., 2008). By having the ability to accelerate digestion, herbs can shorten the time required for the feed to pass through the digestive tract (Platel and Srinivasan, 2001). In addition to their ability to aid in digestion, many herbs contain bioactive compounds that modulate the cellular membrane of microbes (Kamel, 2001). The benefits of feeding antimicrobial diets supplemented with herbs is to counter

pathogenic gut microbes and help maintain a healthy animal; therefore, indirectly maximising its potential for good growth performance.

In vitro studies by Kamel (2001) indicated that minimum inhibitory concentrations (MIC50) and minimum bactericidal concentrations (MBC50) are based on the level of the active substance and the purity of the plant extract. Furthermore, an increase in hydrophobicity of the microbes in response to some plant extracts may influence the surface characteristics of microbial cells and thereby affect the virulence of the microbes. This antimicrobial mechanism may often have implications for the gut because the adhesion of microbes to the intestinal mucosal cells is important for some pathogenic microflora (Pusztai et al., 1990).

With the general shift from synthetic drugs, the use of herbs as an alternative for antibiotic growth-promoters in fish is becoming acceptable (Adedeji et al., 2008). An experiment with *Oreochromis niloticus* (Linnaeus, 1758) suggested that inclusion of herbal growth promoter (Superliv®) in the feed at concentrations ranging from 0 g.kg⁻¹ fishmeal to 10 g.kg⁻¹ feed promoted fish growth (Dada, 2012). The results showed that Superliv® powder treatments enhanced nutrient utilisation which is reflected in improved weight gain, feed conversion ratio and specific growth rate. Olmedo-Sanchez et al. (2009) reported enhanced growth and feed conversion efficiency when supplementing shrimp diets with medicinal herbs. Turan (2006) reported similar results with medicinal herb red clover *Trifolium pratense* (Linnaeus) as a growth-promoting agent in blue tilapia *Oreochromis aureus* (Steindachner, 1864) diets. Juvenile perch *Sander lucioperca* (Linnaeus, 1758) fed diets supplemented with herbs exhibited faster growth than groups fed control diets (Zakes et al., 2008). Faster growths were also reported when *Cyprinus carpio* (Linnaeus, 1758) (Yilmaz et al., 2006), ornamental guppies *Poecilia reticulata* Peters, 1859 (Cek et al., 2007) and red sea bream *Pagrus major* (Temminck & Schlegel, 1843) (Ji et al., 2007) were offered diets containing growth-promoting herbal extracts. Dusky kob juveniles *Argyrosomus japonicus* (Temminck & Schlegel, 1843) fed brewer's yeast and torula yeast based diet, supplemented with four different commercial herbal products produced significant growth (Madibana et al., 2017). Plant materials in fish diets often contain anti-nutritional factors that can induce enteritis (Baeverfjord and Krogdahl, 1996) especially in the distal intestine (Krogdahl et al., 2015; Penn et al., 2010), thereby limiting their inclusion in fish diets. Aquapro® herbal powder was one of the commercial products that produced an FCR of 1.03 compared to 0.99 of the base diet. One of the recommendations from that study was to investigate the effect of this herbal product in dusky kob diets without the two yeast strains. Therefore, the current study was designed to assess the effect of

graded levels of Aquapro® on fish growth performance and intestinal health.

Materials and Methods

Experimental site

This study was carried out from September to November 2018 at the Marine Research Aquarium of the Department of Agriculture, Forestry and Fisheries (DAFF) in Sea Point (33.9169°S, 18.3875°E), Cape Town, South Africa. The experiment was conducted in a recirculating aquaculture system (RAS) consisting of 12 black, high-density polyethylene grow-out tanks, (465 L capacity, 67 cm deep and 94 cm diameter) with flattened conical floors coated with white fibreglass resin to allow for better fish visibility. The seawater temperature and dissolved oxygen were maintained at 25 ± 0.16 °C via a heat pump and 5.8 ± 0.25 mg.L⁻¹ via airlines, respectively. The salinity averaged 34.1 ± 0.12 ‰. The filtration system included the protein skimmer or foam fractionator, the sand filter and the biological filtration. Ultraviolet lights (55 W) were fitted on the water route between the filtration system and the fish holding tanks.

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). An estimated 550 dusky kob fingerlings were sourced from a commercial fish farm based in East London (32°59'S 27°52'E), Eastern Cape Province, off the South African east coast. They were transported in 950 L of seawater at a salinity of 30 ppt. Pure oxygen was injected into the water at a rate of approximately 11 mg.L⁻¹. The water was recirculating through a 50 micron fluted paper filter cartridges and entered the tank through two CO₂ degassing towers. Upon arrival, the fish were acclimatised in four of the experimental tanks for 2 weeks prior to the start of the trial. A high-protein commercial fishmeal diet (SA Feed (Pty) Ltd, South Africa) was offered to the fish during the acclimatisation period. During acclimatisation, 10 fish died due to cannibalism (tail biting), leaving 540 fingerlings, which were used in the feeding trial. Forty-five fish (14.44 ± 0.27 g) were randomly distributed into each of the 12 tanks in preparation for the commencement of the experiment.

