Ethological and Amino Acid Properties of Indigenous Fishing Baits for *Clarias gariepinus* Hatchery Feed Development

[1]Nabulime Margaret, [2]Kahwa David, [3]Rutaisire Justus and [4]Kabasa John David

[1]*Department of Livestock and Industrial Resources, College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University, P.O. Box 7062, Kampala, Uganda*
[2]*Department of Wildlife and Aquatic Animal Resources Management, CoVAB, Makerere University, Kampala, Uganda*
[3]*National Agricultural Research Organization Secretariat (NAROSEC) P.O. Box 295 Entebbe, Uganda*
[4]*Department of Biosecurity, Ecosystem and Veterinary Public Health, CoVAB, Makerere University, Kampala, Uganda*

Key words: Indigenous feed attractants, local bait palatability, larvae fish feeding behavior

Corresponding Author:
Nabulime Margaret
Department of Livestock and Industrial Resources, College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University, P.O. Box 7062, Kampala, Uganda

Abstract: In this study, we envisage that developing suitable feed for fish hatcheries from indigenous feedstuffs reduces production costs and creates income. Whereas such feedstuffs in Uganda are suitable for starter feed, they fail to attract *Clarias gariepinus* (*C. gariepinus*) larvae to feed. Knowledge gaps in feedstuffs' properties and presentation limit intake and consequently stunted larval growth. This study evaluated indigenous fish baits and specifically assessed their potential to induce feeding behavior in *C. gariepinus* larvae, determined amino acid profiles of selected baits and selected the most suitable for further evaluation of feeding attraction to larvae. Methods comprised larvae behavioral feeding trials and profiling of amino acids in indigenous fishing baits including *Zingiber officinale* (ginger), Curcuma longa (turmeric), Abelmoschus esculentus (okra), *Hibiscus sabdariffa* (*malakwang*), Lumbricus terrestris (earthworms), Biomphalaria choanompala (lake-snails) and *Chlorella* species (algae). Data were subjected to hierarchical cluster analysis and scree tests. Results showed significant differences in attraction among baits $p = 0.001$ at 95% confidence level, preference for ginger based rations followed by earthworms, okra and *malakwang* rations. A cluster comprising ginger, earthworms and control was significantly different ($R^2 = 0.9683$) from algae, snail and turmeric and control cluster. Comparison of amino acids concentration in ginger and earthworm gave

p>0.05, for proline, aspartic acid, leucine, valine and glutamic acid. Therefore, behavioral and amino acid properties of ginger and earthworms showed prospects for inclusion in local starter rations for *C. gariepinus*.

INTRODUCTION

Mass production of fry, from Uganda's fish hatcheries, to meet the growing demand of seed is limited by insufficient quantities of affordable and good quality starter feeds[1, 2]. Imported starter feeds proven to be of good quality are costly due to international pricing and cross-border trade delays[3]. Accordingly, research into locally manufactured fish feed from commonly available raw materials is required to address affordability, accessibility and nutritional quality[4-7]. Feeding attraction is very crucial at the larval stage to enhance uptake as well as good growth performance. Compared to imported starter feeds, sole utilisation of dry starter nutritionally balanced feeds prepared from local Ugandan feedstuffs is limited by attractability which greatly affects feed uptake and eventually the profitability of the fish farms[2]. Currently, there is limited information concerning the effectiveness of indigenous fishing bio-baits, in attracting *Clarias gariepinus* larvae to feed and their prospects for incorporation as attractants in starter fish feeds. Therefore, using *C. gariepinus* larvae, in this study, we investigated the attractant properties of seven indigenous fishing bio-baits.

Chemo-attractants such as betaine, inosine monophosphate, DL-alanine and tryptophan exist but contribute to increased feed prices[8]. Studies have been undertaken by various researchers to look for natural attractants as feed supplements and consider different parameters including palatability[9-12]. Commercial feeding attractants and formulations developed from natural materials for key fish species are in use as liquid preparations sprayed on water prior to feeding or added to feed during formulation[13]. Among these are protein hydrolysates used as bio stimulants and condensed fish solubles, algae for herbivorous fish and recently black soldier larvae by Skretting in Norway[14]. Unlocking the potential of indigenous natural (organic) fishing baits as attractants may not only motivate utilization of locally formulated diets and lower cost of fish feeds but also enhance feed uptake by farmed fish.

The study objectives were to: assess the potential of selected indigenous fish baits to induce feeding behavior in *C. gariepinus* larvae determine the amino acid profiles of selected indigenous fish baits and select the most suitable combination of feedstuffs for use in experiments to evaluate attraction of *C. gariepinus* larvae to feed. The identification and incorporation of locally available ingredients as feed attractants shall aid the fish feed industry not only to produce more acceptable starter feed, but minimize costs and subsequently increase profits from feed sales.

MATERIALS AND METHODS

Ethical statement: Prior to start of the study, ethical and legal approval was obtained from Uganda National Council for Science and Technology (UNCST), approval number A 479.

Potential of feedstuffs to induce feeding behavior in fish larvae: To unveil the potential of natural locally used fish baits as fish larvae feed attractants, behavioral responses of the fish larvae towards selected local baits were studied. Feeding behavior as used in the hatchery operations refers to three processes which include detection, capture and ingestion as well as assimilation[15].

