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RESEARCH ARTICLE

SUBSTITUTING FISH MEAL WITH SPIRULINA (ARTHROSPIRA PLATENSIS) MEAL IN DIETS FOR JUVENILE CLARIAS GARIEPINUS

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Abstract

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This study was conducted to evaluate the effects of substituting fish meal with spirulina (*Arthrospiraplatensis*) meal into the diet of juvenilesclarias (*Clarias gariepinus*) in northern Senegal.This work was carried out on juveniles of 15 g raised in 09 aquariums with a volume of 24.3 L each.Three isonitrogenous diets (35% protein) containing different levels of spirulina meal 0%, 50%, 100% noted respectively A, B and C were prepared, each diet was randomly assigned to three groups of fish, fed to satiation, for 60 days.The results of water quality parameters of the different treatments showed that the average water temperature ranged from 26 to 27 °C, the average pH values measured in the different treatments ranged from 6.3 to 6.6.The best growth and feed efficiency performances were obtained with diets B, the one containing 50% spirulina meal, followed by diets C and A.The results obtained show that the specific growth rates (SGR) varied between 1.58%;1.64% and 1.8% respectively in fish fed with diets C, A and B. For the batches of fish receiving diets A, B and C, the feed conversion rate was between 1.01 and 1.38 kg. Regarding survival rates, statistical analyses show that diets B and C obtained the best TS comparedto diet A.The results of the study show that feeding A. platensis increased whole-body protein and ash contents of the fish, but decreased lipid and moisture contents.This study showed that the optimum rate of A. platensis in the fish practical diet is 50% replacement for fish meal in a fishmeal-based diet for of juvenile Clarias gariepinus without any adverse effects on fish growth and proximate composition of carcasses.This information is useful for the proper use of this vegetable protein source in the formulation of inert fish feed, which could reduce production costs and improve farm profitability.

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Introduction:-

Aquaculture is the fastest growing animal feed sector and has great potential to accelerate the growing trend of fish farming.The need for quality fingerlings and feed is the major constraint in this sector.Fish feed typically accounts for 60–70% of operating expenses in intensive farms, with protein sources accounting for 45% of the total feed cost (Singh et al., 2006). Due to its high nutritional value, fishmeal has long been a classic ingredient used as a protein source in fish feed.However, its cost is very high.Therefore, this high cost often translates into higher manufacturing costs, lower profit margins, and higher prices for the products that consumers must purchase (Olsen and Hasan, 2012).

Furthermore, the availability of fishmeal is decreasing day by day and the situation is getting worse when the competition with other industries for fishmeal increases. Fishmeal stocks have been decreasing due to overfishing, rising prices, decreasing natural fish stocks and market volatility (Mamat et al., 2017). In order to limit the use of this raw material from fisheries, the potential of using different plant protein sources has been the subject of many studies (Medale and Kaushik, 2008; Gan et al., 2017). However, the increasing demand, high cost and unstable supply of fishmeal have driven the research towards alternative protein sources including locally available and low-cost plant proteins.

Nowadays, various studies are moving towards the use of proteins of plant (Liu et., al., 2017; LY et al., 2021) and animal origin (Yigit et al., 2006; Robinson and Li. 1999; Fisher et al., 2020) to compensate for the high cost of industrial fishmeal feed.In addition, other cheap protein sources have been investigated in recent years in various research studies to replace fishmeal in Clarias diets.These include Fermented soy pulp roasted soybean meal (Kari, 2021);Black seed (Kotb et al., 2018);sesame flour (Pouomogne et al., 1997; Falaye and Jauncey 1999) ;cottonseed meal (Mbahinzireki et al., 2001) ;moringa leaf flour (Hedji et al., 2014) ;winged bean flour (Psophocarpus tetragonolobus) meal after heat-processed treatment (Fagbenro, 1999);and rocket seed proteins (Eruca sativa Miller) (Fagbenro, 2004).

Clarias gariepinus, a catfish of the family Clariidae of the order Siluriformes, is considered one of the most important tropical catfish species for aquaculture.Its distribution is almost pan-African, from the Nile to West Africa and from Algeria to Southern Africa.The demand and price of this fish are high due to its high protein content and delicious flesh (Talwar and Jhingran, 1991).Farmers are interested in farming this species, but they face problems related to the unavailability of suitable feeds.In this context, for the production of feeds for Clarias gariepinus, A. platensis meal can serve as an alternative protein source to fish meal.Spirulina (Arthrospira platensis) is a good and inexpensive source of protein.It also possesses immune-stimulating capabilities, which may reduce the cost and risk associated with drug use in aquaculture (Eissa et al. 2024; Amer, 2016; Sathasivam et al., 2017).