Experimental feed

Aquapro® powder was donated by Indian Herbs Research & Supply Co. Ltd., Darra Shivrpur, Saharanpur-247 001(U.P), India. The composition of the herbal powder includes extracts of *Boerhavia diffusa* (Linnaeus), *Terminalia chebula* (Retz.),

Terminalia bellirica (Gaertn.) Roxb. and *Eclipta prostrata* (Linnaeus) plants. Performance enhancer, growth promoter, improves feed intake and utilisation, optimises digestive enzymes, and liver function were some of the attributes to the Aquapro® on its packaging. The product is widely used in prawn and fish aquaculture in India (Navin Chandran et al. 2016). Four experimental diets were formulated to contain 0, 50, 100, 150 g Aquapro® kg⁻¹ commercial kob feed (Aqua0, Aqua50, Aqua100, and Aqua150, respectively) (Table 1). The commercial dusky kob feed was sourced from SA Feed (Pty) Ltd, Hermanus (34°25'S 19°15'E), Western Cape, South Africa. The dietary treatments were hand-prepared at the Marine Research Aquarium as follows: The commercial dusky kob pellets (4 mm) were granulated (<0.8 mm) with a Krups Burr grinder (GVX 2, South Africa). The powdered pellets were proportionally mixed with pre-weighed quantities of Aquapro® and multi-vitamins/minerals mix. Water was added to the final dry mixture. The mixture was then kneaded to produce dough, which was then rolled using a kitchen dough roller to make a thin layer on a smooth plastic sheet and subsequently pressure-sprayed with a non-stick agent (Cook'n Bake®, South Africa) that contains sunflower oil and lecithin. The thin layer of paste was then dried (to a constant mass) with a crossflow air current mediated by a household electric floor fan in a ventilated laboratory at ambient temperature. The flaked pieces of dried food were then graded to size using a corn kernel hand grinder with adjustable pressure disc for flake sizing. Flake sizes were predetermined (grinder inter-disc opening size adjustment) for the growth stages of the fish. Flakes were separated by sieving them through stainless steel wire strainers with selected mesh sizes. Prior to fish feeding, the flakes were mixed with cod liver oil at 7 % (m: v) of the ration. Feed treatments were sampled for chemical analysis (dry matter, organic matter, crude protein, crude fat and ash) at the Department of Animal Science, North-West University, South Africa.

Feeding strategy and sampling

Each dietary treatment was offered to three replicate tanks and groups were consistently hand-fed twice a day at 2.8 % of their fish body weight (BW) (Madibana et al. 2017). The 2.8 % BW feeding was also based on our unpublished study that suggested that up to 4.5 % BW feeding, there is no significant weight gain difference and lower feeding rates helps maintain good water quality and lower feed cost. Due to the fish's photophobia tendency, they were not exposed to any artificial light but were exposed to minimal sunlight coming into the building during the day (8:00 a.m.–18:00 p.m.). Minimal light also minimised their customary cannibalistic behaviour. Ten fish from each of the 12 tanks were randomly sampled once a week and the standard length and body mass (Viper SW 15, Mettler Toledo, South Africa) of each individual were recorded to determine the growth performance

and feed conversion ratio/efficiency (FCR/E). A 1 mL dose of 2-phenoxyethanol per 5 L of water was used to anaesthetise fish during the weighing sessions (Brown, 2011). The FCR/E per treatment group was calculated based on the collective feed mass offered for the experimental period. All weekly adjusted feed ration recalculations were based on 2.8 % of the fish population body mass. Tanks were monitored daily before or during feedings for any mortality and for fish behavioural changes. Ammonia was tested twice weekly using Sera ammonium/ammonia test kit (North Rhine-Westphalia, Germany). OxyGuard meter (OxyGuard International A/S, Denmark) was used to monitor both dissolved oxygen and temperature daily. At the termination (day 49), fish were humanely sacrificed by exposure to 2-Phenoxyethanol for 10–20 min, before severing the spinal cord to sample the distal intestine for histological preparations.

Gut histology

Distal intestine (DI) tissues were dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques (Mallory, 1914). Sections of approximately 5–8 µm thick were cut and stained with haematoxylin and eosin (H&E). The tissues were sectioned longitudinally (i.e. perpendicular to the macroscopically visible circular folds). The processing of tissues was done at the Department of Physiological Sciences laboratory, Stellenbosch University (33°55'48.27"S 18°51'53.01"E), Western Cape Province, South Africa. The blind histological examination was done using a light microscope. Tissue morphology was evaluated using a semi-quantitative scoring system of 0 to 10 (Penn et al., 2010). To determine the effect of feed on gut morphology, preselected tissue parameters were scored as follows: mucosal fold height (0 = short, 10 = long); mucosal fold fusion (0 = distinctly single), 10 = highly fused); lamina propria width (0 = thin, 10 = enlarged); lamina propria cellularity (0 = no cells, 10 = high volume of cells); submucosa width (0 = normal size, 10 = enlarged); submucosa cellularity (no cells, 10 = high volume of cells); enterocyte vacuolisation (0 = no vacuoles, 10 = high vacuolisation); enterocyte vacuole size disparity (0 = uniform size, 10 = different sizes), goblet cells (0 = low frequency, 10 = high frequency), and intraepithelial leukocytes (0 = low frequency, 10 = high frequency).