C. gariepinus larvae, at 14 days post hatch, initial weight 1.37±0.26g and of conventional unspecified microbial status, were obtained from a commercial private fish hatchery. Larvae were transported to the study site in clear open-ended, plastic, low density circular polyethene fish bags (size width 8 inches×height 10 inches) supplied with oxygenated water. A total of 600 fish larvae were used in this study and survival during transportation was 100%. On arrival at the experimental site, fish larvae were kept under holding conditions for 1 h. before transfer into the behavioral tank. Larvae were acclimatized to experimental conditions for 4 days before start of the experiment. During that period, they were fed on a commercial standard starter diet from Skretting®, Nutreco, N.V Staatsspoor 17 3994 VD Houten, Netherlands.

Design of the fish feeding behavioural study: The experiment followed a randomized comparative design (Fig. 1) and was set up in six behavioral aquarium tanks. Each tank was stocked with 100 fish larvae initially placed in the front (acclimatization) chamber. The different test bait materials were then randomly assigned to experimental sets in the behavioral tanks. An experimental set consisted of two test materials and the control material was placed in the third partition (Zone 3) of the same chamber at the same time as the test materials were placed in their partitions. This experimental set was repeated two times after cleaning the tank and
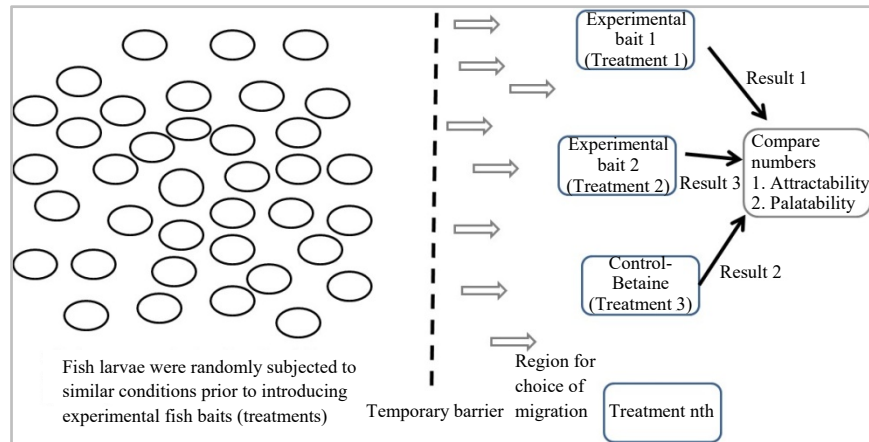
Fig. 1: Pictorial presentation of fish experiment-Randomized comparative design; n = 8 Modified from[16] Fig. 15.2 pg. 274

Table 1: Local fish baits and materials tested as feed attractants for fish larvae

Feedstuff		
Common or local name	Scientific name	Part used
Ginger	*Zingiber officinale Roscoe*	Rhizome
Green algae	*Chlorella*	Whole
Lake snails	*Biomphalaria choanompala*	Whole
Earthworms	*Lumbricus terrestris*	Whole
Turmeric	*Curcuma longa*	Root
Okra	*Abelmoschus esculentus*	Seeds
Hibiscus sabdariffa (Malakwang)	*Corchorus olitorius*	Seeds

interchanging the position of the test materials in the different chambers. Therefore, three sets of data for each test material were obtained and recorded in the Y-maze score sheet.

Quality and source of water: Tap water from a mains supply was collected into a rectangular open reservoir and kept for one day to allow evaporation of chlorine. It was then put in the behavioral aquarium tank and the water quality parameters measured using a Hanna HI 991300 pH /EC/TDS meter, sourced from Hanna instruments, Inc. 584 Park E Dr, Woonsocket, RI 02895, United States. These were recorded as temperature 21.3°C±0.41; dissolved oxygen 57 ppm; pH 7.26 and electrical conductivity 112 µsec. A flow through system was used therefore, no water treatment was needed.

Source of materials for local baits: Seven types of local materials were selected for preparation of the fish baits (Table 1). The samples for water-based bait materials that is, earthworms, lake snails and algae were collected from Ggaba landing site located on the shores of Lake Victoria, in Makindye Division, Kampala district. The plant-based bait samples that included ginger, turmeric, okra and *Hibiscus sabdariffa* were purchased from vendors in a

local market in Nakawa Division, Kampala district. Whole milled *Triticum aestivum* (common wheat) was used as a binder (carrier) substance and served as the negative control.

Preparation of attractant feedstuffs: The feedstuffs were oven dried at 40°C, overnight and each separately ground to a fine powder. The ground powder was weighed using an analytical balance and added to the milled wheat flour in a ratio of 15:75 (w/w), respectively. Following the method developed by researchers at Oregon State University and the Oregon Fish Commission, the pulverized material was transferred into a kitchen mixer and cold tap water was slowly added to the contents while mixing to form a dough[14]. The dough was passed through a stainless-steel electric meat mincer/grinder with a 14 mm die to form thin strands of approximately 0.2 cm pellets long[17]. The resulting moist pellets were kept in plastic bags and frozen at -20°C (wet pellets) for further use. Before availing the pellets to the fish, they were allowed to thaw as recommended by Pitcher[18].

Set up of the behavioral aquarium tank: An aquarium tank designed into a four chambered shuttle maze as described by Conn[19] was used. The type adopted was a modified Y-maze (Fig. 2).

