Open Access Library Journal
2021, Volume 8, e6436
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Growth Performance, Gonadal Weight and Fecundity: A Comparative Study of *Rastrineobola argetea* and Roasted Soybean Meal as Protein Ingredients for Brood Stock African Catfish (*Clarias gariepinus*) in Uganda

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How to cite this paper: Muyinda, R., Emejje, H.F., Zirintunda, G., Kasozi, K.I. and Mawadri, A.P. (2021) Growth Performance, Gonadal Weight and Fecundity: A Comparative Study of *Rastrineobola argetea* and Roasted Soybean Meal as Protein Ingredients for Brood Stock African Catfish (*Clarias gariepinus*) in Uganda. *Open Access Library Journal*, **8**: e6436.
https://doi.org/10.4236/oalib.1106436

Received: May 17, 2020
Accepted: January 26, 2021
Published: January 29, 2021

Abstract

Rastrineobola argetea (fishmeal) was compared to roasted soybean meal as protein ingredients for brood stock African cat fish (*Clarias gariepinus*) in March 2019 on Geossy farm, Uganda. The fish were observed for growth and reproductive indices. Brood stocks of average weight 327 g were fed on different feeds for a month. Feed A contained soybeans as the sole main protein source. Feed B contained fishmeal as the sole protein source. Feed C was the control (usual farm feeds). Growth indices of B were significantly higher than of A ($p = 0.0001$, $P < 0.05$). It is implausible to substitute fishmeal 100% by soybean meal even with heat treatment to remove ant nutritional factors. Fish meal has essential amino acids and fatty acids that are not in soybeans, soybeans can best be used in combination with fishmeal but the most economical ratios need more research. The reproductive indices had no significant differences for feed A, B and control. Replacing fish meal with soybeans had no significant effect on the reproductive indices. Fish meal increases the growth indices and the gonadal weight of *Clarias gariepinus*, soybean meal is good during brood stock management because it increases fecundity although lighter eggs are observed.

Subject Areas

Aquaculture, Fisheries & Fish Science

Keywords

Clarias gariepinus, *Rastineobola argetea*, Soybeans, Growth, Reproductive

1. Introduction

The African cat fish (*Clarias gariepinus*) farming in Uganda is getting popular in the scaling up of food security because they have better adaptability for apparently stressful conditions for other fish species (Abdel-Warith *et al.* 2019) [1]; Oni and Olaleye, 2013 [2]). Fish growth is dependent on factors like the type of feed and the water quality (Amisah *et al.* 2009 [3] ; Otubusin *et al.* 2009) [4]. Exposure to darkness is said to improve the growth of *Clarias gariepinus* especially in conjunction with a good nutrient balance (Almazán-Rueda *et al.* 2004 [5]; Appelbaum & Kamler, 2000 [6]). Growth of *Clarias gariepinus* is the best at low stocking densities (Hengsawat *et al.* 1997 [7]; Hossain *et al.* 1998 [8]). It is recommended that catfish brooders be fed a typical 28%- or 32%-protein feed once daily at a rate of about 0.5% - 1% fish body weight (Robinson and Li 2001) [9].