Due to its nutritional characteristics, Spirulina can enable aquaculture organisms to be resistant to environmental stressors and diseases (Ravi et al., 2010).As a dietary supplement, it has been shown to exert positive effects on growth, reproduction, carcass composition, immune responses, and disease resistance in various fish species, including rainbow trout (Oncorhynchus mykiss) (Teimouri et al., 2013), common carp (Cyprinuscarpio) (Watanuki et al., 2006), and Nile tilapia (O. niloticus) (Al-Deriny et al., 2020).Spirulina therefore appears to be an ideal dietary supplement for fish to improve their growth and immunity.Therefore, the aim of this study is to replace fish meal with A.platensis in the diet of Clarias gariepinus by examining the effects on growth performance and feed utilization.

Materials and Methods:-

An experiment was conducted at the agricultural farm of the Gaston Berger University (UGB) of Saint-Louis which is located in the university city, in Sanar, 16°13' N, 16°18' W, in the rural area of Gandon, Department of Saint-Louis, Region of Saint-Louis.The experiment studied the effect of spirulina (Arthrospira platensis) as a substitute for fish meal in the diet of Clarias gariepinus juveniles on growth performance.The experimentwas lasted for 60 days.

Experimental diets

Spirulina (A. platensis) was obtained from the Ndiafate agronomic farm in the Kaolackregion.The dried leaves were ground and then sieved into fine particles and stored until feed preparation.Before feed formulation, the proximal composition of A. platensis was analyzed and the results were as follows (on a dry matter basis): protein (60.2%), fat (8.9%) and ash (10.1%).The experimental dietwasformulated to be isonitrogenous and contain 35% crude protein, with varying amounts of *A. platensis* replacing fish meal.To obtain a paste, all ingredients were thoroughly mixed with just enough water.The paste was then manually processed using a granulator to create pellets.The pellets were then refrigerated for later use after being sun-dried and placed in plastic storage bags.Fish meal, peanut cake, corn flour and cassava flour were purchased from the Saint-Louis market.Diet A, serving as a control, contained 0% spirulina leaf flour and was compared with diets containing 50% and 100% spirulina leaf flour.The other flours (fish meal, cornmeal, peanut cake flour, and cassava flour) were adjusted to maintain the protein level.Soybean oil, vitamin and mineral blends, and binders were kept the same across diets as shown in Table 1, Figure 1.

Figure 1:-Ingredients used in the formulation.

All flours were finely ground and sieved using a 125 µm mesh sieve.The vitamin and mineral blends and the binder were mixed separately before being added to the main mix.The preparation consisted of manually mixing the quantities of the different raw materials chosen.The ingredients were first mixed in small quantities to obtain a premix to which the ingredients were then added in relatively large quantities in order to have a very homogeneous feed mixture.The semi-moist mixture is poured and then pressed in a meat grinder, giving spaghetti-shaped filaments of 2 mm in diameter.These filaments are dried in the shade, fragmented to the desired size, bagged and stored until distribution.The pellets are sieved before feeding to remove small particles.

Tableau 1:- Formulation des régimes alimentaires testés du Tilapia du Nil (Oreochromis niloticus).

Ingrédients	Régimes		
	A	B	C
Fish meal	39	20	0
Farine de spiruline	0	19	39
Cornmeal	25	25	25
Cassava flour meal	15	15	15
Peanut cake flour	15	15	15
Binder	2	2	2
CMV[1]	2	2	2
Soybean oil	2	2	2

[1]Premix composition (mg/g of mixture): FeSO4•6H_2O, 2.125 mg;$MgSO_4$, 137 mg;KCl, 75 mg;NaCl, 43.5 mg;NaH2PO4, 87.2 mg;KI, 0.15 mg;$AlCl_3$•6H_2O, 0.15 mg;$CuCl_2$•2H_2O, 0.1 mg;$ZnSO_4$•7H_2O, 0.80 mg;$CoCl_2$•6H_2O, 1 mg.Thiamine hydrochloride, 5 mg;riboflavin, 5 mg;calcium pantothenate, 10 mg;nicotinic acid, 6.05 mg;biotin, 0.003 mg; pyridoxine hydrochloride, 0.825 mg;inositol, 10 mg;folic acid, 0.041 mg;L-ascorby1-2-monophosphate-Mg, 2.025 mg;menadione, 4 mg;choline chloride, 44 mg.All ingredients were diluted with alpha-cellulose to 1 g

Experimental fish

Clarias juveniles (Clarias gariepinus), were used in this study with mean average initial weight of 15 g. The juveniles were produced at the Gaston BERGER University of Saint – Louis farm. Juvenilesfish were acclimated to laboratory conditions for one week before the experiment study. During this period, they were fed a commercial food.