The tank consists of a front chamber that serves as an acclimatization section (A) and rear chamber (section B) with three migratory partitions (zone 1, zone 2 and zone 3) separated by two fixed glass slabs (M and N). The front chamber is separated from the rear chamber by a removable glass mid-way between the two chambers which can be lifted up. The behavioral tank was fabricated using clear glass material and measured 27×30×12 inches (length×width×height). The height of water in tank was maintained at two thirds full and throughout the experiment the system was kept aerated using an air stone connected through tubing to the pump.
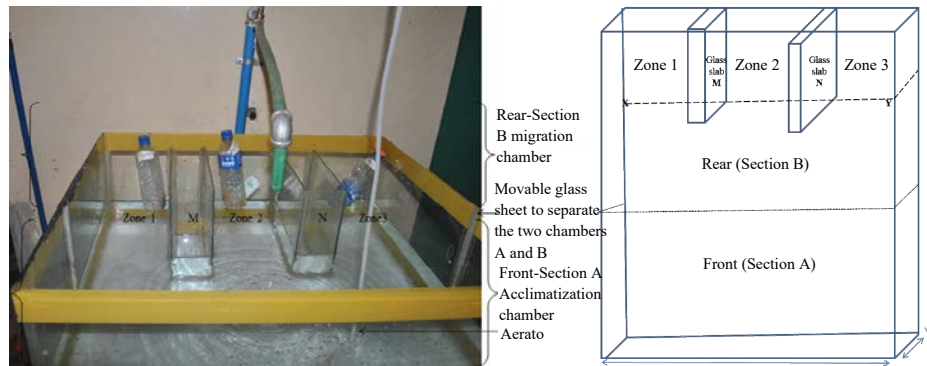
Fig. 2: Behavioural aquarium tank and its schematic presentation

Equipment and procedures used during the feeding behavioral study experiment: Due to photophobia of catfish larvae, the experimental room was excluded from natural lighting by using curtains in the windows and light through the door was eliminated by keeping the room closed all the time. The experimental tanks were covered with black polythene and the water level in the tank was kept at two thirds full. Every morning cleaning was done by suction using a tube in order to remove the water and debris from the bottom of the tank before re-filling it with fresh water.

Procedure for administration of experimental baits: Experimental baits were introduced and placed in the rear chamber at Zone 1-3. By lifting the removable glass between the front and rear chambers, fish larvae were able to move to the three zones where the distinctive choice partitions where test materials were placed (Fig. 3). Each presentation of the bait lasted 15 min during which visual assessment as well as video recording of fish actions was done.

After each run of 15 min, experimental baits were re-arranged in each arm to establish if fish behavior and preference to given bait was influenced by positioning. Every experimental set was repeated twice after cleaning the tank and interchanging the position of the test bait materials in the different partitions of the tank chamber. Therefore, three sets of data (counts of migrated fish larvae) were obtained for each test material and recorded in a Y-maze score sheet.

Only counts of fish larvae that had crossed line X-Y (Fig. 2) into the rear migration zones and within a distance of 2 cm close to the petri dish were recorded corresponding to attractiveness of bait. While counts corresponding to preference included those fish larvae close to the test material by approaching and staying in the petri dish, touching and biting experimental bait. Bias in the observations was controlled through the partitions adopted in tank design as well as video recording during the experiment. The experimental baits randomly assigned

Fig. 3: Close up of migration chamber zones/partitions

into experimental sets tested on the same day using 6 behavioral tanks. The experimental design was the same among the experimental sets in order to minimize the influence of the relative position of the experimental baits on the results.

Observations: Feeding behavior in a fish hatchery set-up refers to three processes which include detection, capture and ingestion as well as assimilation. This experiment measured the feeding behavior of *C. gariepinus* fish larvae, towards the different feed attractant materials by considering their actions following detection of feed. First, feed attractability was linked to search response, selective migration and positioning of the fish larvae. Only counts of fish larvae that had crossed into the rear migration zones and within 2 cm close to the petri dish containing test baits were recorded as corresponding to attractiveness of bait.

Secondly, feed acceptance and palatability were linked to taste responses seemingly noted when fish larvae settled and touched the experimental baits offered. Counts corresponding to preference included those fish larvae close to the test material, approaching and staying in the petri dish as well as touching and biting experimental bait. Bias in the observations was controlled through the partitions adopted in tank design as well as video recording during the experiment.

Amino acid profiling of selected feedstuffs: Accurate determination of the composition and quantity of protein in a sample through analysis of amino acids is important during feed development. Likewise, molecules which can be detected by fish and act as attractants to induce such behavior and prompt feeding need to be analyzed[20]. Therefore, basing on previous studies, test samples were profiled for amino acids to be able to explain their biological activity and narrow down to a biologically meaningful feeding stimulant.

As an output from the study to check attraction of fish larvae to feed, five of the local fishing baits were selected. These included ginger, turmeric, algae, lake snails and earthworms which had enticed good searching and positioning, settling and biting (i.e., feeding.) responses by the fish *C. gariepinus* larvae. Samples of these were dried using convectional hot-air at 40°C, cooled and then milled to a particle size of 0.025 mm. Such processed samples were packed in air tight plastic containers and dispatched to an ISO accredited analytical laboratory offering services in food analysis and protein characterization. The amino acid analysis was carried out in compliance with ISO 17025:2005 and ISO 9001:2008 following standard operating procedure number 270, "Scope of amino acid analysis". The laboratory used a Waters 2487 Dual Absorbance Detector with a Waters 2695 Separations Module (Waters, Milford, MA, USA). Each sample was analyzed in duplicate and the principle of the method involved release of amino acids from the feed matrix through acid hydrolysis, separation of the individual amino acids by Ion Exchange chromatography and post column ninhydrin (Indanetrione Hydrate) derivatization; and lastly detection and quantification according to method 982.30 AOAC[21]. Data was submitted from the external analytical lab in the form of chromatograms presenting elution profiles of amino acids and concentration tables for amino acids.

