Aquaculture 489 (2018) 70–79

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Growth performance and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on live and dry feeds

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ARTICLE INFO

Keywords:
Catfish larvae
Live feed
Intestinal morphology
Artemia

ABSTRACT

Effects of live and dry feeds on intestinal morphology and growth of African catfish, *Clarias gariepinus*, larvae (Burchell, 1822) were investigated from 2 days post hatching (dph) to 6 dph and 11 dph, respectively. Feeding trials were carried out at Fleuren and Nooijen hatchery, Netherlands, in glass tanks connected to a RAS system. Five test diets (*Artemia* nauplii combined with dry feed (A), decapsulated *Artemia* cysts combined with dry feed (B), *Artemia* nauplii only (C), decapsulated *Artemia* cysts only (D) and commercial dry feed (E)) were assigned randomly in triplicate. The proportion of live feed in the combined diets was gradually reduced until 6 dph. Thereafter, all treatments were fed on the dry feed. Histological parameters were analysed in the proximal, middle and distal part of the intestine using standard H and E staining methods. At the end of the experiment, final wet weight (17.90 ± 0.38 mg) and specific growth rate (SGR) (24.12 ± 0.35%/day) were significantly higher ($P < 0.05$) in larvae fed nauplii combined with dry feed. A diet of decapsulated *Artemia* cysts resulted in the lowest values for these parameters (8.90 ± 0.44 mg and 17.18 ± 0.28%/day, respectively). FCR was best in the diets using nauplii only (0.53 ± 0.12) or nauplii combined with dry diet (0.70 ± 0.30). Feeding decapsulated *Artemia* cysts or its combination with dry feed resulted in the poorest (1.62–1.66) FCR values. Microscopic observation of the intestinal morphology demonstrated a decrease of mucosal folds, mucosal fold height, perimeter ratio (inner/outer perimeter) and wall thickness from the proximal to the distal intestine at 6 dph. Generally mucosal morphometric parameters were significantly ($P < 0.05$) higher when feeding nauplii combined with dry feed, than when feeding other diets. Goblet cells counts relative to PAS staining decreased from the proximal to the distal intestine. Nauplii and its combination with dry feed resulted into significantly ($P < 0.05$) higher counts of goblet cells in all intestinal parts. The highest goblet cell count was on 4 dph (range 57–254) before decreasing by 6 dph (32–54) in all diets. A gradual reduction of nauplii daily ration in its combination with dry feed stimulated morphological development that resulted in improved growth performance. Different starter feeds thus had an impact on intestinal morphological development and on growth in the larval phase, but could also affect further rearing results.

1. Introduction

The growth and survival of fish is dependent on many factors, including the nutritional composition of the diet, the environmental conditions, and the organism's digestive capacities. The development of an efficient digestive capacity is reflected in the anatomy of the gastrointestinal tract, of accessory digestive organs and in the production of digestive enzymes (Ikpegbu et al., 2013). The ontogenetical development of the gastrointestinal tract is similar in all teleosts and is initiated by endogenous feeding at hatching (Govoni et al., 1986; Grosell et al., 2010; Wilson and Castro, 2010). However, there are differences between species in the presence or rate of stomach development, the shape of the gut, intestinal differentiation and functionality, which reflect differences in trophic levels (Khojasteh, 2012). Thus, understanding the progressive changes in the gastrointestinal tract of fish larvae is important in defining a proper larval feeding and

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https://doi.org/10.1016/j.aquaculture.2018.01.046
Received 21 June 2017; Received in revised form 30 January 2018; Accepted 31 January 2018
Available online 02 February 2018

weaning strategy (Osman et al., 2008; Gisbert et al., 2008).

The complexity of the intestinal morphology in fish varies with age and is also influenced by the quantity and quality of the feed (Gisbert et al., 2004, 2008; Rønnestad et al., 2013). Studies on the intestinal morphology of larvae have been conducted in various species of catfish, such as *Ompok bimaculatus* (Pradhan et al., 2014), *Pseudoplatystoma punctifer* (Gisbert et al., 2014), *Rhamdia quelen* (Silveira et al., 2013) and *Clarias gariepinus* (Verreth et al., 1992), showing that the intestinal morphology affects the digestibility and absorption of nutrients (Khojasteh, 2012).

C. gariepinus Burchell, 1822, is an indigenous African fish species, cultivated globally because it is hardy and easy to produce in captivity (Solomon et al., 2015). It is considered a delicacy in Africa, is used as bait for *Lates niloticus* fishing in Lake Victoria and as a regulator of *Oreochromis niloticus* population in ponds (Musa et al., 2013; Alfaro et al., 2014). The production of this species is increasing globally both in quantity and in value (Moffitt and Cajas-Cano, 2014). However, its production potential in Africa is not fully exploited because of the shortage of quality larvae, the low quantity and quality of available fish feed, as well as high feed prices (Munguti and Ogello, 2014). Additionally, its larval stage, the most sensitive life period, shows slow gut maturation and radical shifts in dietary demands in relation to changes in gut morphology and functioning (Verreth et al., 1994; Kolkovski, 2001; Adriaens et al., 2001; Grosell et al., 2010).

In *C. gariepinus*, the larval stage starts with exogenous feeding on the third day post-hatching (Verreth et al., 1992; Olaniyi and Omitogun, 2014). Different studies have suggested that the larval stage might end at different larval lengths with the development of the fully differentiated median fin-fold (Balon, 1975), or with the development of a functional stomach (Stroband and Kroon, 1981; Verreth et al., 1992). As such, the duration of this stage is thus not clearly defined for this species (Chepkirui-Boit et al., 2011).

The larval stage of *C. gariepinus* has incomplete digestive organs at first feeding and thus relies on live feeds (Verreth et al., 1992). Earlier studies have shown that different starter feeds may be used for the species with success (Uys and Hecht, 1985; Verreth and Van Tongeren, 1989; Hecht, 1996; Awaiss and Kestemont, 1998; El-Sebaie et al., 2014; Adewumi, 2015). *Artemia* nauplii are often preferred over other types of live feed because they result in better larval growth and reduced mortalities (Hecht and Appelbaum, 1987). However, the hatching of *Artemia* cysts and handling (harvesting and storage) of the nauplii is time consuming and requires specific equipment and skills. Also, the nutritional value of this live food may vary with the strain, the batch of cysts and the developmental stage of the nauplii (Van Stappen, 1996). This leaves hatchery operators with the alternative of using decapsulated *Artemia* cysts that combine the benefits of the nutritional value of *Artemia* nauplii and the practical advantages of dry feeds (Verreth and Den Bieman, 1987). However, decapsulated *Artemia* cysts are unavailable to most African hatchery managers and sink relatively fast in freshwater, thus becoming unavailable for ingestion and utilization by catfish larvae (Verreth and Den Bieman, 1987), that feed by sucking their prey, detected by chemosensory stimuli, from the water surface or column (Bruton, 1979; Hossain et al., 1998; Freyhof, 2014). On the other hand, dry feeds are generally available year round, although high quality commercial dry feeds are sometimes unavailable and always expensive in Africa (Munguti and Ogello, 2014; Musa et al., 2013). In order to work out an optimal but also affordable larval feeding strategy, research efforts have thus been conducted focusing on combined feeding with both live and dry feeds (Awaiss and Kestemont, 1998; Chepkirui-Boit et al., 2011).