Experimental unit
Fish were stocked in 09 aquariums with a volume of 24.3 L eachand were randomly divided into three equal experimental groups (20 fish each aquarium, three replicate/treatment). Each aquarium was supplied with air blowers. The tank water was exchanged with fresh water 3time/week. Tanks were washed and changed withfresh water every two weeksin order to reduce the bio-film and eliminate certain accumulated waste.

The fish were subjected to the same photoperiod and all the aquariums had identical lighting conditions.

Experimental design
At the start of the experiment, the fish were weighed and counted, then deprived of food for 24 hours. Each experimental diet was randomly assigned to 3 groups of 20 fish (mean weight: 15±0.02 g) per aquarium. Five fish were randomly sampled fromeach experimental group. Fish were used forchemical analysis of the whole body. Each experimental unit was constantly aerated and its water level kept constant. Each diet was fed to visual satiation, 2 times per day at 10:00 and 16:00 for 8 weeks. They were fed at 6% of their biomass and the daily rations were readjusted after each control fishing.

Water temperature and pH were measured using a multifunction pH meter every day and their respective averages were 6.5 mg/L and 27°C. The fish in each aquarium were weighed every fifteen (15) days, the fish were deprived of food 12 hours before and 12 hours after the weighing operations in order to minimize the stress linked to handling. The aquariums were cleaned and all water renewed during control fishing.

Growth performance parameters
To estimate the growth of fish during this study and characterize the efficiency of use of the foods tested, the following different parameters and zootechnical indices were calculated

Survival rate (SR)
The survival rate is calculated from the total number of fish at the end of the experiment and the number at the beginning of rearing, according to the relationship 1:

$$\mathrm{SR}\ (\%) = \frac{\mathrm{Nt}}{\mathrm{N0}} \times 100 \qquad (1)$$

Nt : Total number of fish survived in aquarium at the end of experiment
N0 : Total number of fish in aquarium at the beginning of experiment

Absolute average weight gain
The absolute average weight gain is used to assess the weight gain of fish in farming.It is determined from the relationship below:

$$\mathrm{WGA(g)} = \mathrm{Afw} - \mathrm{Aiw} \qquad (2)$$

Specific Growth Rate (SGR)
This coefficient is used to assess the weight gained by the fish each day, as a percentage of its live weight

$$\mathrm{SGR}\ (\mathrm{g\%j^{-1}}) = \frac{[\ln \mathrm{wt1} - \ln \mathrm{wt0}]}{\mathrm{t}} \times 100 \qquad (3)$$

Ln : normal log ; wt 0 :initial weight (g) ; wt 1 : final weight (g) ; t : number of days

Feed conversion ratio (FCR)
This coefficient commonly used to characterize the efficiency of feed utilization is a ratio between feed ingested and body mass gain.

$$\mathrm{FCR} = \frac{\mathrm{FC}}{\mathrm{Wg}} \qquad (4)$$

FC : being the quantity of food ingested in grams andWg :weight gain in grams

Proximate composition of experimental fish

The proximal composition (moisture, crude protein, crude lipid, ash) of the tested ingredients (Arthrospira platensis meal) and fish was determined on a dry matter basis using the A O A C method (1990). An initial analysis of the carcasses was carried out at the start and at the end of the experiment for each treatment.

Statistical Analysis

Results are presented as mean ± SEM.Results are statistically compared by one-way analysis of variance (ANOVA) to test the effect of 3 dietary formulas after prior verification of homogeneity of variances and normality of the data to be analyzed.Treatment effects were considered significant at a 5% level ($P < 0.05$).Tukey and Duncan tests were used for multiple comparisons of means when effects were significant.All statistical analyses were performed using SAS/PC statistical software (SAS Institute Inc).

Resultats:-

The results of water quality parameters of the different treatments studied in this study are summarized in Table 2. During the experiment, the average water temperature ranged from 26 to 27 °C, the average pH values measured in the different treatments ranged from 6.3 to 6.6.Statistical analyses revealed that temperature and pH have no significant difference ($p > 0.05$) with increasing inclusion of spirulina in the diet of Clarias gariepinus.Nevertheless, the temperature and pH values obtained are within the optimal range for the growth and survival of Clarias gariepinus.

Table 2:- Water quality parameters (Mean ± SD).