Statistical analysis

The mean of measurements from multiple fish per tank was calculated before analysis, such that each replicate tank had one value. Weekly growth performance data were analysed using repeated measures analysis (SAS, 2010) according to the following model:

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

Where, Y_{ijk} = dependant 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, E_{ijk} = random error associated with observation ijk , assumed to be normally and independently distributed.

The general linear models procedure of SPSS 14.0 (2006) was used to analyse histological evaluation 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 , assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$. Least squares means were compared using Tukey's-b option in the Post Hoc Multiple Comparisons of SPSS.

Calculations

$$\text{Specific growth rate} = \left[\frac{(\log n \text{ Final fish weight} - \log n \text{ Initial fish weight})}{\text{Time interval (days)}} \right] \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Weight gained (g)}}{\text{Feed consumed (g)}}$$

$$\text{Feed conversion efficiency} = \frac{\text{Weight gained (g)}}{\text{Feed consumed (g)}}$$

$$K = \frac{W \times 100}{L^3}$$

Where, K = condition factor; W = weight of fish (g), L = length of fish (cm)

Results

Experimental diets

The increment of the herbal product did not have a particular trend (increasing or decreasing) on any of the proximate composition parameters of the experimental diets (Table 1). The highest numerical dry matter value was recorded for Aqua0 diet (916.9 g.kg⁻¹) and the lowest was recorded for Aqua150 diet (890.5 g.kg⁻¹). Aqua150 diet recorded the highest numerical protein content of 493.8 g.kg⁻¹, with Aqua50 diet recording the lowest protein content of 477.7 g.kg⁻¹. The crude fat content did not significantly vary among the diets, with Aqua150 recording slightly superior numerical content of 130.1 g.kg⁻¹, with Aqua0 diet recording the lowest content of 115.1 g.kg⁻¹.

Feed intake, growth performance, feed conversion ratio and feed conversion efficiency

All the feed offered (2.8 % body weight per day) was consumed immediately upon contact with the water surface. This was confirmed by the lack of refusals 20 minutes after feeding. Test kit indicated that

ammonia level was maintained at 0 ppm. Repeated measures analysis revealed a non-significant ($P > 0.05$) interaction between the diets and the fish age (weeks) on both the weight and standard length of the experimental fish. At the initiation of the experiment, fish had similar weight and length ($P > 0.05$), but the overall weight and length gain after 7 weeks of feeding was significantly different ($P < 0.05$). The fish weight gain decreased significantly with an increase in Aquapro® inclusion in the diets. The highest weight gain of 36.97 ± 1.57 g and standard length gain of 5.47 ± 0.09 cm were recorded for the control diet after 7 weeks. The highest Aquapro® inclusion level diet produced the least weight gain of 24.57 ± 2.44 g and a standard length of 4.01 ± 0.33 cm (Table 2). Condition factor of fish fed different treatments did not differ significantly ($P > 0.05$) (Table 2). Weekly growth trends showed that at the final week of weight measurements, only Aqua10 fed group had a slight weight gain and the rest had a weight decline from the previous week (Fig. 1).

Table 1. Ingredients and chemical composition of the experimental diets for juvenile *Argyrosomus japonicus* supplemented with Aquapro® herbal product (g.kg⁻¹ dry matter).

	Diets ¹ Aqua0	Aqua50	Aqua100	Aqua150
Ingredients				
Fishmeal(g)	700	694	687	681
Aquapro® herbal product(g)	0	50	100	150
Cellulose(bulk agent)(g)	200	160	125	93
Vit/min mix(g)	7	7	7	7
Fish oil(mL)	93	89	81	69
Proximate(g.kg ⁻¹)				
Dry matter	903.0	916.9	900.0	890.5
Crude protein	493.8	481.2	489.4	477.7
Crude fat	115.1	125.1	124.6	131.0
Organic matter	786.4	757.8	775.5	788.0
Ash	116.6	112.5	115.0	112.0

¹Diets: Aqua0 = Control; Aqua50= 50 g.kg⁻¹ Aquapro®; Aqua100 = 100 g.kg⁻¹ Aquapro®; Aqua150 = 150 g.kg⁻¹ Aquapro®; Vit/min mix = The vitamins/minerals mix was composed of procaine HCl (15mg) methylsulphonylmethane (MSM) (300 mg) lecithin (300 mg) alpha-tocopherol (vitamin E) (30 mg) thiamine HCl (vitamin B1, 10 mg) riboflavin (vitamin B2, 3 mg) pyridoxine HCl (vitamin B6, 3 mg), nicotinamide (10 mg), calcium pantothenate (10 mg), choline (40 mg), magnesium (100 mcg), chromium (25 mcg), zinc amino acid chelate (10 mg), inositol (30 mg), manganese (75 mcg) and iron (5 mg).