Different starter feeds have however diverse effects on fish larvae digestive ontogeny. These effects have been analysed by techniques such as histochemistry, morphometry, immune-histochemistry and stereology (Gisbert et al., 2008; Zambonino-Infante et al., 2008; Rašković et al., 2011; Rønnestad et al., 2013). In the *C. gariepinus* larvae, the ontogeny of the gastrointestinal tract, especially the multifunctional intestine (Jutfelt, 2011; Grosell et al., 2010), has been described histologically (Kolkovski, 2001; Osman et al., 2008; Olaniyi and Omitogun, 2014). The effects of different starter feeds on the intestinal morphological parameters (such as the number and height of the mucosal folds, the goblet cells counts) have been described qualitatively through immune histochemical methods (Verreth et al., 1992). However, literature on other intestinal parameters such as perimeter ratio, mucosal thickness and general intestinal morphometrics is scarce despite the dependence of the intestinal morphology and morphometrics on the diet (Ikpegbu et al., 2013; Pradhan et al., 2014). The aim of the present study was thus to get a better understanding of the effects of different starter feeds in relation to the development of the intestinal morphology and growth in *C. gariepinus* larvae. To assess the effect of the use of live feed as starter feed, both *Artemia* nauplii and decapsulated *Artemia* cysts were used, either supplied as sole diet, or in combination with dry feed.

2. Materials and methods

2.1. Experimental fish larvae and experimental design

The larval feeding test took place at Fleuren and Nooijen B.V., Someren, the Netherlands, in a recirculation system (RAS) comprising 15 glass aquaria of 150 L each, a sedimentation tank, biofilter and UV unit, with a rearing temperature of 27.5 ± 0.5 °C and pH of 7.0–7.1. Aeration in each aquarium was provided by a single air stone and water flow was maintained at 2.6 L min^{-1}. A gravid female was stripped, eggs fertilized and incubated at 29 °C as described by De Graaf and Janssen (1996). On the morning of the second day post-hatching (2 dph), each glass aquarium was randomly stocked at 26 larvae L^{-1}. The aquaria were randomly assigned to one of the 5 diets (Table 1) in triplicate and feeding started the same evening. The following dietary sources were used: commercial dry feed (Skretting "Gemma micro 150" of 100–200 μm particle size); nauplii and decapsulated cysts of Great Salt Lake-type *Artemia franciscana* Kellogg 1906 (Ocean Nutrition, Belgium). These were fed alone, or in combination. The nutritional composition of these feed sources was provided by the respective suppliers (Table 2), and their dry weight was analysed at the Laboratory of Aquaculture & *Artemia* Reference Center, Ghent University, Belgium. In the treatments with a combination of *Artemia* and dry diet (treatments A and B), the amount of *Artemia* nauplii (A) and decapsulated *Artemia* cysts (B) was decreased by 20% daily, while the amount of dry feed increased by 20% daily, until 6 dph. The percentage increase or decrease was based on the dry weight of each diet. Thereafter, all larvae were fed on 100% dry feed until the end of the experiment at 11 dph (Table 1). Diets were broadcasted in the respective tanks at 25% wet body weight per day (WBW d^{-1}) until 6 dph and at 20% WBW d^{-1} from 7 dph until the end

Table 1
Dietary treatments and feeding protocol for *C. gariepinus* larvae from 2 dph (stocking) to 11 dph.

Test diet	Days post-hatching					
	2	3	4	5	6	7–11
A	100% C	80% C + 20% E	60% C + 40% E	40% C + 60% E	20% C + 80% E	100%E
B	100% D	80% D + 20% E	60% D + 40% E	40% D + 60% E	20% D + 80%E	100%E
C	100%	100%	100%	100%	100%	100%E
D	100%	100%	100%	100%	100%	100%E
E	100%	100%	100%	100%	100%	100%E

Test diets: *Artemia* nauplii + commercial dry feed (A), decapsulated *Artemia* cysts + commercial dry feed (B), 100% *Artemia* nauplii (C), 100% decapsulated *Artemia* cysts (D), 100% commercial dry feed (E).

Table 2
Nutrient composition (as provided by commercial suppliers of feed products) and dry weight of experimental feeds.

Type of feed	% Nutrient composition				Dry weight (%)
	Protein	Lipid	Fibre	Ash	
Artemia nauplii	60	24	-	4.4	7.27
Decapsulated *Artemia* cysts	54	9	6.0	4.0	95.28
Dry feed (Skretting 'Gemma micro 150')	59	14	0.2	13.0	91.43

Dash (-) = not analysed.

of the experiment. Larvae were weighed daily to adjust the feeding rate.

Each day, freshly hatched *Artemia* nauplii were stored at +4 °C according to the procedures of Van Stappen (1996), and the daily ration fed to catfish larvae (treatments A and C) was calculated based on the individual dry weight of an *Artemia* nauplius and the density of nauplii in the stored suspension. *C. gariepinus* larvae were fed 6 times a day between 9 am and 9 pm. Excess feeds at the bottom of the aquaria were siphoned daily before feeding. Dissolved oxygen, NO_2^- and NH_4^+ were measured every 2 days.

2.2. Growth parameters

Sampling for growth measurement was done on days 2, 4, 6 9 and 11 (dph). For this purpose, a total of 30 *C. gariepinus* larvae were weighed to the nearest 0.1 g, using a mechanical balance (KB 360-3N). Total length was measured to the nearest 0.01 mm under a KD 3320 microscope fitted with a Nikon camera and digital graphics Clemex vision PE. The larvae were dried to a constant weight in a Heraeus D6345 oven at 103 °C for 4 h and their dry weight was determined to the nearest 0.01 mg (Sartorius balance). The specific growth rate (SGR) and food conversion ratio (FCR) were calculated as follows:

$$FCR = \frac{\text{Amount of dry feed supplied}}{\text{Wet weight gain}} \qquad SGR = \frac{\ln Wt - \ln W0}{t} * 100$$

With ln = natural log; W_t = weight at time t and W_o = initial weight.