	Treatment		
	A	B	C
Temperatures mean (°C)	26,97±0,24	27,03±0,27	27,03±0,13
pH means	6,5±0,13	6,65±0,1	6,39±0,04

The results of the zootechnical parameters in Clarias gariepinus during the experiment are reported in Table 3. The best growth and feed efficiency performances were obtained with diets B and the one containing 50% spirulina meal, followed by diets C and A.The results obtained show that the specific growth rates (SGR) varied between 1.58%;1.64% and 1.8% respectively in fish fed with diets C, A and B.No significant difference between the diets was observed ($P > 0.05$).The weight gain (WG) varied from 11.5 to 14.47.For the batches of fish receiving diets A, B and C, the feed conversion index (FC) was between 1.01 and 1.38 kg of dry feed per kg of fresh weight produced.Fish groups fed with diets A and B obtained the best TCA compared to diet C ($P < 0.05$).The difference between TCA of diets A and B is not significant ($P > 0.05$).Regarding survival rates, statistical analyses show that diets B and C obtained the best TS compared to diet A ($P < 0.05$).The difference between TS of diets B and C is not significant ($P > 0.05$).

Tableau 3:-Growth performances of Clarias gariepinus juveniles fed diet with differentslevels of spirulina.

Items	Treatment		
	A	B	C
Initial weight (g)	14.3 ± 0,25	15.4 ± 0,13	15.6 ± 0,37
Weight gain (g)	11,5 ±0,09	14,47 ±0,12	12,42 ±0,09
Specific growth rate (g.%/j)	1,64 ± 0,09	1,8 ± 0,12	1,58 ± 0,09
Food conversion ratio	1,01 ± 0,06a	1,08 ± 0,09a	1,38 ± 0,31b
Survival rate	33.3 ± 0,02a	77.3 ± 0,02b	88.8 ± 0,02b

Means±SD in the same letters in the row is not significantly different at $P<0.05$.

The effect of substituting fishmeal with spiruline leaf meal on fish survival as shown in Table 3 shows that the survival rate was affected throughout the experimental period regardless of the feeding regime.

Tableau 4:- Proximate composition of experimental fish before and after experiment.

(g kg^{-1} wet weight) of the whole bodyofclarias fed diets with different level of spirulina (Arthrospira platensis) meal				
	Initial	A	B	C
Protein	30,21	46,31	47,28	48,02

Lipides	11,12	18,12	17,27	16,67
Ash	3,82	12,36	13,22	14,33
Dry matters	80,19	90	86	83

The results of the study show that feeding A. platensis increased whole-body protein and ash contents of the fish, but decreased lipid and moisture contents.

Discussion:-

The recorded temperature and pH values obtained are within the optimal growth and survival range of Clarias gariepinus.Temperature and pH were therefore not a limiting factor for the expression of the growth potential of C. gariepinus during the test.Spirulina belongs to the group of cyanobacteria and it is these bacteria that carry out photosynthesis.At the end of the test, the survival rate obtained is low and varies between 33.3% and 88.8%.The mortalities recorded during the test are partly linked to the stress of handling during control fishing.The low survival rates of treatments A and B can be explained by the cannibalism observed in the clarias of these batches after one month of testing.Indeed, the species Clarias gariepinus is predisposed to cannibalism, most often due to growth heterogeneity(Martins et al., 2005).Indeed, a hierarchy is most often observed in clarias, the largest fish become socially dominant and ensure their access to food while the small ones are subordinate and have difficulty accessing resources (Martinset al., 2005).

In the same species in basins and ponds.Similar results were observed in a study on a trial of adaptation of mass production of juveniles of Clariasgariepinus in rural conditions.However, juveniles fed with C, a feed containing 100% spirulina flour replacing fishmeal, obtained the best survival rate of 88.8% and which could indicate that spirulina leaf flour could have had a positive effect on juvenile survival.Algae have shown beneficial effects, positively modulated immune parameters and improved resistance to pathogens in various fish species.Microalgae including spirulina (Arthrospira platensis) improved survival and immune responses of European sea bass.

The best growth and feed efficiency performances were obtained with diet B, the one containing 50% spirulina flour, followed by diets C and A.This increase of up to 50% could be attributed to spirulina due to its high nutritional value in terms of protein content, minerals, growth performances.However, the decrease in growth observed in the batch of fish fed with diet C,can be explained by the presence of some antinutritional factors that can reduce growth in C. gariepinus.According to many authors, it has been shown that heat treatment improves the dietary utilization of plant proteins by modifying their structure (Alonzo et al., 2000; Vodouhe et al., 2012).Similar results, performed on other species, have shown that growth performance is poor when fish are fed vegetable protein at higher inclusion level due to the effect of an antinutritional factor Falaye et al. 1998.