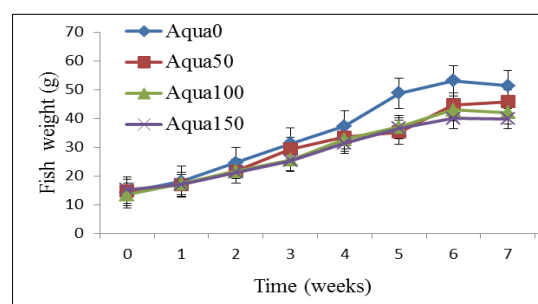


Fig. 1. Weekly growth trend of juvenile dusky kob (*Argyrosomus japonicus*) fed Aquapro® supplemented diets over 7 weeks. Diets: Aqua0 = Control; Aqua50 = 50 g.kg⁻¹ Aquapro®; Aqua100 = 100 g.kg⁻¹ Aquapro®; Aqua150 = 150 g.kg⁻¹ Aquapro®.

The four experimental diets produced varying ($P < 0.05$) fish-specific growth rate (SGR) over the 49 days feeding period. The control diet conspicuously produced the highest SGR of 2.61 ± 0.06 g.day⁻¹, with Aqua200 diet producing the least SGR of 1.96 ± 0.16 g.day⁻¹ over the feeding period (Table 2). All the diets produced good feed conversion ratio (FCR) and feed conversion efficiency (FCE) which were not significantly different from each other ($P > 0.05$). The lowest FCR value was recorded for the control diet (1.48 ± 0.05) and the highest was recorded for the Aqua200 diet (1.83 ± 0.16). A similar trend observed for the FCR was also evident with regards to FCE whereby the control diet recorded the highest FCE value (0.680 ± 02) and the Aqua200 recorded the least FCE value (0.55 ± 0.05). The FCR and FCE data is shown in Table 2.

Histological evaluation

The ANOVA performed on the data obtained from the semi-quantitative analysis of the preselected intestinal histology parameters, revealed that the effect of the experimental diets on these parameters did not differ ($P > 0.05$). The normal structure of the distal intestine (DI) was characterised by long simple and complex mucosal folds, thin width of the submucosa and lamina propria, clear vacuolization of the enterocytes, few leukocytes were visible in the submucosa and lamina propria section, no fusion between the mucosal folds. Few goblet cells were noted. However, reduced mucosal folds with a slightly enlarged width of the submucosa and lamina propria and visibly increased goblet cell numbers were evident from the DI of fish fed the control diet. The DI evaluation scores are shown in Table 3. The different morphology of the DI of fish fed different experimental diets is shown in Figures 2, 3, 4 and 5.

Table 2. Dusky kob (*Argyrosomus japonicus*) growth performance (weight $\pm$ SE), feed utilization (FCR/FCE $\pm$ SE) and condition factor (K $\pm$ SE) when fed diets containing graded levels of commercial herbal product Aquapro® in a 7 week feeding trial (n = 3).

Diets ¹	Initial mass (g)	Mass gain (g)	Initial length (cm)	Length gain (g)	SGR (g.day ⁻¹)	FCR	FCE	K
Aqua0	14.29 $\pm$ 0.49 ^a	36.97 $\pm$ 1.57 ^a	9.56 $\pm$ 0.09 ^a	5.47 $\pm$ 0.09 ^b	2.61 $\pm$ 0.06 ^b	1.48 $\pm$ 0.05 ^a	0.68 $\pm$ 0.02 ^a	1.73 $\pm$ 0.45 ^a
Aqua50	14.77 $\pm$ 0.19 ^a	31.04 $\pm$ 3.61 ^{ab}	9.76 $\pm$ 0.06 ^a	4.72 $\pm$ 0.32 ^{ab}	2.30 $\pm$ 0.17 ^{ab}	1.56 $\pm$ 0.13 ^a	0.65 $\pm$ 0.05 ^a	1.77 $\pm$ 0.15 ^a
Aqua100	13.49 $\pm$ 0.44 ^a	28.56 $\pm$ 2.10 ^{ab}	9.45 $\pm$ 0.13 ^a	4.71 $\pm$ 0.33 ^{ab}	2.31 $\pm$ 0.09 ^{ab}	1.61 $\pm$ 0.10 ^a	0.63 $\pm$ 0.04 ^a	1.89 $\pm$ 0.11 ^a
Aqua150	15.25 $\pm$ 0.44 ^a	24.59 $\pm$ 2.44 ^a	9.76 $\pm$ 0.13 ^a	4.01 $\pm$ 0.33 ^a	1.96 $\pm$ 0.16 ^a	1.83 $\pm$ 0.16 ^a	0.55 $\pm$ 0.05 ^a	1.82 $\pm$ 0.89 ^a

¹Diets: Aqua0 = Control; Aqua50 = 50 g.kg⁻¹ Aquapro®; Aqua100 = 100 g.kg⁻¹ Aquapro®; Aqua150 = 150 g.kg⁻¹ Aquapro®; SGR = specific growth rate; FCR = Feed conversion ratio; FCE = feed conversion efficiency. K = Condition factor. ^{a,b} Means along the same column with different superscripts denote significant differences ($P < 0.05$).

Table 3. Semi-quantitative histology evaluation of dusky kob (*Argyrosomus japonicus*) distal intestine (parameter value $\pm$ SE) fed diets containing graded levels of commercial herbal product (Aquapro®) over 7 week feeding period (n = 3).