2.3. Histological sampling and analysis

The effects of different test diets on the intestinal morphological parameters of the *C. gariepinus* larvae were assessed at 2, 4 and 6 dph. Three live fish larvae were randomly sampled at 2 dph to serve as a control. This was done in the morning, before stocking them in the aquaria, and well before the start of exogenous feeding that took place in the evening of the same day. At 4 and 6 dph, one live larva was randomly sampled from each replicate, quickly fixed in Bouin solution for 12 h before it was preserved in 70% ethanol for analysis at the Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Belgium. All preserved samples were dehydrated in a graded series of ethanol in a tissue processor STP 420D over 15 h, embedded in paraffin and cooled to blocks on EC 350-1 microm and EC 350-2 microm as described in Gurcan et al. (2009).The relative position of section 1 (proximal), 2 (middle), and 3 (distal) of the intestine on the blocks was obtained based on Holden et al. (2013). For each of these three sections, five slices of 5 μm size were cut per fish larva using a HM 360 microtome for viewing on the same slide. This was followed by Hematoxylin and Eosin (H and E) and PAS (Periodic Acid Schiff) staining and mounting following the description in Fischer et al. (2008). Out of these five, three field views were randomly taken for observation under the light microscope for morphometric measurements and counts (Table 3), totalling nine measurements per section (3 views per larva, with one larva for each of the three replicates per treatment). Goblet cells were counted for the entire field view, and calculated for sake of standardization per 100 μm length of the inner perimeter. Photomicrographs of selected fields were captured by a DP50 digital camera fitted to a BX61 Olympus light microscope. The histological measurements and counts were analysed according to Dimitroglou et al. (2011), using the imaging analysis software Cell^Fare.

2.4. Statistics

Statistical analysis was conducted using SPSS statistics version 20. One-way analysis of variance (ANOVA) was performed to test the effects of test diets on all parameters of water quality, growth and intestinal morphology. Significant differences between means were tested by Tukey's HSD and considered significant at $P < 0.05$. Prior to data analysis, all data sets were subjected to normality and homogeneity of variance tests using Kolmogorov Smirnov's (Zar, 1999) and Levene's (Levene, 1960) tests, respectively. All data passed the tests for normality and homogeneity. Water quality parameters (DO, NO_2^-, NH_4^+), growth parameters (wet weight, daily growth, total length, SGR, FCR) and intestinal morphological parameters (mucosal folds, mucosal perimeter, mucosal thickness and goblet cells) were expressed as mean and standard error (SE).

3. Results

3.1. Water quality

The results of measurements of DO, NO_2^- and NH_4^+, taken every 2 days, are summarized in Table 4. There were no significant differences (one-way ANOVA, $p > 0.05$) in water quality parameters among the different treatments. The water quality parameter values remained rather stable throughout the culture period, and were well within the tolerance range of *C. gariepinus* larvae (Boyd, 1990).

3.2. Growth

At the end of the feeding experiment, growth in terms of final wet weight, daily growth and SGR was significantly ($P < 0.05$) higher in diet A (*Artemia* nauplii + dry feed), than in all other treatments (Table 5). Diet C (nauplii only) was second best for these growth parameters. In terms of final body length, there was no significant difference between treatments A and C, and these treatments were significantly better than the three others. Feeding decapsulated *Artemia* cysts only (D) resulted almost always in significantly lower growth values compared to all other dietary treatments. Also decapsulated *Artemia* cysts combined with dry feed (diet B) resulted in lower values than the dry feed diet alone (E). A similar pattern was found for FCR, with the best values found when feeding *Artemia* nauplii alone (C) or nauplii combined with dry feed (A), whereas offering decapsulated *Artemia* cysts (B and D) recorded the most unfavourable FCR values. Feeding the dry diet alone (E) gave an intermediate FCR (Table 5).

3.3. Histology

3.3.1. Mucosal folds

The microscopic investigation of the proximal, middle and distal intestine revealed effects of the diets tested on the larval intestinal morphology. At 2 dph, different sections of the intestine were not clearly differentiated (Fig. 1). The number of mucosal folds decreased from proximal to distal intestine but the numbers increased over time from 2 dph (Fig. 1) to 4 dph and to a limited extent from 4 to 6 dph (Table 6). Larvae fed with *Artemia* nauplii in diets A and C had almost always significantly ($P < 0.05$) more mucosal folds than larvae fed all other diets. Larvae fed decapsulated *Artemia* cysts only (D) produced significantly lower values than all other diets, whereas diets B and E resulted in intermediate values (Table 6). The mucosal folds of the best performing diet (A), the worst performing diet (D) and an intermediate

Table 3
Intestinal mucosal parameters of *C. gariepinus* larvae measured after 5 days of feeding on different diets.

parameter	Mucosal folds		Mucosal perimeter		Mucosal thickness		Goblet cell
	N	H	IP	OP	IA	OA	
Unit	Counts	μm	μm	μm	μm^2	μm^2	Entire view counts

H = height; N = number; IP = inner perimeter; OP = outer perimeter; IA = inner area; OA = outer; mucosal fold height = distance between the base of the fold and its apical tip in the lumen; IP = total length of mucosal epithelium lining the lumen; OP = total length of serosal epithelium; IA = total area within the IP (=luminal area); OA = total area within the OP.

diet (E) are shown in Fig. 2.

3.3.2. Mucosal height

The mucosal fold height steadily increased from 2 to 6 dph but decreased from the proximal to the distal part of the intestine in all diets (Table 7). Significant differences between the diets were only found at the proximal part, both at 4 and 6 dph. Diet A (nauplii and dry feed) produced the highest values for mucosal fold height, although the differences with the other diets were not always significant. Feeding decapsulated *Artemia* cysts only (D) produced significantly lower mucosal folds heights compared to diets A, C and E. Feeding dry diet (E) resulted in intermediate values (Table 7).

3.3.3. Mucosal perimeter ratio (IP/OP)

The perimeter (IP/OP) ratio increased over larval development, with a general decrease from proximal to distal in all diets investigated. Significant differences between diets were only discerned in the proximal and middle intestine (Table 8). Generally, larvae fed nauplii combined with dry feed (A) had significantly ($P < 0.05$) higher IP/OP ratio compared to all other diets. At 6 dph, larvae fed decapsulated cysts combined with dry feed (B) or decapsulated cysts alone (D) generally had significantly lower IP/OP ratio compared to the rest. Diet C and E fed larvae showed intermediate values (Table 8).