Protein is one of the most essential nutrients for fish growth and maintenance of physiological functions (Amoah, 2011) [10]. Farmers prefer animal protein sources because of their superior amino acid composition and digestibility (Hecht, 2013 [11]; Masette & Bamwirire, 2013 [12]; Ugwoke, 2013 [13]). Protein composition should range between 45% - 50% in which the optimal being 46.1% for catfish (Hien *et al.* 2018) [14]. It must consist of various amino acids required for normal body functions (Hien *et al.* 2018) [14]. Protein ingredients should contain more than 20 percent crude protein, while energy ingredients usually have less than 20 percent crude protein and less than 18 percent crude fiber (Gatlin, 2010) [15]. The quality of proteins used determines the survival, growth, reproduction, feed efficiency and chemical composition of catfish (Mohanta *et al.* 2013 [16]; Ugwoke, 2013 [13]). In immature fish, a well-balanced diet with high protein levels is essential during the laying down of germinal tissue containing future egg stock and egg development during breeding (Khan *et al.* 2003 [17]; Mwanja *et al.* 2015 [18]). The female fish also require adequate proteins for egg development, formation of follicles and other ovarian tissues and in general reproduction (James and Sampath, 2003) [19]. However, the expanding demand for fish meal (*Rastrineobola argetea*) for aquaculture, poultry and livestock feed production (Huntington and Hasan, 2012) [20] has made it unacceptably too expensive (Mohanta *et al.* 2013) [16]. Fish meal contains DM (88%), CP (60%), CF (1%), Ca (4.37%), P (2.53%), ME (2310 Kcal/kg), Lysine (4.08%) and Methionine (1.70%); soybean meal contains DM (88%), CP (43%), CF (6%), Ca (0.53%), P (0.64%), ME (2800 Kcal/kg), Lysine (2.84%) and Methionine (0.65%); maize bran contains DM (88%), CP (9.4%), CF (13%), Ca (0.04%), P (1.03%), ME (2200 Kcal/kg), Lysine (0.18%) and Methionine (0.21%); (UNBS, 2019) [21].

2. Problem Statement

The scarcity and cost of fish meal may lead to over 65% of the operating costs of aquaculture (Nwanna *et al.* 2014) [22]. This narrows the profit margin of fish farmers at the end of the culture period (Eyo *et al.* 2014) [23]. Overdependence on fishmeal makes commercial production of catfish capital intensive as it accounts for between 30% and 60% of variable operating costs (Amisah *et al.* 2009 [3]; Huynh & Portfolio, 2010 [24]). However plant protein source like soybean meal contains (43 - 47)% crude protein, (1.5 - 1.9)% crude fat (UNBS, 2019) [21]. Soybeans are good protein sources but have anti-nutrients (protease and trypsin inhibitor activity, lectins, phytates, glucosinolates, saponins, and tanins) which are harmful to fish (Bueno *et al.* 2018) [25] despite the low cost and availability. Soybeans have low histidine, lysine, methionine and threonine amino acids (Zarate & Lovell, 1997) [26]. There are many formulations of *Clarias gariepinus* feeds on the Uganda market (Hecht, 2013 [11]; Munguti, 2012 [27]) but studies haven't come up with reasonable economical formulas making farmers incur losses helplessly. There are many locally available materials that could be used to come up with efficient diets (Munguti, 2012) [27] but research to develop effective *Clarias gariepinus* feeds for the local market is still limited. There is need for innovations that enable successful use of plant ingredients like soybeans which are known to contain anti-nutrients. Heat treatment of the soybeans removes the anti-nutritional factors which are harmful (Anderson, 1992 [28]; Liener & Tomlinson, 1981 [29]; Peres *et al.* 2003 [30]).

The purpose of this study was to compare the growth performance and the reproductive performance of *Clarias gariepinus* fed on roasted soybean protein source to those that were fed on fish meal (*Rastrineobola argetea*) protein source. The parameters of growth performance were weight gain, specific growth rate and protein efficiency ratios. The parameters of reproductive performance were gonadal weight, gonadal somatic index and fecundity.

3. Materials and Methods

3.1. Study Area

The study was carried out in Asinge village off the Tororo-Malaba highway at the Aquaculture Geossy fish farm 633222.19˚E 71866.65˚N UTM 36N zone in Tororo district in the Eastern Uganda from April to May 2019.

3.2. Sample Preparation and Treatment

Mono sex female juvenile catfish used for the study were obtained from the National Fisheries Resources Research Institute Entebbe. A total of 30 fishes were acclimatized without food for 3 days prior to the commencement of the feeding trial at the farm. At the end of the 3 days of acclimation period, juvenile fish of mean weight 327 g were randomly distributed into groups of 10 in 3 circular cemented tanks of 4 cubic meter capacity. The experimental set up was supplied with water through a flow system and oxygen was augmented with a one horsepower aerator. Fecal wastes and feed remnants were removed daily by siphoning using a vacuum pump. Water changes were done during sampling days.