Diets ¹	Mucosal fold height	Mucosal fold fusion	Lamina propria width	Lamina propria cellularity	Submucosa width	Submucosa cellularity	Enterocyte vacuolization	Vacuole disparity	Goblet cells	Intraepithelial leukocytes
Aqua0	8.33 $\pm$ 0.88 ^a	2.33 $\pm$ 0.67 ^a	2.00 $\pm$ 0.58 ^a	1.33 $\pm$ 0.33 ^a	2.00 $\pm$ 1.00 ^a	1.67 $\pm$ 0.67 ^a	8.67 $\pm$ 0.88 ^a	2.00 $\pm$ 1.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.67 ^a
Aqua50	9.33 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	9.67 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a
Aqua100	9.67 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	2.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	9.67 $\pm$ 0.33 ^a	1.33 $\pm$ 0.33 ^a	1.67 $\pm$ 0.67 ^a	1.67 $\pm$ 0.00 ^a
Aqua150	9.67 $\pm$ 0.33 ^a	0.67 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a	1.33 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	10.00 $\pm$ 0.00 ^a	0.67 $\pm$ 0.33 ^a	1.00 $\pm$ 0.00 ^a	1.00 $\pm$ 0.00 ^a

¹Diets: Aqua0 = Control; Aqua50 = 50 g.kg⁻¹ Aquapro®; Aqua100 = 100 g.kg⁻¹ Aquapro®; Aqua150 = 150 g.kg⁻¹ Aquapro®; ^a Means along the same column with similar superscripts denote no significant differences ($P < 0.05$).

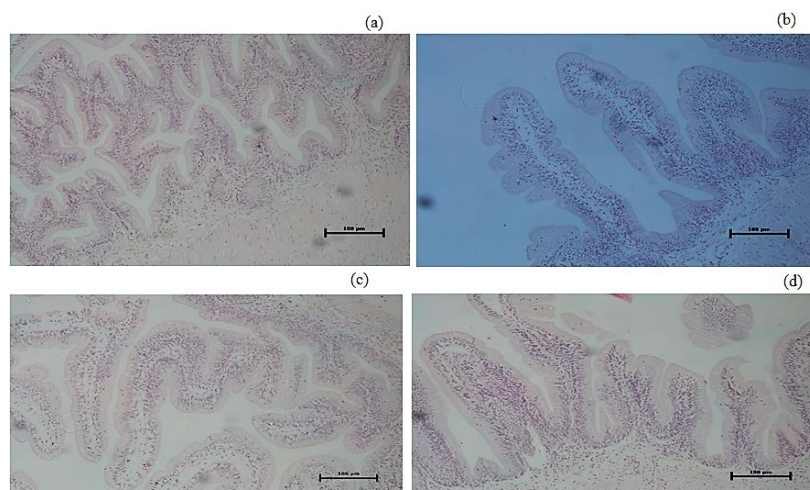


Fig. 2. Distal intestinal histology in dusky kob (*Argyrosomus japonicus*) as influenced by the Aqua0 (control, no Aquapro® supplementation) diet; (a) = The complex mucosa folds can be observed; (b, c, d) = Reduced mucosal folds and slightly enlarged lamina propria and submucosa width are evident. Clear enterocyte vacuolization and minimal goblet cells can be observed (H&E). Scale bar represents 100 µm.