3.3.4. Mucosal thickness

In all diets mucosal thickness increased with the days post hatching and generally decreased from the proximal to the distal part of the intestine. A significant effect of the diets was found in every section (Table 9). At 4 and 6 dph, larvae fed on decapsulated *Artemia* cysts (D) and its combination with dry feed (B) often had significantly ($P < 0.05$) higher mucosal thickness compared to the other diets. In most cases there were no significant differences at 4 and 6dph between *Artemia* nauplii only (C), its combination with dry feed (A) and dry feed only (E) (Table 9).

3.3.5. Goblet cell counts

PAS staining of the mucosa epithelium revealed the presence of magenta stained goblet cells scattered on the intestinal mucosa (Fig. 3). The number of goblet cells per 100 μm IP length increased from 2 to 4 dph before decreasing on 6 dph in all sections (Table 10). The goblet cells generally increased from the proximal to the distal intestine except in the larvae fed for *Artemia* nauplii only (C). Except for the proximal intestine, larvae fed on nauplii combined with dry diet (A) generally had the highest counts of goblet cells per 100 μm IP at 4 and 6 dph, compared to the other diets, and the differences were mostly significant ($P < 0.05$). Feeding decapsulated *Artemia* cysts only (D) often gave significantly ($P < 0.05$) lower goblet cell counts compared to all other diets evaluated. Larvae fed diets B (cysts and dry diet), C (nauplii) and E (dry diet) generally gave intermediate goblet cell counts (Table 10).

4. Discussion

Live and dry feeds have been used as starter feed for *C. gariepinus* larvae in fish hatcheries (Uys and Hecht, 1985; Verreth and Van Tongeren, 1989; El-Sebaie et al., 2014; Adewumi, 2015). *Artemia* is a standard starter feed as it generally results in improved larval performance. However, it is expensive and may not be available as and when required. Therefore, for practical and financial reasons, efforts have been directed in seeking alternatives to *Artemia* use (Agadjihouèdé et al., 2012; Brüggemann, 2012). The current study investigated the effects of different starter diets on *C. gariepinus* larvae performance through analysis of the intestine's morphological development and assessment of the larvae growth.

The development of the digestive tract is similar among all teleost fish and proceeds in three major stages (Zambonino-Infante et al., 2008). The first stage begins with hatching and ends with the end of endogenous feeding, during which the larva depends on yolk sac and oil globules for all energy and nutritional requirements. In this stage the larva shows a relatively undifferentiated digestive system, with closed mouth and anus. The second stage starts with exogenous feeding (which is approximately 48 h after hatching when the larva is reared at 28–30 °C). During this stage the digestive tract has distinct regions, including a clear and functional intestine (Verreth et al., 1992), with different sections of the intestine acquiring distinct histological features. The intestine forms the main site for absorption and digestion (Gisbert et al., 2008). The digestive system is fully functional, with the exception of the stomach (Segner et al., 1993). The second stage is considered critical for larval survival because of increased metabolic rate and because improper feeding at this stage may affect subsequent performance of the animal. The third and last stage is marked by a drop of the gastric pH about 5 days after the start of exogenous feeding. It marks the onset of a functionally mature digestive tract (Govoni et al., 1986), including a functional stomach with the stomach epithelium becoming structurally differentiated (Rønnestad et al., 2013). Feeding trials started the evening of 2 dph, when larvae would be in the second stage of digestive development, according to the classification described above. Histological sections were all taken in larvae in the second stage (2–6 dph): the progressive changes observed in these

Table 4
Water quality parameters (mean ± SE of three replicates, measured every 2 days) in the *C. gariepinus* larvae rearing tanks over the 10 days experimental period.

Water parameter	Diet				
	A	B	C	D	E
DO (mg/l)	6.54 ± 0.03	6.34 ± 0.01	6.51 ± 0.13	6.53 ± 0.05	6.10 ± 0.02
NO_2^- (mg/l)	0.03 ± 0.02	0.03 ± 0.00	0.03 ± 0.03	0.02 ± 0.02	0.03 ± 0.01
NH_4^+ (mg/l)	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.04	0.10 ± 0.01

For explanation of diets A–E, see Table 1.

Table 5
Growth parameters (mean ± SE, $n = 9$) of *C. gariepinus* larvae after 10 days of feeding on different diets.

Parameter	Diet				
	A	B	C	D	E
Final wet weight (mg)	17.90 ± 0.38^{a}	10.80 ± 0.59^{d}	16.20 ± 0.84^{b}	8.90 ± 0.44^{e}	13.40 ± 0.67^{c}
Daily growth (mg d^{-1})	1.67 ± 0.02^{a}	1.22 ± 0.02^{d}	1.51 ± 0.05^{b}	0.75 ± 0.01^{e}	1.39 ± 0.01^{c}
Final body length (mm)	12.35 ± 0.30^{a}	10.43 ± 0.36^{b}	11.88 ± 0.34^{a}	9.95 ± 0.29^{c}	11.13 ± 0.32^{b}
SGR (%/day)	24.12 ± 0.35^{a}	19.39 ± 0.35^{cd}	22.81 ± 0.29^{b}	17.18 ± 0.28^{d}	21.60 ± 0.30^{c}
FCR	0.70 ± 0.30^{a}	1.62 ± 0.10^{c}	0.53 ± 0.12^{a}	1.66 ± 0.21^{c}	1.03 ± 0.12^{b}

Values in the same row with different superscripts are significantly different ($P < 0.05$). For explanation of diets A–E, see Table 1.

larvae are an important indicator of their ability to cope with different food sources. Further, adjusting first feeding with ontogenetic changes at this stage optimizes the feeding process and nutritional assimilation (Osman et al., 2008).

As exogenous feeding started, larvae were observed swimming within the water column, capturing the prey and ingesting it. At this stage, larvae show improving musculature for locomotion activities, the mouth aperture has opened and there is increased development of barbels with taste buds around the mouth (Olaniyi and Omitogun, 2014). As the larva also has developed vision by 3 dph (Adriaens et al., 2001; Osman et al., 2008; Olaniyi and Omitogun, 2014), these functions allow for increased efficiency in prey detection (Hecht and Appelbaum, 1987; Mukai and Lim, 2011; Prokešová et al., 2017).