These results are consistent with those of Teimouri et al.(2013), who noted that rainbow trout fed 5% S. platensis had significantly lower growth performance than those fed 7.5% and 10% S. platensis, and in particular that the diet containing 2.5–10% S. platensis increased the percentage of weight gain from 113.1 ± 4.8% to 131.4 ± 7.7%.Furthermore, Akter et al.(2021) observed that replacing fishmeal with S. platensis in the diet of Ompokpabda resulted in the best growth performance at a level of 15% compared to the control.S. platensis contains high-quality proteins and bioactive compounds that play a critical role in improving growth (Da Silva et al.,

The feed conversion ratio (FCR) ranged from 1.01 to 1.38.Fish groups fed diets A and B had the best FCR compared to diet C ($P < 0.05$).These results are consistent with those obtained by Dawah et al.(2002) and Jha et al.(2009).They found that the FCR was better when fish were maintained on artificial diets containing 10% and 20% dried seaweed.Similarly, Badwy et al.(2008) showed that incorporation of 50% seaweed replacement resulted in significantly higher FCR (2.03 ± 0.08 and 1.76 ± 0.05), respectively.

Duncan and Klesius (1996)reported that Spirulina alga was a good source of protein for animal feed, being containing high amounts of vitamins and minerals, in addition, Nakono et al. (2003) recorded that the lack of cellulose from the cellular structure of Spirulina render it easily digestible, thus, increase fish appetite, improve feed intake and nutrient digestibility and in turn enhance the health of fish, increasing the ability to fight off infections through the reduction of stress levels

According to James et al.(2006), S. platensis also improved the gut flora of fish, by breaking down indigestible feed components to extract more nutrients and promoting the synthesis of enzymes that transport lipids for metabolism rather than storage.The higher feed utilization pattern in the present study is also justified by this claim.Previous research suggests that the high content of vitamins, minerals, essential amino acids, linoleic acid, and linolenic acid in S. platensis in the diet improves growth performance and feed utilization (Cao et al., 2018a, Cao et al., 2018b, Roohani et al., 2018).

The results of the study show that feeding A. platensis increased the whole-body protein and ash contents of fish, but decreased the lipid and moisture contents.This finding aligns with the finding of Mohammadiazarm et al., 2021, who also documented an increase in protease levels in fish fed with **S. platensis**, suggesting its role in improving protein utilization. Moreover, the current results revealed a decrease in lipid content in the groups fed with S. platensis-supplemented diets. This is in congruence with Mohammadiazarm et al., (2021) who attributed that lipid decrease to the polyphenol content of the algae like β-carotene or phycocyanin, which are known as fat reducers (Kim et al., 2013, Hassaan et al., 2021). According to Abdel-Tawwab et al., 2008; these changes could be related to changes in their synthesis, muscle deposition rate, and/or different growth rate.These results are in agreement with those of (Almulhim et al., 2023; Cao et al., 2018b, Cao et al., 2018a, Kim et al., 2013, Roohani et al., 2018, Teimouri et al., 2016, Velasquez et al., 2016).The reduction in lipid content with increasing A. platensis levels was confirmed by Balasundram et al.(2006), who reported that S. platensis contains polyphenolic compounds that act as fat reducers and antioxidants.Similarly, Mamun et al., 2023, in their study, fat content was significantly reduced with increasing levels of S. platensis in the diet of M. cavasius.

Spirulina is the most widely used microalgae in aquatic animal feed due to its richness in proteins, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Asghari et al., 2016; Almulhim et al., 2024).It can also be used to improve the color, flavor and quality of meat (Al-Badri, 2010).

Conclusion:-

This study determined the effect of A. platensison the diet of juvenile Clarias gariepinusin northern Senegal.This study showed that the optimum rate of A. platensis in the fish practical diet is 50% replacement for fish meal in a fishmeal- based diet for of juvenile Clarias gariepinus without any adverse effects on fish growth and proximate composition of carcasses.The use of A.platensis in the diet can thus reduce the amount ofincorporated fishmeal, which presently is the mainprotein source for the culture of most fish species

This information is useful for the appropriate use of this plant protein source in the formulation of inert fish feeds.These feeds will have the advantage of being locally available, relatively cheaper and accessible to fish farmers, unlike industrial commercial feeds.

Contribution of the authors:

MA LY, F PREIRA, B SANE and M GUEYEparticipated in the design of the theme, the collection and organization of the scientific information provided. F PREIRA and MA SENECollected the data, MA LY Performed the data analysis and wrote the paper. MA LY, CT BA participated in the reading and correction of the article.

Conflict of Interest Declaration:

The authors declare that there are no conflicts of interest.

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