Identification of appropriate combinations of attractant feedstuffs: Identification of desirable combinations of feedstuffs was done using statistical approaches. Information from the feeding behavior experiments and the amino acid profiling was exerted to correlation studies in order to identify relatively homogeneous combinations of selected attractant feedstuffs.

Raw data of experimental counts from actions of *C. gariepinus* larvae including migration (searching and positioning) as well as feeding (settling and biting) was recorded in a Y-maze score sheet and eventually transcribed into SPSS Inc., PASW (power of advanced statistical analysis) statistics 18 software. Mean counts of fish migrated and mean counts of fish feeding were established and bait preference was computed as:

$$Preference\% = \frac{\text{Mean larvae in petridish}}{\text{Mean larvae migrated}}$$

Hierarchical cluster agglomeration algorithm test statistic was used to assess differences in bait attractability and palatability by identifying combinations of Nearest Neighbors (NN). The two steps involved calculation of first, a similarity based on the information provided in the data sheets and secondly formation of clusters by merging cases. The assumption was that they possess signaling molecules, possibly amino acids which have the potential to induce feeding behavior in *C. gariepinus* larvae. Clustering was done a step at a time, until all cases were joined in one big dendrogram. Euclidean distance was the measure considered as corresponding to the type of interval data analyzed and is given by the square root of the sum of the squared differences between values for the items[22].

Data and statistical analyses
Data analysis: To determine statistically significant attractant clusters, a Scree test was used[23, 24]. Agglomeration coefficients were plotted against clustering stages and the point where the steep slope and levelling off occurred was determined. The "bend-elbow" rule[12, 4] was followed in selection. The values to the left of the inflection or the steep slope in the graphical plot were considered as the most homogenous groups of attractants and retained while those to the right of the inflection were eliminated. This fact, together with cohesiveness of the clusters observed from the dendrogram was used to decide potential attractants retained. This analysis portrayed a hierarchy of clusters from highest to lowest similarity.

Raw data of individual amino acids in each selected attractant feedstuff were entered into an Excel sheet and computed to get the absolute concentration for each of the amino acids as a percentage of total amino acid concentration. Descriptive statistics were used to compare the composition among profiles of amino acids and data was presented in form of histograms. Specific differences in patterns of amino acids between different experimental attractants were obtained by statistical analysis (ANOVA, $p < 0.05$).

RESULTS

Indigenous fish baits inducing feeding behaviour in *C. gariepinus* **larvae:** Of the seven baits tested, only algae, ginger, earthworm, snail and turmeric elicited noticeable movement and aggregation in above 50% of *C. gariepinus*, similar to the control. Preference of indigenous baits was evaluated by positioning and feeding of *C. gariepinus* larvae and it decreased in the order earthworms>ginger>control>algae>lake>snails>turmeric
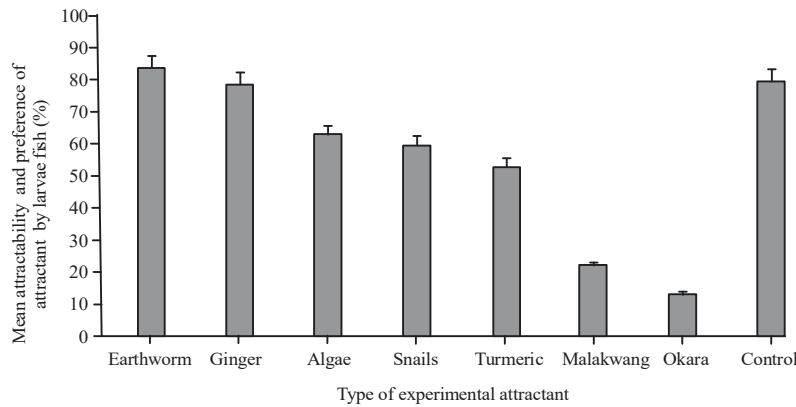
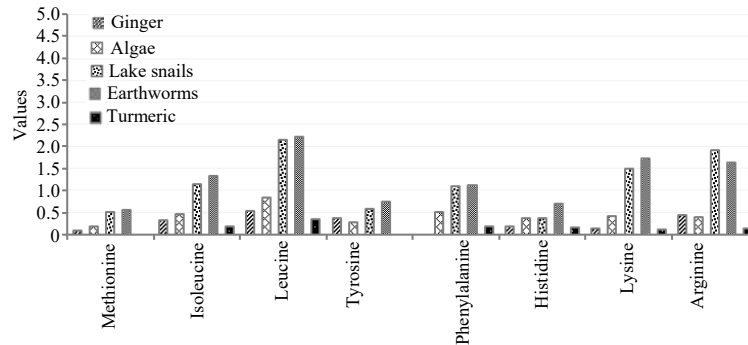Fig. 4: Mean attractability and preference of indigenous fish bait attractants by fish larvae

Fig. 5: Parameters of amino acid

(Fig. 4). Statistical analysis for counts of *C. gariepinus* larvae attracted by different baits gave p = 0.001 at 95% confidence level. The results indicated statistically significant differences in attraction between the baits. Relative to betaine (control), earthworms and ginger exhibited highest and second highest respectively induction of feeding behaviour in *C. gariepinus* larvae

Amino acid profiles of selected attractant feedstuffs:
The data set collected comprised of 17 free amino acids which are hydroxyproline, aspartic acid, threonine, serine, glutamine (estimated as glutamic acid), proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, tyrosine and arginine, Fig. 5. Hence, the data considered comprised of 85 profiles from five potential attractant feedstuffs. The mean value of each amino acid was used to estimate differences between and within the group.