All growth parameters investigated (Table 5) were significantly higher ($P < 0.05$) when feeding nauplii combined with dry feed. This could be attributed to the advantage of combined protein proportions and vitamins from these diets (Awaiss and Kestemont, 1998). In addition, the involvement of exogenous digestive enzymes from *Artemia* nauplii could have enhanced digestion and absorption (Aderolu et al., 2010). The results of the current study are in line with previous studies (Appelbaum and McGeer, 1998; Awaiss and Kestemont, 1998; Chepkirui-Boit et al., 2011) that showed a significantly higher growth when using combined diets, consisting of nauplii and dry feed.

Larvae fed decapsulated *Artemia* cysts performed poorer (Table 5) compared to all other diets, although this is reportedly an ideal starter feed for *C. gariepinus* larvae (Verreth et al., 1992; Garcia-Ortega et al., 1998; Adewolu et al., 2009; Olurin et al., 2012; Ngupula et al., 2014). Similar results, however, have also been reported in Asian catfish *Hemibagrus wyckioides* (Hung et al., 2002). Verreth and Den Bieman (1987) suggested that low performance of the decapsulated *Artemia* cyst diet can be linked to its fast sedimentation during feeding, making it unavailable for ingestion and utilization. In addition, the African catfish larva is reported to prefer larger prey sizes, possibly to optimize energy (Prokešová et al., 2017), and this might disfavour the uptake of decapsulated *Artemia* cysts, which are about half the size of instar I nauplii (Van Stappen, 1996). Further, decapsulation and drying procedures might have affected the cyst protein quality which is essential for its use as a starter feed (Pector et al., 1994; Van Stappen, 1996; Garcia-Ortega et al., 2000). Dry feed fed larvae recorded a better performance compared to those fed on either a combination of decapsulated *Artemia* cysts and dry feed, or decapsulated *Artemia* cysts only. This suggests a better feed quality, digestibility, bioavailability and ease of assimilation properties of the dry feed.

The SGR of *C. gariepinus* larvae ranged between 17.2% d^{-1} and 24.1% d^{-1} (Table 5). Larvae fed *Artemia* nauplii combined with dry feed had a significantly ($P < 0.05$) higher SGR compared to other diets investigated. However, the SGR range found was lower compared to earlier studies (Vandecan et al., 2011). This observation may be attributed to differences in feeding frequency and feeding level which affects feed availability and chances of feed uptake (Verreth and Den Bieman, 1987; Aderolu et al., 2010; Al Zahrani et al., 2013). A similar pattern was observed in mean FCR values with a range of 0.53–1.66. Larvae fed on *Artemia* nauplii or its combination with dry feed had an FCR in the range 0.53–0.70 (Table 5). These results are in line with those of Vandecan et al. (2011) who, using *Artemia* of the same origin and the same dry feed as in this study showed that nauplii and its combination with dry feed reported better FCR compared to dry diets alone.

Earlier studies have described nutritionally induced changes in fish larvae based on histological analysis of the intestine (Govoni et al., 1986; Verreth et al., 1992; Pradhan et al., 2014). The intestine may show variable but reversible morphology in relation to food quality and quantity. In the current study, morphological investigation demonstrated dietary effects on the intestinal histology of the *C. gariepinus* larvae. The mucosal fold counts, height, perimeter ratio and thickness decreased from the proximal to the distal intestine in all diets (Tables 6-9), suggesting gradual mucosal cell development. Intestinal mucosal morphometric parameter values increased with age in all diets investigated, as observed in other teleosts (Zambonino-Infante et al., 2008). Larvae fed with nauplii combined with dry feed showed significantly higher mucosal fold counts, height and perimeter ratio values, indicating increased surface area for digestion and absorption. This observation can be attributed to increased amounts of nutrients in the intestinal lumen, provided by this diet, which directly stimulated positive development of the mucosa for efficient digestive function (Rios et al., 2004). Furthermore, high protein and lipid proportions in

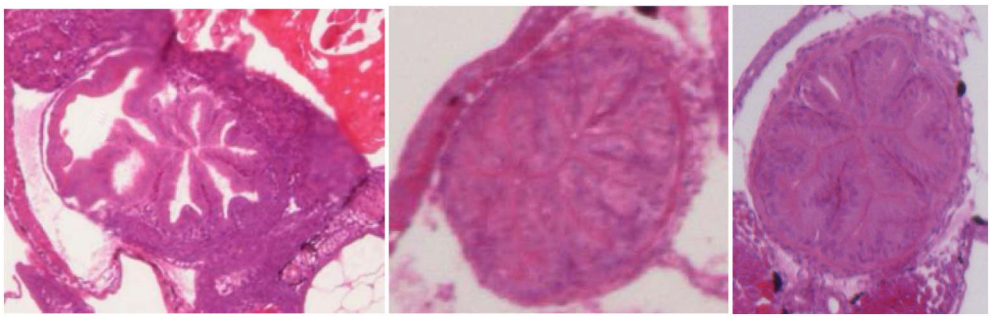

Fig. 1. Microphotographs of *C. gariepinus* larvae intestinal mucosa folds, obtained at 2 dph before start of feeding, from sections 1 (proximal), 2 (middle) and 3 (distal) (left, middle and right pictures, respectively; H and E staining, objective × 40).

Table 6
Mucosal fold counts (mean ± SE, n = 9) at 2, 4 and 6 dph for section l (proximal), 2 (middle) and 3 (distal) of the intestine in *C. gariepinus* larvae fed on different diets.

Section	DPH	Diet				
		A	B	C	D	E
1	2	9.56 ± 0.30	9.56 ± 0.30	9.56 ± 0.30	9.56 ± 0.30	9.56 ± 0.30
	4	30.88 ± 0.61^{a}	21.00 ± 87^{c}	29.52 ± 0.52^{a}	21.49 ± 0.55^{d}	26.00 ± 0.61^{b}
	6	31.87 ± 0.50^{a}	28.12 ± 0.60^{b}	30.45 ± 0.40^{ab}	25.78 ± 0.60^{c}	28.51 ± 0.40^{b}
2	2	8.00 ± 0.30	8.00 ± 0.30	8.00 ± 0.30	8.00 ± 0.30	8.00 ± 0.30
	4	16.78 ± 0.87	13.78 ± 0.63	14.66 ± 1.30	14.67 ± 0.97	16.00 ± 1.00
	6	20.01 ± 1.20^{a}	17.34 ± 1.30^{b}	16.00 ± 1.20^{b}	14.85 ± 0.93^{c}	16.11 ± 1.50^{b}
3	2	4,92 ± 35	4.92 ± 0.35	4.92 ± 0.35	4,92 ± 0.35	4.92 ± 0.35
	4	9.57 ± 0.57^{a}	8.00 ± 0.87^{b}	9.00 ± 0.30^{a}	5.77 ± 0.57^{c}	8.33 ± 0.30^{b}
	6	10.44 ± 0.60^{a}	8.00 ± 0.40^{b}	9.79 ± 0.34^{a}	5.87 ± 0.60^{c}	8.00 ± 0.40^{b}

Values in the same row with different superscripts are significantly different ($P < 0.05$). Values in the same row without superscripts are not significantly different ($P \geq 0.05$). For explanation of diets A–E, see Table 1.