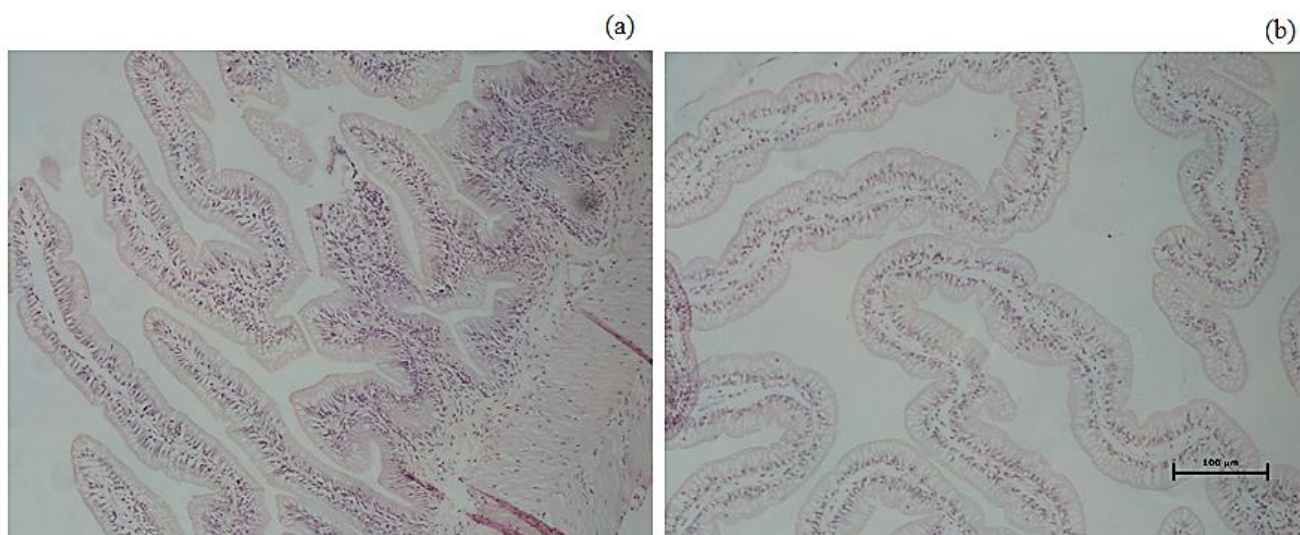


Fig. 3. Distal intestinal histology in dusky kob (*Argyrosomus japonicus*) as influenced by the Aqua50 (50 g.kg⁻¹ Aquapro®) diet; (a) = Long mucosal folds with clear enterocyte vacuolization, nucleus at the base of the cell, one cell lamina propria can be noted; (b) = Long and normal complex mucosal folds, no intraepithelial leukocytes is seen (H&E). Scale bar represents 100 µm.

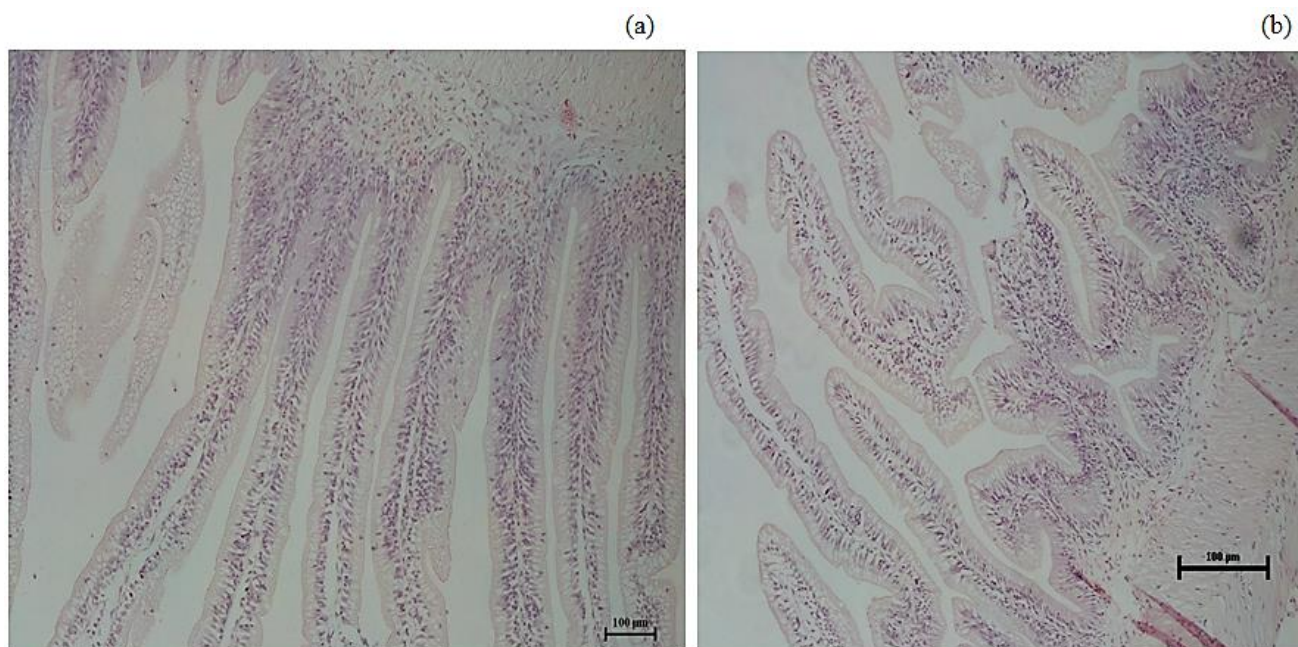


Fig. 4. Distal intestinal histology in dusky kob (*Argyrosomus japonicus*) as influenced by the Aqua100 (100 g.kg⁻¹ Aquapro®) diet; (a, b) = Normal long mucosal folds, thin submucosa with minor leukocytes infiltration is note (H&E). Scale bar represents 100 µm.