3.3. Diet Formulation and Preparation

The ingredients to be used were submitted to the college of Agriculture and Environmental Sciences, Makerere University for determination of crude protein content and it was found to be 43.2% for the soybean meal, 61.37% for *Rastrineobola argetea* (fish meal), 10.48% for the maize bran and 12.71% for the Geossy farm feeds (feed treatment **C**) which was the control. Ingredients were weighed following calculations of Pearson square method using a CP of 46.1%. The control feed was a farm formulated whose records stated that 100 kg contained 25 kg of wheat pollard, 35 kg of bread waste, 40 kg of *Rastrineobola argetea* and 100 g of mineral-vitamin premix.

Feed treatment **(A)** was composed 7.4 kg soybean (crude proteins-43.2%), 0.6 kg maize bran (crude proteins-10.48%) and 0.04 kg of mineral and vitamin premix to make an approximately 8 kg sample of 46.1% crude proteins. Feed treatment **(B)** was composed of 5.6 kg of *Rastrineobola argetea* (crude proteins-61.37%), 2.4 kg of maize bran (crude proteins-10.48%) and 0.04 kg of mineral and vitamin premix to make an approximately 8 kg sample of 46.1% crude proteins. They were uniformly mixed and warm water was added to make a dough. The material was pelleted manually using a meat-mincing machine and dried at 60˚C for 48 hours in a hot air oven. The pellets were ground into bits and stored under room temperature in labelled airtight containers.

Fish were fed a mass 5% their body weight twice a day (Ahmed *et al.* 2019) [31] (daily (08:00-09:00 am, 05:00-6:00 pm) throughout the week including weekends for a 30 day growth period. The standard body weight of all the fish under each of the dietary treatment was weighed fortnightly using a digital balance (0.1 g) (Ali & Jauncey, 2005) [32]. Fish were starved overnight prior to weighing. Adjustment of feed was done at the end of every weighing to feed 5% of the new body weight.

Weight Gain (WG), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Protein Intake (PI), gonadal weight and fecundity were calculated using methods described below by Ahmed *et al.* (2019) [31];

Equation 1;

$$\text{Weight gain}(\text{WG}) = \text{Final body weight}(g) - \text{Initial body weight}(g)$$

Equation 2;

$$\text{Specific growth rate}(\text{SGR}) = \frac{\text{In}(\text{Final body weight}) - \text{In}(\text{Initial body weight})}{\text{Time}(\text{days})} \times 100$$

Equation 3;

$$\text{Protein efficiency ratio}(\text{PER}) = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Amount of protein fed}}$$

Equation 4;

$$\text{Gonadosomatic index}(\text{GSI}) = \frac{\text{Weight of ovary}}{\text{Weight of fish}} \times 100$$

Equation 5

$$\text{Fecundity} = \frac{\text{Number of eggs in sub sample} \times \text{Gonad weight}}{\text{Weight of sub sample}}$$

3.4. Selection of Brood-Stock

The fish used was captured using a net from the pond. After selection, breeders were then put in separate concrete tanks filled with .well oxygenated and clean water. Only females were selected and the selection was done in the evening quickly and carefully to avoid oxygen deprivation and minimize injuries.

3.5. Brood-Stock Management

Brood stock management involved caring for the brood stock from the time the fish was captured and brought for breeding to the time of, stripping and return to the pond. Cleaning of the tanks, changing of the water and feeding the fish three times a day on 5% of their body weights for feed treatments A, B and C. Feeding was started 12 hours after the experimental setup to allow the fish rest. Feeding was done for four weeks.

3.6. Hormone Processing and Administration

Just prior to hormone administration on the 28th day of the experiment, the pituitary gland was extracted from seven donor catfish (Andrus *et al.* 1992) [33]. The extracted pituitary gland was then placed in a micro vial and sterile double stilled water added. After grinding it using micro-pestle and more distilled water was added in order to achieve concentration of 40 mg/ml of pituitary extract and mixed thoroughly. This was then followed by centrifuging the extract for about 1 minute to separate the tissue debris from the extract. A required amount of clear supernatant was then withdrawn into a syringe and the recipient fish injected via the intramuscular route in the anterior caudal region (Haniffa & Sridhar, 2002) [34]; the fish were injected depending on weight at a dosage rate of 0.5 ml/kg body weight (Abubakar and Ipinjolu, 2019) [35]. Each was weighed before hormone administration to obtain the final weight after treatment to help calculate the weight gain. After hormone administration, each fish was put in a separate well labelled plastic can tank containing water where they stayed overnight for the latency period.