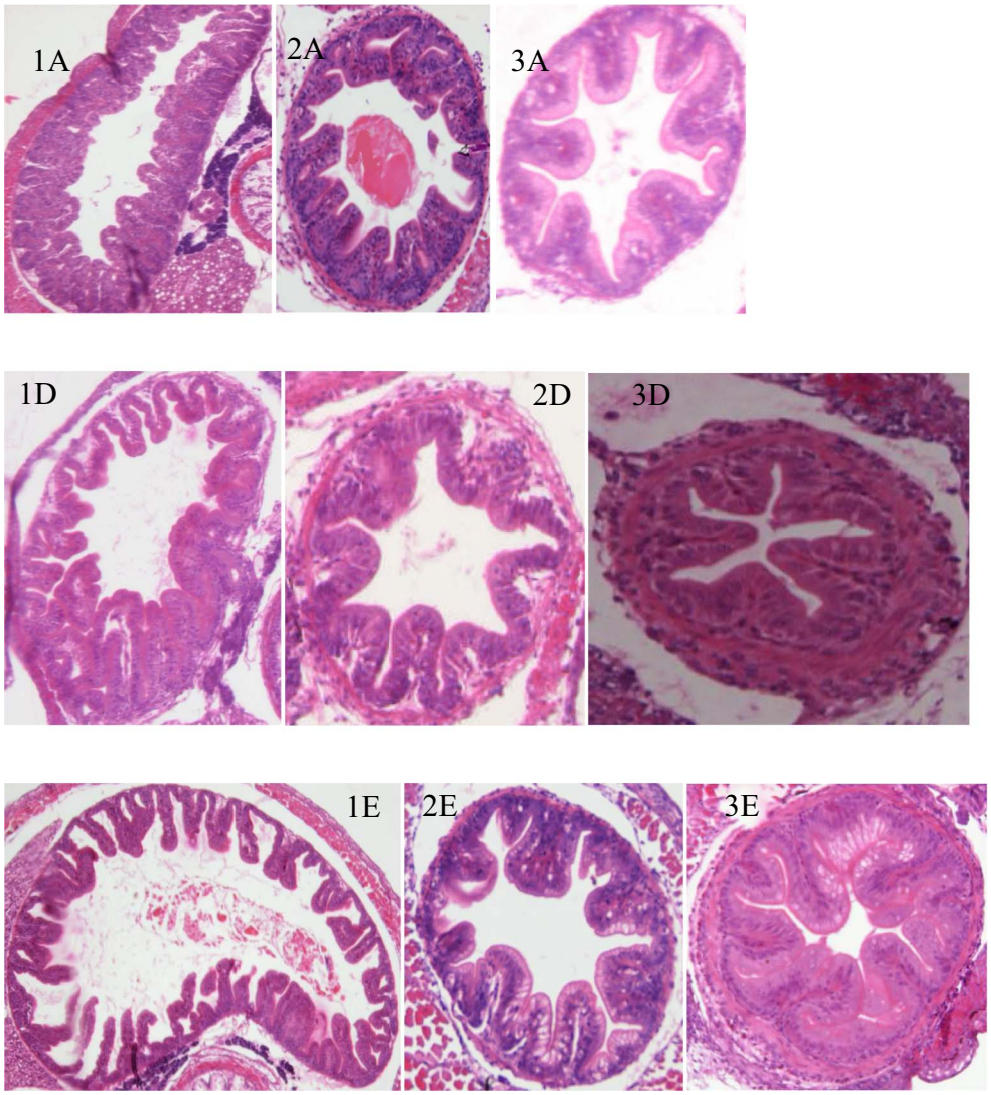

Fig. 2. Microphotographs of *C. gariepinus* larvae intestinal mucosa folds obtained at 6 dph with diets A (*Artemia* and dry feed; top row), D (decapsulated cysts only; middle row) and E (dry feed only, bottom row) from sections 1 (proximal), 2 (middle) and 3 (distal) (left, middle and right pictures, respectively; H and E staining, objective × 20).

the nauplii and its combination with dry feed could have increased protease activities or prolonged cytosolic enzyme activities compared to decapsulated *Artemia* and its combination with dry feed (Garcia-Ortega et al., 1998; Lazo et al., 2007). Increased growth when using the diet with nauplii can be related to improved diet contact time with the absorptive area and increased protein digestion, thus increased nutrient utilization (Rønnestad et al., 2013; Fang et al., 2015). These findings deviate from those reported on *Pseudoplatystoma punctifer* by Gisbert et al. (2014), probably because of differences in feeding history (Grosell et al., 2010). Poorer mucosal morphometrics with decapsulated *Artemia* cysts and its combination with dry feed (Tables 6–10) was an indication of partial starvation, possibly due to unavailability of this diet for

Table 7
Mucosal fold height (μm, mean ± SE, n = 9) at 2, 4 and 6 dph for section l (proximal), 2 (middle) and 3 (distal) of the intestine in *C. gariepinus* larvae fed on different diets.

Section	DPH	Diet				
		A	B	C	D	E
1	2	33.89 ± 1.52	33.89 ± 1.5	33.89 ± 1.52	33.89 ± 1.52	33.89 ± 1.52
	4	57.44 ± 0.50^{a}	51.63 ± 1.12^{bc}	54.24 ± 1.10^{ab}	47.91 ± 1.01^{c}	55.07 ± 1.01^{ab}
	6	84.12 ± 1.40^{a}	71.04 ± 1.20^{bc}	76.23 ± 1.30^{ab}	68.23 ± 1.61^{c}	78.20 ± 0.51^{ab}
2	2	32.86 ± 1.51	32.86 ± 1.51	32.86 ± 1.51	32.86 ± 1.51	32.86 ± 1.50
	4	48.70 ± 3.12	47.90 ± 3.10	46.59 ± 3.12	43.05 ± 3.13	45.05 ± 3.14
	6	63.32 ± 5.22	54.57 ± 3.41	56.19 ± 2.80	55.94 ± 3.41	61.91 ± 4.83
3	2	30.86 ± 1.50	30.86 ± 1.50	30.86 ± 1.50	30.86 ± 1.50	30.86 ± 1.50
	4	34.88 ± 3.00	33.87 ± 3.21	35.18 ± 2.53	31.38 ± 3.01	34.94 ± 3.33
	6	46.32 ± 2.00	46.73 ± 3.01	48.78 ± 2.50	48.43 ± 2.20	50.36 ± 3.21

Values in the same row with different superscripts are significantly different ($P < 0.05$). Values in the same row without superscripts are not significantly different ($P \geq 0.05$). For explanation of diets A–E, see Table 1.