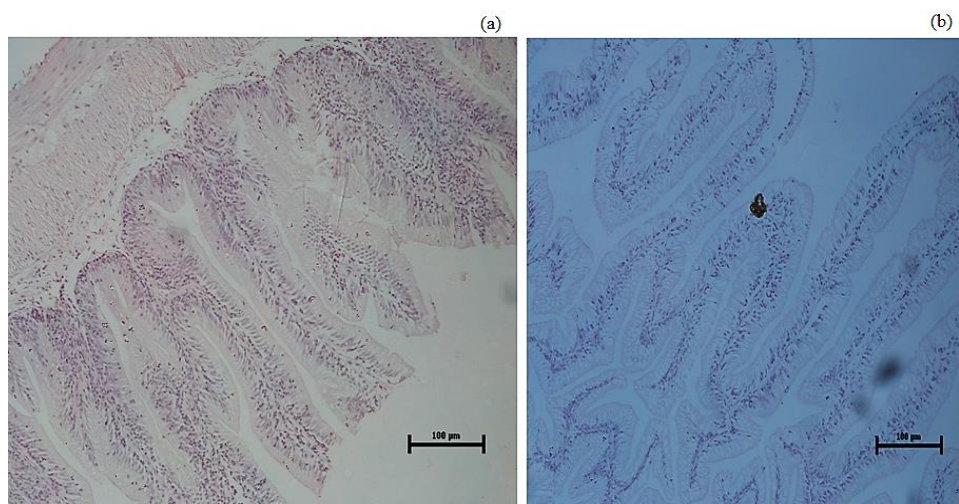


Fig. 5. Distal intestinal histology in dusky kob (*Argyrosomus japonicus*) as influenced by the Aqua150 (150 g.kg⁻¹ Aquapro®) diet; (a) = Thick submucosa and lamina propria width with minimal leukocyte; (b) = clear enterocyte vacuolization, basal nucleus, long simple and complex mucosal folds can be observed (H&E). Scale bar represents 100 µm.

Discussion

Experimental diets

The protein content of the Aquapro® diets in the current study was 485.53 g.kg^{-1} on average, which was an insignificant difference from 473.7 g.kg^{-1} recorded in an earlier study for a diet with Aquapro® as an additive (Madibana et al., 2017). The control diet in the current study and Madibana et al. (2017) recorded a protein content of 916.9 g.kg^{-1} and 482.6 g.kg^{-1} , respectively. Protein content was singled out for comparison because it is the largest component of carnivorous fish diets, and it is the most expensive nutrient. In fish, proteins and their amino acids play a significant role in many functions, including growth processes and cell renewal (Sanz et al., 2000). The inclusion of Aquapro® as an additive did not in any way compromise the protein content of diets in the current study. Using a similar commercial herbal product as used in this study, Dada and Alugbemi (2013) recorded a protein content of 422.5 g.kg^{-1} and 425.3 g.kg^{-1} for Aquapro® diet and the control, respectively. Different basal diets (fishmeal) and formulations may explain significant varying protein content of our earlier study (Madibana et al., 2017) and the current study with that of Dada and Alugbemi (2013). Madibana et al. (2017) and the current study used local South African fishmeal which is comprised of pelagic fish such as anchovy *Engraulis capensis* Gilchrist, 1913, pilchard *Sardinops ocellata* (Pappe, 1853), red eye *Etrumeus whiteheadi* Wongratana, 1983, Atlantic horse mackerel *Trachurus trachurus* (Linnaeus, 1758) and lantern fish *Lampanyctodes hectoris* (Günther, 1876). Dada and Alugbemi (2013) used local Nigerian fishmeal which is predominately made of ray-finned fishes such as herring *Clupea harengus* Linnaeus, 1758 and menhaden *Brevoortia tyrannus* (Latrobe, 1802) (Ahmad and Ibrahim, 2016).

Growth performance

Aquapro® used in this study was primarily used as an additive with the desire that its growth promoting properties and its bioactive compounds would aid in fish growth performance. Frankić et al. (2009) highlighted that feed supplements such as herbs that possess growth-promoting activities can beneficially influence the animal's gastrointestinal ecosystem by means of inhibiting pathogenic microorganism growth. Even though the current study did not investigate the effect of Aquapro® on pathogenic microorganisms, this physiological benefit of inhibiting their growth in animals was one of the factors to investigate Aquapro®'s effect on dusky kob growth and feed utilisation. Initial weight measurements as the feeding trial commenced indicated that fish were of uniform weight and length. However, at the termination of the trial, weight and length measurements showed a decreasing trend with an increase of Aquapro® in the diets. Feeding

African catfish fingerlings at 3 % body weight, Dada (2012) reported that of the two herbal products tested, the other being Aquabooster®, Aquapro® (0.5 % of the diet) produced superior growth performance, even outperforming the control diet with no herbal product. Fish in the current study were fed at 2.8 % of their weekly BW and in contrast to Dada and Alugbemi (2013) who fed fish at 3 % BW, the control diet outperformed all the groups fed Aquapro® diets. Different fish species, water quality (sea water versus freshwater), varying feeding strategies employed by the two studies might explain the difference in the growth performance results.

As indicated earlier, the Aquapro® product was donated by an Indian pharmaceutical research company. Using the same product as the current study, Navin Chandran et al. (2016) tested 250, 500, 750 and 1000 mg.g^{-1} diet in diets for giant tiger prawn *Penaeus monodon* (Fabricius, 1798) post larvae diets. Similar to Dada and Alugbemi (2013), the Aquapro® fed group (1000 mg.g^{-1}) produced superior weight gain as compared to the control diet. The previous studies used relatively small quantities of Aquapro® in their experimental diets. The current study is one of the few to test this product at a relatively higher dosage in fish diets. Our earlier study, (Madibana et al. 2017) which also tested lower inclusion levels (1 % of the diet) and the effect on growth, was masked by the two yeast strains which constituted the basal composition of the diet.