3.7. Harvesting of Ripe Eggs by Stripping

After the latency period Procedures of FAO (1994) were done with minor modifications, the fish was ready for stripping as the following signs indicated readiness for stripping; a well-rounded and soft abdomen, eggs also showed clear nucleus at the center and the genital opening was swollen and for some reddish in colour. Each fish; one at a time was then placed in a bucket, disinfected and cleaned using a towel before transferring to the stripping bowl. The fish was held at the base of the tail with the left hand by one person and the head placed using the left hand by another person with the fish belly facing down. The fish head was then slightly elevated so that the eggs flow towards the vent by gravity. The fish was then caught with the right hand and the thumb put on one side of the belly and fingers on the other side just ahead of the pelvic fins. It was then gently squeezed as the hand was being slide back towards the vent. The milking process continued until the eggs stopped flowing and the eggs were weighed.

3.8. Determining the Fecundity

After stripping the eggs, the eggs from each fish was weighed to get the weight of the entire egg mass from one fish, a one (1) gram sample from each egg mass was then measured separately in plastic plates (Dadebo *et al.* 2011) [36]. The eggs from each sample were then mixed with potassium permanganate to separate the eggs from one another in order to facilitate easy counting which was done physically. The number of eggs stripped from each fish was then calculated by multiplying the weight of the egg mass (from each female) by the number of eggs present in sampled one gram of the respective egg masses from the different fishes.

3.9. Data Analysis

Primary data was determined from the fish, other variables were determined from the Equations (1-6) above and entered into a table (**Table 1**). Tukey's multiple comparison test on weight changes, growth rates, PER, gonadal weight and fecundity in fish exposed to the three feeds was done. Graph pad prism was used to make graphs to confirm the comparisons.

4. Results and Discussion

Data from MS-excel was used to generate data in **Table 1**.

4.1. Feed Effects on Growth Performance

The mean weight gain, specific growth rate and protein efficiency ratios were greater in group **B&C** and lowest in group **A** (**Figure 1**, graph 1-3). There was a significant difference in weight gains in fish on feed treatment **B** and **A** (**Table 2**) possibly because of the amino acid profile of feed **B**. There was a significant difference in weight gains in fish on feed treatment **C** (control) and **A** (**Table 2**) possibly because of the ingredients in wheat pollard and bread waste in **C**. Mean weight gain in group **B** was higher than in **A** (**Figure 1**, graph 1) because the diet with the highest protein leads to higher body weight gain (Degani *et al.* 1989) [37]. Higher weight gain in treatment **B** than in **A** could have been due to the better balance of amino acid profile and digestibility of the protein ingredient in *Rastrineobola argetea* and anti-nutritional remnants in soybean. This observation is in agreement with (Abdel-Warith *et al.* 2019 [1]; Amisah *et al.* 2009) [3] who reported that every time fishmeal was left out in diet formulation, it led to low weight gain. Soybean meal contains low levels of essential amino acids (methionine and lysine) resulting in poor growth and feed utilization in fish. (Yang *et al.* 2009) [38]. The findings agree with Goda *et al.* (2007) [39] about the possibility of better growth with partial replacement of fishmeal with soybean. However, the findings of the study disagree with Adewumi, (2006) [40] who proposed that heat treatment of a certain level could make soybean an absolute replacement of fish meal. Equivocally the findings disagree with Ajani *et al.* (2016) [41] who partially inferred that soybean can replace fish meal by 100% as protein sources and make *Clarias gariepinus* attain acceptable growth performance. This indicates that Soybeans can best be used in combination with *Rastrineobola argetea* (Goda *et al.* 2007) [39] and not as a sole protein source regardless of the methods of removing the anti-nutritional factors. Phytase treatment will only improve the digestibility of phosphorus (Weerd *et al.* 1999) [42] and this will also not make soybeans absolute fish meal replacers. Group **C** (control feeds) had the highest mean weight gain because it contained wheat pollard and bread waste and not maize bran as the experimental feeds. Bread waste is known to produce better growth performance compared to maize (Fakunmoju, 2014) [43].