Table 8
Perimeter ratio IP/OP (mean ± SE, n = 9) at 2, 4 and 6 dph for section l (proximal), 2 (middle) and 3 (distal) of the intestine in *C. gariepinus* larvae fed on different diets.

Section	DPH	Diet				
		A	B	C	D	E
1	2	0.48 ± 0.02	0.48 ± 0.02	0.48 ± 0.02	0.48 ± 0.02	0.48 ± 0.02
	4	1.70 ± 0.03^{a}	1.18 ± 0.02^{b}	1.20 ± 0.03^{b}	1.19 ± 0.02^{b}	1.40 ± 0.02^{b}
	6	1.79 ± 0.03^{a}	1.43 ± 0.02^{c}	1.48 ± 0.03^{b}	1.36 ± 0.03^{c}	1.50 ± 0.01^{b}
2	2	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02
	4	1.36 ± 0.03^{a}	0.89 ± 0.03^{c}	1.29 ± 0.02^{ab}	0.91 ± 0.00^{c}	1.20 ± 0.03^{b}
	6	1.52 ± 0.04^{a}	1.39 ± 0.03^{bc}	1.38 ± 0.04^{bc}	1.25 ± 0.03^{c}	1.43 ± 0.03^{ab}
3	2	0.34 ± 0.01	0.34 ± 0.02	0.34 ± 0.00	0.34 ± 0.02	0.34 ± 0.02
	4	1.12 ± 0.02^{a}	0.87 ± 0.01^{a}	0.95 ± 0.02^{a}	0.98 ± 0.00^{a}	1.31 ± 0.03^{a}
	6	1.15 ± 0.02^{a}	1.04 ± 0.02^{a}	1.02 ± 0.03^{a}	0.79 ± 0.04^{a}	1.05 ± 0.01^{a}

Values in the same row with different superscripts are significantly different ($P < 0.05$). Values in the same row without superscripts are not significantly different ($P \geq 0.05$). For explanation of diets A–E, see Table 1.

Table 9
Mucosal wall thickness (in 1000 μm^2; mean ± SE, n = 9) at 2, 4 and 6 dph for section l (proximal), 2 (middle) and 3 (distal) of the intestine in *C. gariepinus* larvae fed on different diets.

Section	DPH	Diet				
		A	B	C	D	E
1	2	8.06 ± 0.14	8.06 ± 1.36	8.06 ± 1.36	8.06 ± 1.36.	8.06 ± 1.36
	4	24.11 ± 0.48^{a}	27.52 ± 0.55^{a}	20.37 ± 0.39^{a}	25.70 ± 0.47^{a}	26.60 ± 0.53^{a}
	6	60.44 ± 0.34^{b}	70.54 ± 0.87^{a}	49.72 ± 0.83^{c}	64.68 ± 0.36^{ab}	60.72 ± 0.42^{b}
2	2	6.59 ± 0.10	6.59 ± 0.10	6.59 ± 0.10	6.59 ± 0.10	6.59 ± 0.10
	4	19.77 ± 0.52^{b}	25.28 ± 0.49^{a}	13.95 ± 0.32^{c}	29.81 ± 0.71^{a}	21.17 ± 0.44^{b}
	6	31.46 ± 0.33^{b}	43.52 ± 0.72^{a}	34.37 ± 0.57^{b}	42.83 ± 0.71^{a}	39.34 ± 0.66^{b}
3	2	6.10 ± 0.11	6.10 ± 0.11	6.10 ± 0.11	6.10 ± 0.11	6.10 ± 0.11
	4	19.82 ± 0.40^{b}	25.23 ± 0.42^{a}	19.28 ± 0.32^{b}	22.14 ± 0.37^{ab}	19.59 ± 0.43^{b}
	6	30.61 ± 0.50^{b}	43.46 ± 0.51^{a}	28.92 ± 0.48^{b}	33.21 ± 0.55^{ab}	28.19 ± 0.49^{b}

Values in the same row with different superscripts are significantly different ($P < 0.05$). Values in the same row without superscripts are not significantly different ($P \geq 0.05$). For explanation of diets A–E, see Table 1.

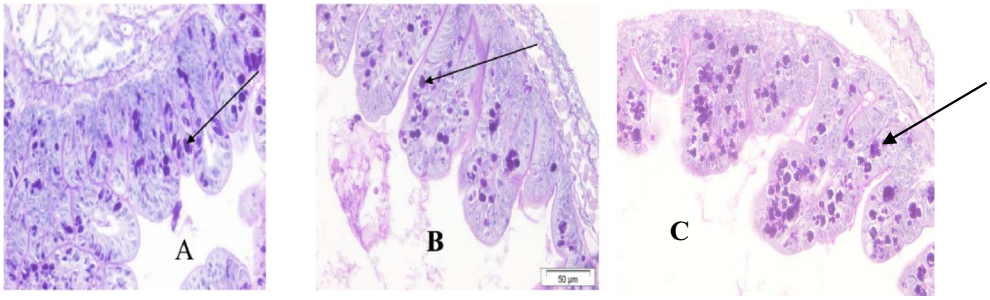

Fig. 3. Microphotographs of *C. gariepinus* intestinal mucosa folds obtained from section 1 at 4 dph with diet A (picture A), D (picture B) and E (picture C), PAS staining, objective × 20. The arrows point to goblet cells.

Table 10
Goblet cell counts per 100 μm inner perimeter (mean ± SE, n = 9) at 2, 4 and 6 dph for section l (proximal), 2 (middle) and 3 (distal) of the intestine in *C. gariepinus* larvae fed on different diets.