Of all the five studied potential attractant feedstuffs, water-based baits had the highest value of total amino acid, earthworms with 25.3 g/100 g, lake snails 24.9 g/kg and algae 9.20 g/kg protein. In contrast, total amino acids in the land-based baits were 7.6 and 3.1 g/kg protein in ginger and turmeric, respectively.

The concentration of methionine one of the essential amino acids for fish was found to be highest in

earthworms at 0.567 g/kg, followed by lake snails 0.504 g/kg, algae 0.190 g/kg protein and ginger 0.093 g/kg protein. The main essential amino acids for fish in water based baits were arginine and proline[25, 26]. The highest content of arginine was found in algae 1.91 g/kg and earthworms 1.63 g/kg while lake snails had the lowest 0.389 g/kg. The concentration of proline was highest in algae 1.33 g/kg; earthworms contained 0.982 g/kg while lake snails had 0.412 g/kg. The main non-essential amino acids were glutamic acid and aspartic acid with g amino acid/100 g values of 4.3 and 2.63 in lake snails; 4.21 and 2.7 in earthworms and 1.26 and 1.03 in algae, respectively.

On the other hand, land-based baits ginger and turmeric had the essential amino acids arginine and proline in ginger as 0.434 and 1.92 g/100 g while turmeric contained 0.143 and 0.0585 g amino acid/100g of protein, respectively.

The non-essential amino acids, glutamic acid and aspartic acid were 0.815 g/100g and 1.3 g/100 in ginger while 0.488 g/100 g and 0.613 g/100 g of protein were found in turmeric, respectively.

Depending on the type and protein source of attractant feedstuffs, there are differences in the type and amount of amino acids. In decreasing amounts, ginger had proline>aspartic acid>glutamic acid>leucine>valine and
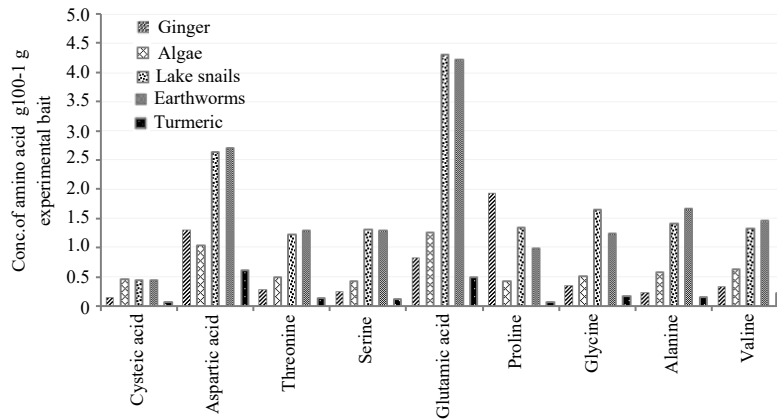
Fig. 6: Concentration of amino acids among the experimental baits

Table 2: Agglomeration schedule for the hierarchical cluster analysis

Stage	Cluster combined			Stage cluster first appears		
	Cluster 1	Cluster 2	Coefficients	Cluster 1	Cluster 2	Next stage
Dimension						
1	2	8	0.038	0	0	3
2	3	4	0.113	0	0	5
3	1	2	0.188	0	1	6
4	6	7	0.338	0	0	7
5	3	5	0.376	2	0	6
6	1	3	1.164	3	5	7
7	1	6	2.666	6	4	0

there were significant differences in concentration (p = 0.022) among the amino acids. Alternatively amino acid content in earthworms varied from glutamic acid>aspartic acid>Leucine> arginine>valine>phenylalanine with significant differences in concentration (p = 0.003).

A comparison of individual amino acids in ginger and earthworm revealed significant differences in the concentration of proline (p = 0.190), aspartic acid (p = 0.214), glutamic acid (p = 0.378), leucine (0.351) and valine (p = 0.363). But no significant difference was observed in the concentration of phenylalanine (p = 0.50) found in ginger and earthworms.

Combination of feedstuffs with potential to attract *C. gariepinus* larvae to feed: From the dendrogram in Fig. 6, two useful clusters were identified and the distance between the two clusters joined corresponds to the coefficient value. The salient interrelationships are that given Euclidean Distance 2.5, a collection of items 1, 8 and 2 have similar features and strong associations to each other than they are to the collection of items 3, 4 and 5 which form another cluster.

In the first collection of items 1, 8 and 2, ginger and the control emerged first and have the smallest distance (coefficient 0.038). While earthworms and ginger coefficient (0.188) emerged third but ties with ginger in the first place. This implies that the two experimental

baits and the control (betaine) have closely similar characteristics. The items 3, 4 and 5 include algae, snails and turmeric and emerged second forming a second cluster. *Hibiscus sabdariffa* (*malakwang*) and Okra in position four have closely similar characteristics (coefficient 0.338) but ties furthest to the control and is completely separate from the other two clusters, indicating that the two were also not among the five that induced feeding movement during the behavioral feeding study (Fig. 7).