Table 1. Growth and Reproductive indices of *Clarias gariepinus* on different feed treatments from Geossy fish farm, Tororo in March 2019.

Sample	Initial weight (g)	Final weight (g)	Gonadal weight (g)	No of eggs in 1 g sample	weight gain (g)	In (weight gain)	Specific growth rate	Protein efficiency ratio	Gonadal somatic index	Fecundity (Eggs)	Fecundity in 1000
A	447	456	18	30,000	9	2.20	7.86	0.193	3.947	540,000	540
A	371	378	14	29,120	7	1.95	6.96	0.150	3.704	407,680	407.68
A	364	372	15	31,200	8	2.08	7.43	0.172	4.032	468,000	468
A	326	342	31	64,480	16	2.77	9.89	0.343	9.064	1,998,880	1998.88
A	291	310	6	12,480	19	2.94	10.50	0.408	1.935	74,880	74.88
A	287	291	32	66,560	4	1.39	4.96	0.086	10.997	2,129,920	2129.92
A	285	288	22	45,760	3	1.10	3.93	0.064	7.639	1,006,720	1006.72
B	400	440	19	35,000	40	3.69	13.18	0.858	4.318	665,000	665
B	345	392	24	18,024	47	3.85	13.75	1.009	6.122	432,576	432.576
B	317	372	29	21,779	55	4.01	14.32	1.180	7.796	631,591	631.591
B	304	364	36	27,036	60	4.09	14.61	1.288	9.890	973,296	973.296
B	304	338	39	29,289	34	3.53	12.61	0.730	11.538	1,142,271	1142.27
B	270	320	6	4506	50	3.91	13.96	1.073	1.875	27,036	27.036
B	261	298	34	25,534	37	3.61	12.89	0.794	11.409	868,156	868.156
C	365	398	54	46,008	33	3.50	12.50	0.708	13.568	2,484,432	2484.43
C	366	391	40	34,080	25	3.22	11.50	0.536	10.230	1,363,200	1363.2
C	328	382	35	29,820	54	3.99	14.25	1.159	9.162	1,043,700	1043.7
C	325	377	33	28,116	52	3.95	14.11	1.116	8.753	927,828	927.828
C	308	372	23	19,596	64	4.16	14.86	1.373	6.183	450,708	450.708
C	301	351	24	20,448	50	3.91	13.96	1.073	6.838	490,752	490.752
C	299	338	40	34,080	39	3.66	13.07	0.837	11.834	1,363,200	1363.2

Table 2. Tukey's multiple comparisons test on weight changes, growth rate, protein efficiency, gonadal weight and fecundity in fish exposed to 3 feed types from Geossy farm in Asinge, Tororo in March 2019 (Tukey test; $p < 0.05$, df = 6).

Tukey's multiple comparisons test	Feed A vs. Feed B	Feed A vs. Feed C	Feed B vs. Feed C
Weight change	<0.0001	<0.0001	0.9864
Specific growth rate	<0.0001	<0.0001	0.9824
Protein efficiency ratio	<0.0001	<0.0001	0.9862
Gonadal weight	0.4443	0.0294	0.2826
Eggs per gram	0.0841	0.4129	0.5965
Gonadal somatic index	0.6113	0.1219	0.5128
Fecundity	0.7233	0.8141	0.3675