Superliv®, a Nigerian herbal product, was tested at inclusion levels of 0, 2.5, 5, 7.5 and 10 g.kg^{-1} on Nile tilapia *Oreochromis niloticus* diets (Dada 2012). The author reported the highest specific growth rate (SGR) of $1.3 \pm 0.03 \text{ \%.day}^{-1}$ for the group fed on the 10 g.kg^{-1} Superliv® diet as compared to the other three groups fed Superliv® diets and the control group. The SGR results from the current study followed a similar pattern as the weight gain, with the control (Aqua0) diet producing a superior SGR of 2.61 g.day^{-1} , Aqua50 and Aqua100 fed groups producing significantly similar SGR of 2.30 and 2.31 g.day^{-1} , respectively and Aqua150 at 1.96 g.day^{-1} . A herbal combination of velvet beans *Mucuna pruriens* (Linnaeus) and Gokhru-big *Pedalium murex* (Linnaeus) were included in diets for rohu *Labeo rohita* (F. Hamilton, 1822) at graded levels 0.0, 0.06, 0.08 and 0.1 g.100 g^{-1} diet (Ojha et al., 2016). The supplemented diets significantly improved the growth and SGR, especially the 0.06 g.100 g^{-1} diet with an SGR of 1.474 ± 0.002 . Similar to the literature on Aquapro®, both Dada (2012) and Ojha et al. (2016) supplemented their herbal products at relatively lower amounts as compared to the current study. The general consensus from these studies is that the inclusion of herbal products in fish diets does not significantly compromise fish growth.

The herbal product used in the current study did not influence fish condition factor. Similar results were observed when sterlet sturgeon *Acipenser ruthenus*

Linnaeus, 1758 was fed diets supplemented with garlic extract (Lee et al., 2012).

Feed utilisation

For animals that are raised using commercial feeds and are confined to an intensive production systems, their feed conversion ratios are as follows: beef cattle: 6.0–10.0, pigs: 2.7–5.0, chickens: 1.7–2, farmed fish and shrimp: 1.0–2.4 (Tacon and Metian, 2008; Smil, 2013; Zuidhof et al., 2014). The three herbal diets from the current study had a combined best FCR of 1.67 and 0.61 feed conversion efficiency (FCE) as compared to the 1.48 FCR and 0.68 FCE recorded for the control diet. Madibana et al. (2017) also recorded a lower FCR of 1.03. Naylor et al. (2009) and Torrissen et al. (2011) attributed the lower FCRs in aquatic animals to less energy expenditure for locomotion, ability to regulate their body temperature due to buoyancy and their ectothermic properties as compared to larger terrestrial animals. Lower FCR (0.74) when feeding Aquapro® containing diets for African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings was reported by Dada and Alugbemi (2013). However, Navin Chandran et al. (2016) reported an average of 1.66 FCR and 0.06 FCE when feeding giant tiger prawn *P. monodon* larvae with Aquapro® herbal product diets. Ojha (2016) reported a slightly higher FCR (2.6) for fish when feeding rohu *L. rohita* fingerlings with herbal supplemented diet. The current study and these few cited literature suggest that herbal products do not negatively affect feed utilisation in fish. Thus an expansion of aquaculture is viewed worldwide as an opportunity to meet annual rising demands for animal products using less feed as compared to pig and cattle farming (Béné et al., 2015).

Histology

Gut inflammation or enteritis induced by feed is mostly observed on the distal intestine of the fish (Baevefjord and Krogdahl, 1996; Penn et al., 2010; Chikwati et al., 2012; Krogdahl et al., 2015). Histological analysis of the distal intestine did not indicate any pathological changes for fish which were fed the three Aquapro® diets, however unusual slightly shorter mucosal folds were observed for the control fed group in the current study. Thin lamina propria, narrow submucosa due to few leukocytes infiltration, high enterocyte vacuolization as seen in the current study, usually symbolises a healthy gut, therefore shorter mucosal folds observed for fish fed the control diet may be due to artefacts during histological sectioning of the samples. A linear effect on the intestinal mucosal folds, fold width, absorptive surface area and muscle was reported in yellow tail tetra *Astyanax altiparanae* Garutti & Britski, 2000, when fed with oregano oil supplemented diets (Ferreira et al., 2016). Increased number of goblet cells was also observed in Ferreira et al. (2016) study, which normally suggests inflammation in the gut. Ayotunde et al. (2011) observed no visible histological changes in

Nile tilapia *O. niloticus* that were exposed to an aqueous extract of *Moringa oleifera* (Lam.) seeds powder. Also, Agbebi et al. (2013) did not observe any gut pathological changes in *C. gariepinus* fed on dietary garlic source. There is a general consensus among these studies that most herbal products in fish diets do not cause any gut morphological alteration.

Conclusion

An ideal herbal additive should not compromise the fish growth, feed utilization and health. Therefore, based on the SGR, FCR/E and gut histology results of the current study, Aquapro® herbal product may be beneficial if included in future dusky kob commercial diets. Care should be taken when including Aquapro® in future dusky kob diets not to exceed 100 g.kg⁻¹ feed as growth rate and weight gain decreased beyond this inclusion level. As the aquaculture grows and the use of dietary antimicrobial agents continue to decline due to restrictions, future trials should test the combination of different herbal additives to determine the right combination to assist in both fish growth and health. Aquapro® did not cause any inflammation in the gut which suggests that nutrient absorption was not affected.

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