During hierarchical clustering, the distance between each cluster formed is denoted as a coefficient value and seven agglomeration coefficients resulted from the hierarchical cluster statistical test Table 2.

A scree test was then applied to the data by plotting agglomeration coefficients against clustering stages to obtain a scree plot Fig. 8. The scree plot suggests that there is a clear break in homogeneity among clusters of attractants at the first noticeable large difference between coefficients (coefficient at stage 5 minus coefficient at stage 6 equal to 0.788) where the steep slope and levelling off occurred.

Two useful clusters of attractants that are more homogeneous were revealed and these formed at stages 1, 2, 3, 4 and 5 corresponding to coefficients 0.038, 0.113, 0.188, 0.338 and 0.376 (Table 2). Implying that the collection of items 1, 2, 8 and items 3, 4, 5 in Fig. 7 were
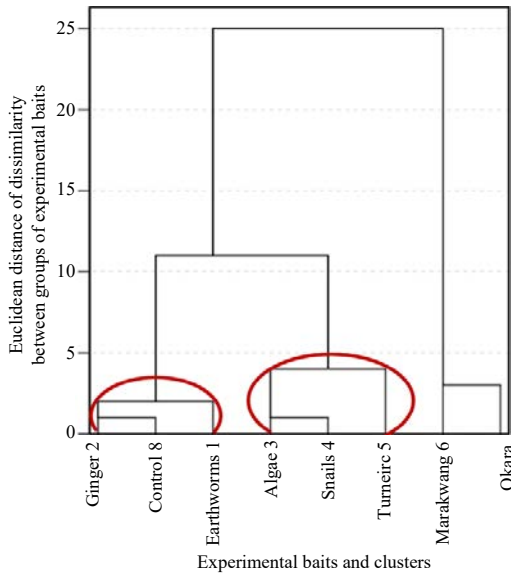
Fig. 7: Dendrogram showing complete linkage clusters of potential attractant feedstuffs and the control (betaine)
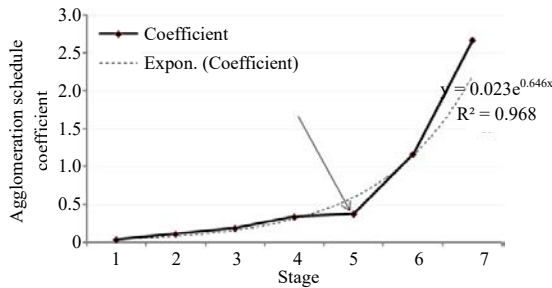

Fig. 8: Scree plot presenting the coefficients and exponential trend lines of clustering

thus, considered as the most suitable from which to select the attractant feedstuffs for the next fish larvae feed formulation experiments. The two clusters were significantly different (coefficient of determination R^2 = 0.9683) from the cluster formed at stages 6 and 7 meaning that 96.8% of the points fall on the regression line.

Formatting of mathematical components:

$$Preference\% = \frac{Mean\ larvae\ in\ petridish}{Mean\ larvae\ migrated} \qquad (1)$$

DISCUSSION

The current study establishes that ginger, turmeric, earthworm, snail and algae have the potential to attract *C. gariepinus* larvae to feed. In fact *C. gariepinus* larvae had higher preference for ginger than the control

betaine (a commercial attractant). The potential of betaine in improving feed attractability was previously studied by 15. The fish larvae demonstrated selective feeding behavior in agreement with previous studies that illustrated differences depending on taste or smell of food offered[27]. However, despite the remarkable smell of turmeric compared to betaine, fish larvae showed lower preference for it. Relatedly the fact that earthworms and betaine (without smell) were most highly preferred implies that as noted by Kasumyan *et al.*[16], there are possibly other factors besides smell contributing to attractability.

There were significant differences in total amino acid content between earthworms and lake snails compared to algae, ginger and tumeric which are plant materials. However, all the five had levels of glutamic acid, proline, glycine, lysine, alanine, leucine, arginine and aspartic acid, above 1.5 g amino acid/100 g. The current study reported histidine as one of the essential amino acids in the same amount range reported by Isea-Leon *et al.*[27] while a higher value (4.21) compared to 1.5 g/100 g of glutamic acid was obtained. Ginger was found to be rich in glutamic acid, aspartic acid and proline as well as leucine reported to be important feeding stimulants in several fish species[28, 19]. Previous studies reported glycine and lysine[29, 30] as well as glutamic acid, proline and glycine[15, 12, 31, 32] to cause stimulatory effect for fish feeding behavior.

Two most homogenous clusters one of which comprised ginger, earthworms, control and the other with algae, lake snails and turmeric were identified, but chemical analysis showed that ginger and earthworm were the most suitable. In fact ginger is among the herbal species reported to have an additive benefit in growth performance and enhanced immunity when supplemented in fish diets[33-35]. On the other hand, earthworms have been used for formulation of high lysine fish feeds, as a protein supplement and for fish meal replacement in common carp (*Cyprinus carpio*) and Indian major carp[36, 3, 2, 8]. Earthworm's have also been evaluated for a source of high fatty acid PUFA content[37, 38].

Although, ginger and earthworm are readily available, there are minimally documented findings of their effects, especially in fish feeding attraction. This cluster is thus worth considering for inclusion in eventual experimental evaluation of ration formulations for inducing *C. gariepinus* larvae to feed.