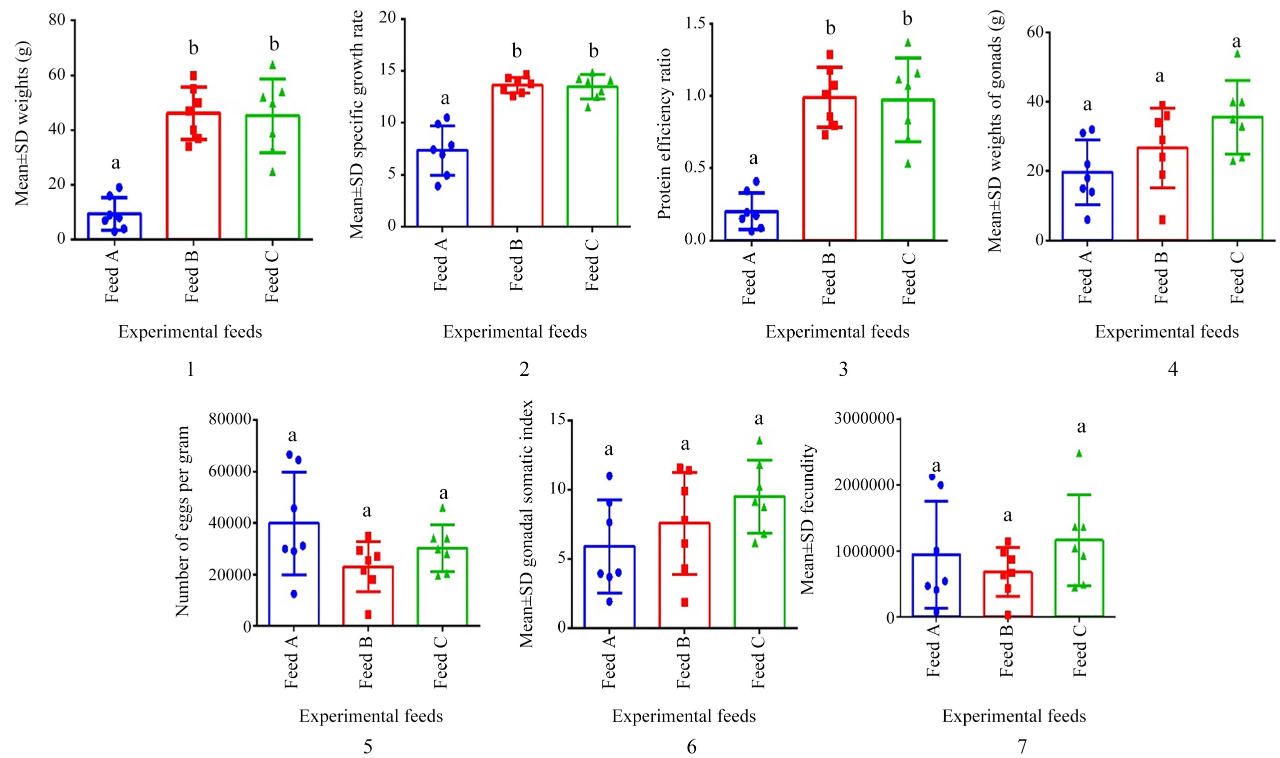

Figure 1. Shows the Feed effects on growth and reproductive performance indices. Graph (1) shows that feed **B** caused a significantly higher mean weight than feed **A** and feed **B** didn't cause a significantly higher mean weight than the control (feed **C**). Feed **B** and Feed **C** are all represented by "b" over the bars implying that they had similar effects on mean weight gain but Feed A is represented by letter "a" meaning that it is different from Feed B and C. Graph (2) shows that feed **B** caused a significantly higher specific growth rate than feed **A** and feed **B** didn't cause a significantly higher specific growth rate than the control (feed **C**). Feed **B** and **C** are all represented by "b" over the bars implying that they had no significant differences in effects on growth rate but Feed A is represented by "a" meaning that it had a different effect from that of Feed **B** and **C**. Graph (3) shows that feed **B** caused a significantly higher PER than feed **A** and feed **B** didn't cause significantly higher PER than the control (feed **C**). Feed **B** and **C** denoted by "b" over the bars caused a significantly higher PER than that of Feed **A** denoted by "a" over the bars. Graph (4) shows that there wasn't any significant differences in gonadal weights for feed **A**, **B** and the control (**C**); all denoted by "a" over the bars. Graph (5) shows that there wasn't any significant differences in number of eggs due to the feeds although the soybean protein source (feed **A**) caused more eggs; all denoted by "a" over the bars. Graph (6) shows that there wasn't any significant differences in the GSI due to the feeds **A**, **B**, **C**; all denoted by "a" over the bars. Graph (7) shows that there wasn't any significant differences in fecundity due to the feeds **A**, **B**, **C**; all denoted by "a" over the bars.