Section	DPH	Diet				
		A	B	C	D	E
1	2	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01
	4	4.32 ± 0.08^{b}	4.37 ± 0.68^{b}	6.38 ± 0.77^{a}	2.28 ± 0.22^{c}	4.45 ± 0.42^{b}
	6	1.85 ± 0.13^{ab}	1.24 ± 0.10^{b}	2.21 ± 0.19^{a}	1.57 ± 0.25^{b}	2.53 ± 0.49^{a}
2	2	0.80 ± 0.04	0.80 ± 0.04	0.80 ± 0.04	0.80 ± 0.04	0.80 ± 0.04
	4	8.49 ± 0.58^{a}	4.49 ± 0.65^{b}	5.99 ± 0.55^{b}	2.50 ± 0.29^{c}	4.70 ± 0.28^{b}
	6	4.41 ± 0.35^{a}	1.74 ± 0.33^{c}	2.48 ± 0.22^{b}	1.80 ± 0.20^{c}	3.22 ± 0.12^{ab}
3	2	1.39 ± 0.07	1.39 ± 0.07	1.39 ± 0.07	1.39 ± 0.07	1.39 ± 0.07
	4	9.57 ± 0.57^{a}	4.79 ± 0.29^{b}	4.01 ± 0.45^{b}	2.59 ± 0.34^{c}	5.91 ± 0.47^{b}
	6	4.60 ± 0.33^{a}	2.53 ± 0.28^{c}	3.67 ± 0.30^{b}	3.11 ± 0.43^{b}	4.86 ± 0.21^{a}

Values in the same row with different superscripts are significantly different ($P < 0.05$). Values in the same row without superscripts are not significantly different ($P \geq 0.05$). For explanation of diets A–E, see Table 1.

ingestion as it sediments fast in water (Verreth and Den Bieman, 1987). The low growth performance of the larvae fed with decapsulated *Artemia* cysts and its combination with dry feed (Table 5) may also be explained by nutrient deprivation, a consequence of tissue degeneration following their disuse (Mackerel, 1986). However, feeding decapsulated *Artemia* cysts and its combination with dry feed resulted in a significantly thicker (Table 9) mucosal wall compared to all other diets, which may be attributed to differences in the dietary protein structure and to intestinal restructuring as an adaptation to reduced feed intake (Garcia-Ortega et al., 2000; Gisbert et al., 2008; Fang et al., 2015), as also found in Asian catfish (*Pangasius bocourti*) by Hung et al. (2002). However, the proximal intestine in all diets showed a thick mucosal wall, maybe because of increased mucosal muscle development as a function of food type and quantities present (Ikpegbu et al., 2013). The proximal intestine receives large quantities of chyme; high muscle energy is needed to propel it along by intestinal contraction (Rao et al., 2010).

Gisbert et al. (2014) compared goblet cell counts in the larval development of different Siluriformes (however not including *C. gariepinus*). In our study a PAS staining of embedded *C. gariepinus* larvae was conducted to test the influence of the diet on digestive efficiency, important in larval rearing procedures and in studying digestive physiology. Goblet cells were present before exogenous feeding, an indication of absorptive preparedness. Earlier studies also report on the presence of trypsin, chymotrypsin, amino-peptidase and esterase activities in *C. gariepinus* larvae before exogenous feeding (Verreth et al., 1992; Garcia-Ortega et al., 2000). Except for the larvae fed *Artemia* nauplii only (diet C), goblet cell counts per inner perimeter unit length (100 μm) increased from the proximal to the distal part of the intestine (Table 10). When feeding nauplii alone, goblet cell counts were higher in the proximal intestine, a section with increased protein and lipid digestion due to pancreatic secretions as compared to the remaining sections (Hamre et al., 2013), suggesting increased uptake and transportation of amino acids and free fatty acids from protein and lipid digestion, respectively (Grosell et al., 2010). Decapsulated *Artemia* cysts and *Artemia* nauplii are reported to be nutritionally indifferent and with biomolecules that stimulate enzyme secretion (Garcia-Ortega et al., 2000). However, their combination with dry feed resulted into different counts of goblet cells (Table 10). Different protein and lipid levels in these diets (Table 2) might have resulted in different goblet cell abundance (Kozarić et al., 2008; Hlophe and Moyo, 2014). Increased goblet cells towards the distal intestine are in line with findings by Pradhan et al. (2014) on butter catfish (*Ompok bimaculatus*) larvae fed on nauplii combined with dry feed. More goblet cells in the anterior end (as found when feeding *Artemia* nauplii only) was also reported in earlier studies on green catfish (*Mystus nemurus*) by El Hag et al. (2012), and tiger catfish (*Pseudoplatystoma apunctifer*) by Gisbert et al. (2014). Differences in goblet cell counts when feeding different dry and live diets may be related to the protein structure of the feeds used (Hlophe and Moyo, 2014). The low values for goblet cell counts with larvae fed decapsulated *Artemia* cysts was an indication for partial starvation and reduced mucosal surface area for digestion and absorption. Accordingly, poor growth was found for larvae fed with decapsulated cysts in this study (Table 5).

Generally, goblet cell counts in teleosts increase with the days post hatching (Gisbert, 1999). In the current study, goblet cell counts decreased from 4 dph to 6 dph (Table 10) in all diets investigated, implying compromised absorptive efficiency. However, a reduction of goblet cell counts may not just mean reduced numbers of cells, but could also imply staining variability and differentiation of goblet cells types (Kozarić et al., 2008; Zambonino-Infante et al., 2008; Uc, 2014). PAS staining is only positive with neutral goblet cells, located near the apical end of the mucosal fold. As such, a double staining (PAS for neutral and blue alcin for acid goblet cells) is thus recommended to fully ascertain goblet cell densities, types and their differentiation during *C. gariepinus* larval development.

5. Conclusions and recommendations

Growth and intestinal morphology responded differently to different starter feeds. A gradual reduction of *Artemia* nauplii in its combination with dry feed improved intestinal morphological development and growth. There was no observed compensatory growth in larvae fed decapsulated *Artemia* cysts, after feeding them with dry feed, to match growth results obtained with other diets. Therefore, the impact of starter feeds on intestinal morphological development affects larval growth and may affect further rearing results after the starter feed period. Future studies on intestinal microbial abundance in relation to starter diets are recommended so as to assess their influence on growth and intestinal development. Further, a cost-benefit analysis is important in establishing the most economical starter feed, considering both the costs of the respective feeds and the culture success obtained with them.

Acknowledgements

Bert-Jan Roosendaal of catfish hatchery Fleuren & Nooijen B.V., Someren, the Netherlands, is warmly acknowledged for allowing us to use his hatchery for our experiments. This study was financially supported by Nuffic project NICHE/KEN/158 with South Eastern Kenya University.

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