Simple summary: Acess to dry starter feeds for rearing newly hatched African catfish (fish larvae) in hatcheries, together with high cost of imported starter feed in Uganda are factors limiting maximum fish growth and boosting profits. This study evaluated the ethological and amino acid properties of indigenous fishing baits as an interventional measure to develop domestic starter feeds

and reduce the hish cost of feeds. Seven indigenous baits were identified and behavioral feeding trials carried out. This prompted insight into probable molecules causing the differed effect. Amino acids were molecules chosen for the study basing on literature and these were profiled in five of the best feedstuffs. Results from the trials demonstrated that baits used to attract fish during fishing, have significant differences in attraction, p = 0.001 at 95% confidence level. Correlation studies of the results from the amino acid composition and behavioral studies pointed at ginger and earthworms having potential for inducing attraction. These results suggest performing digestibility experiments to establish other outcomes which shall lead to development of starter feed prototypes.

CONCLUSION

This work establishes that local fishing baits, ginger and earthworms demonstrate great potential to elicit feeding response in *C. gariepinus* larvae. These feedstuffs contain substantial amounts of glutamic and proline amino acids and in a similar manner to betaine, an existing commercial feed attractant are capable of attracting *C. gariepinus* larvae to feed.

This evidence can inform future studies including *in-vivo* trials of potential attractant feedstuffs in fish larvae starter feed formulations. The output of such studies would contribute to improving *C. gariepinus* fish larvae feed quality and thus, benefit fish feed manufacturers and farmers.

Author contributions: Ms. Nabulime Margaret participated in research conceptualization, methodology, project administration, formal analysis of data and authorship of drafts and final manuscript. Dr. Kahwa David participated in research conceptualization, methodology, supervision project administration design and supervision of experimental work. Dr. Rutaisire Justus participated in funding acquisition research design and supervision of experimental work editing manuscript, review and editing of manuscript. Prof. Kabasa John David participated in conceptualization validation supervision research design and supervision of experimental work editing manuscript.

REFERENCES

01. Hassanin, M.E., Y. Hakim and M.E. Badawi, 2014. Dietary effect of ginger (*Zingiber officinale* Roscoe) on growth performance, immune response of Nile tilapia (*Oreochromis niloticus*) and disease resistance against *Aeromonas hydrophila*. Abbassa Int. J. Aqua., 7: 35-52.

02. Martin, W., 2018. A research agenda for international agricultural trade. Applied Econ. Perspect. Policy, 40: 155-173.

03. Irungu, F.G., C.M. Mutungi, A.K. Faraj, H. Affognon and C. Tanga *et al.*, 2018. Minerals content of extruded fish feeds containing cricket (*Acheta domesticus*) and black soldier fly larvae (*Hermetia illucens*) fractions. Int. Aquat. Res., 10: 101-113.

04. Felix, N. and M. Sudharsan, 2004. Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquacult. Nutr., 10: 193-197.

05. Musiba, M.J., G.W. Ngupula, B.B. Kashindye, M. Elison, A.P. Shoko *et al.*, 2014. Performance of locally formulated feeds for rearing of African catfish in Tanzania. Afr. Crop Sci. J., 22: 979-986.

06. Lim, L.S., J.S.K. Lai, A.S.K. Yong, R. Shapawi and G. Kawamura, 2015. A preliminary study on the taste preferences of marble goby (*Oxyeleotris marmoratus*) for amino acids. Songklanakarin J. Sci. Technol., 37: 397-400.\

07. Bhilave, M.P., 2018. Study of shelf life of formulated fish feed. Int. J. Fish. Aquat. Stud., 6: 174-176.

08. Nunes, A.J.P., V.C.S. Marcelo, F.F. Andriola-Neto and D. Lemos, 2006. Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 260: 244-254.

09. Chamberlain, G.W., 1995. Frontiers in Shrimp Nutrition Research. In: Swimming through Troubled Water, Browdy C.L. and J.S. Hopkins (Eds.)., The World Aquaculture Society, Baton Rouge, Louisiana, pp: 108-117.

10. Moore, D.S., 2010. Essential Statistics. W.H. Freeman, New York, USA.,.

11. Polat, A. and G. Beklevik, 1999. The importance of betaine and some attractive substances as fish feed additives. Ciheam-Iamz, 37: 217-220.

12. Olsen, K.H., N. Sukovich, J. Backman and T. Lundh, 2018. Chemical foraging stimulation in the omnivorous species crucian carp, *Carassius carassius* (Linnaeus 1758). Aquacult. Rep., 10.1016/j.aqrep.2018.09.003

13. Bai, S.C., K. Katya and H. Yun, 2015. Additives in Aquafeed: An Overview. In: Feed and Feeding Practices in Aquaculture, Davis, D.A. (Ed.)., Woodhead Publishing, Oxford, England, UK., pp: 171-202.

14. Gabriel, U.U., O.A. Akinrotimi, D.O. Bekibele, D.N. Onunkwo and P.E. Anyanwu, 2007. Locally produced fish feed: Potentials for aquaculture development in Subsaharan Africa. Afr. J. Agric. Res., 2: 287-295.

15. Nyina-Wamwiza, L., B. Wathelet, J. Richir, X. Rollin and P. Kestemont, 2010. Partial or total replacement of fish meal by local agricultural by-products in diets of juvenile African catfish (*Clarias gariepinus*): Growth performance, feed efficiency and digestibility. Aquacult. Nutr., 16: 237-247.