4.2. Feed Effects on Reproductive Performance

Gonadal weight was highest in Feed **C** (**Figure 1**, graph 4), and number of eggs per gram were highest in Feed **A** (**Figure 1**, graph 5) while gonadal somatic index and fecundity were the highest in Feed **C** (**Figure 1**, graph 6-7), however, no significant differences were found for all the reproductive indices between Feeds **A**, **B** and **C** (**Figure 1**, graph 4-6). Tukey's multiple comparison test found a significant difference in effects on gonadal weights caused by the control feed C and feed A ($P = 0.0294$, $P < 0.05$) but the results of the graph pad prism graphs were used (**Table 2**). However, there was only a very small difference between the mean gonadal weight in treatment **C** and that of treatment **B.** This is because increasing protein levels of the diet increase eggs size which is in agreement with (Quintero & Davis, 2016) [44]. The findings are in agreement with (Çek & Yilmaz, 2009) [45] who found that gonadal weight was increasing with the increasing energy in the feed. The studies in other geographical zones have stated that gonadal development is seasonal (Nyina-wamwiza *et al.* 2012 [46]; Romanova *et al.* 2018 [47]) but such studies haven't been done for Uganda; the seasonal effects on the reproductive indices are not known. The observed trend of gonadal weight is possibly the trend of n-3 fatty acids in fish as compared to soybean meal (Kubiriza *et al.* 2020 [48]; Mwanja *et al.* 2015 [18]). Egg size is associated with higher total number of n-3 fatty acids as these fatty acids play an important structural role as components of phospholipids in fish bio-membranes (Izquierdo, 2001) [49].

There was no significant difference in the reproductive indices for the feed treatments; replacing fish meal with soybean didn't affect mean gonadal weight, number of eggs, GSI and fecundity. The findings are in agreement with Nyina-wamwiza *et al.* (2012) [46] who found out that replacing fish meal didn't affect the GSI. The findings don't agree with (Effiong *et al.* 2014) [50] who found that GSI increases with increased proteins in the diet. However, the study agrees with Ahmad (2008) who found that GSI may be increased by the right balance between crude proteins and lipids as was the control feed. The low average fecundity in treatment **B** (677,132 eggs) is most likely to be associated with high levels of n-3 highly unsaturated fatty acids (HUFA), which was present in the fishmeal which was used as a protein source. Lipid and fatty acid composition of broodstock diet have been identified as major dietary factors that determine successful reproduction (Barua, 2011) [51]. The findings are in agreement with (Izquierdo, 2001) [49] who reported that high levels of dietary n-3 HUFA which is in fish reduce the total amount of eggs produced. Soybeans contain n-6 HUFA which increases the average fecundity as was observed in feed **A** (5,763,177 eggs) compared to treatment **B** (677,132 eggs) and the control **C** (1,160,546); the finding is in agreement with (Izquierdo, 2001) [47]. The findings are not in agreement with Ekanem *et al.* (2013) [52]; Eyo *et al.* (1992) [53] whose findings associated fecundity directly with weight gain and gonadal weight of fish. This was possible because feeds were a major variable in this study.

5. Conclusion and Recommendations

Feeding broodstock on fishmeal as a protein source increases in body weight and gonadal weight of *Clarias gariepinus* while soybean meal as a protein source increases fecundity. It's imperative to use soybeans during broodstock management but if it's to be used earlier then should be supplemented with lysine and methionine amino acids. However, research should be done on the hatchability and survivability of the fries from *Clarias gariepinus* brood that use soybean meal protein source because of the low egg weights that were observed. Replacing fish meal with soybean is a good economic strategy but the appropriate ratios for the various stages of *Clarias gariepinus* need further research.

Acknowledgements

The management of Geossy fish farm for allowing us to use your facilities to run our experiments.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

DM (Dry matter), **CP** (Crude Protein), **CF** (Crude fibre), **ME** (Metabolizable energy), **UNBS** (Uganda National Bureau of Standards), **FAO** (Food and Agriculture organization), **HUFA** (Highly unsaturated fatty acids)