16. Kasumyan, A.O., E.A. Marusov and S.S. Sidorov, 2003. Feeding behavior of the ruffe *Gymnocephalus cernuus* triggered by olfactory and gustatory stimulants. J. Ichthyol., 43: S247-S254.

17. Goddard, S., 2012. Feeds in Intensive Aquaculture Feed Management in Intensive Aquaculture. Springer, Boston, Massachusetts,.

18. Pitcher, T.J., 1992. Behaviour of Teleost Fishes. 2nd Edn, Springer, Netherlands, Europe, Pages: 708.

19. Conn, P.M., 2008. Sourcebook of Models for Biomedical Research. Humana Press, Totowa, New Jersey,.

20. Katunzi, E.F.B, 2014. Performance of locally formulated feeds for rearing of African catfish in Tanzania. Afr. Crop Sci. J., 22: 979-986.

21. Hecht, T., 2013. A Review of on-Farm Feed Management Practices for North African catfish (*Clarias gariepinus*) in Sub-Saharan Africa. In: On-Farm Feeding and Feed Management in Aquaculture, Hasan, M.R. and M.B. New (Eds.)., FAO, New York, USA., pp: 463-479.

22. Ronnestad, I., M. Yufera, B. Ueberschar, L. Ribeiro, O. Sale and C. Boglione, 2013. Feeding behaviour and digestive physiology in larval fish: Current knowledge and gaps and bottlenecks in research. Rev. Aquacult., 5: S59-S98.

23. Dmitrienko, A., C. Chuang-Stein and R.B. D'Agostino Sr, 2007. Pharmaceutical Statistics Using SAS: A Practical Guide. SAS Institute, Cary, North Carolina, Pages: 315.

24. Beavers, A.S., J.W. Lounsbury, J.K. Richards, S.W. Huck, G.J. Skolits and S.L. Esquivel, 2013. Practical considerations for using exploratory factor analysis in educational research. Pract. Assess. Res. Eval., Vol. 18,

25. Molnar, C. and J. Gair, 2015. Concepts of Biology-1st Canadian Edition. 1st Edn., OpenStax College, Houston, Texas.

26. Cowey, C.B., 1994. Amino acid requirements of fish: a critical appraisal of present values. Aquaculture, 124: 1-11.

27. Isea-Leon, F., V. Acosta-Balbas, L.B. Rial-Betancoutd, A.L. Medina-Gallardo and B.M. Celestin, 2019. Evaluation of the fatty acid composition of earthworm *Eisenia Andrei* meal as an alternative lipid source for fish feed. J. Food Nutr. Res., 7: 696-700.

28. Carr, W.E.S., J.C. Netherton, R.A. Gleeson and C.D. Derby, 1996. Stimulants of feeding behavior in fish: Analyses of tissues of diverse marine organisms. Biol. Bull., 190: 149-160.

29. Rawat, P., S.M.S. Siddhnath, R. Bharti, N. Verma and B. Chirwatkar, 2018. Ompok bimaculatus rearing potential with feed attractants used in aquaculture. Int. J. Pure App. Biosci., 6: 621-634.

30. Silva Neto, J.F., A.J.P. Nunes, H. Sabry Neto and M.V.C. Sa, 2012. Spirulina meal has acted as a strong feeding attractant for Litopenaeus vannamei at a very low dietary inclusion level Aquacult. Res., 43: 430-437.

31. Tesser, M. B. and M.C. Portella, 2011. Feeding stimulants for pacu lavae. Braz. J. Anim. Sci., 40: 1851-1855.

32. Hamid, S.N.I.N., M.F. Abdullah, Z. Zakaria, S.J.H.M. Yusof and R. Abdullah, 2016. Formulation of fish feed with optimum protein-bound lysine for African Catfish (*Clarias gariepinus*) fingerlings. Proc. Eng., 148: 361-369.

33. Dugenci, S.K., N. Arda and A. Candan, 2003. Some medicinal plants as immunostimulant for fish. J. Ethnopharmacol., 88: 99-106.

34. Apines-Amar, M.J.S., E.C. Amar, J.P. Faisan Jr., R.V. Pakingking Jr. and S. Satoh, 2012. Dietary onion and ginger enhance growth, hemato-immunological responses and disease resistance in brown-marbled grouper, *Epinephelus fuscoguttatus*. Aquacult. Aquarium Conserv. Legislation, 5: 231-239.

35. Hara, T.J., 2012. Fish Chemoreception. Springer, Netherlands, Pages: 324.

36. Gonzalez, C. and G.L. Allan, 2007. Preparing Farm-Made Fish Feed. NSW Department of Primary Industries, Australia, ISBN: 9780734718020, Pages: 21.

37. Farah, K., I.R. Gunawan, G.B. Putra, W.P. Lokapirnasari, M. Lamid, E.D. Masithah and T. Nurhajati, 2018. Effect of earthworm (*Lumbricus rubellus*) in feed formulation to improve fatty acids profile in Eel (*Anguilla bicolor*) meat. IOP Con. Ser. Earth Environ. Sci., Vol. 137,

38. Rate, F.O.S.G., 2019. Effectiveness of earthworm (lumbricusrubellus) substitution flour in feed formulation on specific growth rate, feed conversion ratio and feed efficiency of *Nile tilapia* (*Oreochromis niloticus*). Technology, 10: 1022-1029.