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Inclusion of *Sargassum muticum* and *Parkia biglobosa* in diets for African Catfish (*Clarias gariepinus*) elevates feed utilization, growth and immune parameters

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The use of antimicrobial agents and antibiotics as remedial measures against fish diseases has been questioned. The huge amounts of antibiotics used in animal husbandry has exerted a very strong selection pressure on the resistance among bacteria, which have adapted to this situation, mainly by a horizontal and philandering flow of resistance genes. Prebiotics, probiotics and symbiotics have been proposed as strategic means to enhance feed utilization, growth and immunity in fish production. In the present trial, a total of 180 fish was divided into 4 groups of 3 replicates each. Each replicate contained 15 fish. The formulated diets were supplemented with prebiotic (*Sargassum muticum*), probiotic (*Parkia biglobosa*) and symbiotics (a combination of *Parkia biglobosa* and *Sargassum muticum*). Efficiency of the inclusion of prebiotics, probiotics and symbiotics in formulated diets was evaluated in African catfish, *Clarias gariepinus* fingerlings (mean weight 2.53±0.05g). Formulated diet was fed 5% body weight to a group of 15 fish (in 3 replica) for 12 weeks, compared to fish fed control pellet containing similar ingredients but was not supplemented. Results showed on the skin of fish fed probiotics diet recorded improved GST and SOD activity and less CAT activity whereas in the liver fish fed prebiotic and symbiotic diet showed improved GST and CAT activity relative to the control. There were significant ($p<0.05$) differences between fish fed the control diet and all treatments (prebiotic, probiotic, symbiotics). It may be concluded based on the results recorded in this study that prebiotics, probiotics and symbiotics supplementation in diets has positive effect on antioxidant enzyme activity in African catfish, *Clarias gariepinus* to improve resistance against bacterial infections.

Key words: African catfish, antioxidant enzyme, feed utilization, growth, diets.

INTRODUCTION

African catfish is useful in diet of most populace, with optimum nutritional value with complete wide range of

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amino acids, vitamins and minerals (Akinrotimi et al., 2007), and it's a vital source of animal protein for both man and livestock in third world countries. Report from FAO (2014) shows that African catfish contributes above 60% of the world's supply of protein, especially in third world countries.

Though aquaculture is the fastest growing food sector, diseases most especially bacterial infection tends to hold back the expansion of aquaculture (Abd El-rhman et al., 2009; Pieters et al., 2008). Bacterial, viral and other types of diseases are usually treated with antimicrobials. Prevention and control of diseases have led to a substantial increase in the use of veterinary medicines in the recent years. However, the utility of antimicrobial agents and antibiotics as a remedial measure has been questioned. These huge amounts of antibiotics have exerted a very strong selection pressure on the resistance among bacteria which have adapted to this situation, mainly by a horizontal and philandering flow of resistance genes (Cabello et al., 2013; Yousefian and Amiri, 2009; Cabello, 2006). This is transmitted to the consumer and can cause allergy and diseases, cause an imbalance in the intestinal mucosa due to elimination of useful microbes in the gastro intestinal tract (Rombout et al., 2010).

WHO and EU regulations proscribing the use of antimicrobials as growth promoters in animal husbandry has led various researchers to seek alternative methods. Several researches have proposed alternatives methods which include Phytobiotics, Antimicrobial peptides (AMP), Inhibitors for bacterial Quorum sensing (QS), Feed enzymes, immunomodulatory agents, Bacteriophages and their Lysins, Biofilm and virulence, Antibacterial vaccines, Prebiotics, Probiotics and Symbiotics (Antache et al., 2013; Panigrahi and Azad, 2007).

Thus, the use of measures like prebiotics (*S. muticum*), probiotics (*P. biglobosa*) and symbiotics (a combination of *P. biglobosa* and *S. muticum*) has been of great value as alternative therapy in aquaculture, which appears to be strategic biological control and a necessary step for aquaculture practices for enhancing growth and disease resistance (Rombout et al., 2010). Widanarni and Tanbiyaskur (2015) and Fuller (1989) defined probiotic as live microbial feed supplements which are of benefits to the host by improving its intestinal microbial balance. Probiotica also strengthen the host immune system, improve the host's living environment and enhance nutritional value of the feed. Supplementation of probiotic has become successful based on the foundation of other concepts like prebiotic and symbiotic. Prebiotics are non-digestible food ingredients which selectively stimulates the growth and the physiology of health-promoting bacteria in the intestinal tract, thus improving an organism's gastrointestinal balance (Widanarni and Tanbiyas, 2015; Gibson and Roberfroid, 1995), whereas symbiotics are a combination of prebiotics and probiotics in a form of synergy.

Therefore, the objective of this trial is to mitigate these challenges by improving the aquaculture systems, the diet as well as the immunity state of the fish in order to arrest issues that may lead to their mortalities.

MATERIALS AND METHODS

Experimental diets

In the formulation used, fishmeal served as principal protein source whereas cornstarch was the energy source for all diets. Tapioca powder was used as binder. Other ingredients include vegetable oil as lipid source, mineral premix and vitamin C as sources of minerals and vitamins, respectively. After preparing the ingredients, it was weighed (OHAUS) and mixed in appropriate proportions to give the desired protein level required by the fish. Four experimental feeds were formulated at varying percentage inclusion of *S. muticum* (prebiotic 0.5%), *P. biglobosa* (probiotic 2%), symbiotic (prebiotic and probiotic 2.5%) and control with no inclusion.

The adopted feed formulation calculated for the trial showed that, for each 100g of feed contained approximately 46% cornstarch, 40% fishmeal, 7% vegetable oil, 4% mineral premix, 1% vitamin C and 2% tapioca(binder).

Feeding, fish rearing conditions

The experimental tanks used for the research were twelve (12) 20L capacity plastic aquaria filled with water. The fingerlings of *C. gariepinus* were purchased at a Commercial fish farm (Mallam Farms) in Niger State; a total of 180 fingerlings was purchased and stocked at the rate of 15 fingerlings per tank using 20 L capacity plastic aquaria. The aquaria were kept in a complete randomized design (CRD), fish were acclimated to the experimental facility conditions and fed with control feed for one week. The fingerlings were fed at 5% body weight twice daily at 7.00 am and 5.00 pm except on sampling days. Feeding trial lasted for 12 weeks.

Feed efficiency and growth parameters

Feed efficiency and growth parameters were calculated by applying the appropriate formulae where necessary, from the following:

Feed efficiency

Feed intake (FI) = total feed intake/number of fish
Feed conversion ratio (FCR) = total feed intake (g)/total wet weight gain (g)
Protein intake (PI) = feed intake (g) x percent protein in diet
Protein efficiency ratio (PER) = total wet weight gain (g)/ total feed intake (g)

Growth parameters

$$\text{Weight gain (WG)} = (W_f - W_i)/W_i$$

$$\text{Specific growth rate (SGR \%)} = [(\ln W_f - \ln W_i)/T] \times 100$$

Table 1. Growth parameters and feed efficiency of *C. gariepinus* (African catfish) fed formulated diets.

Treatment	W_i (g)	W_f (g)	WG (g)	SGR	FI	FCR	PI	PER
Control	2.54±0.05^{a}	17.31±0.02^{b}	14.77±0.01^{b}	0.16±0.01^{ab}	14.46±0.005^{b}	2.03±0.008^{a}	429.35±0.67^{b}	0.01±0.00^{a}
Prebiotics	2.27±0.05^{a}	15.54±0.05^{a}	13.26±0.01^{a}	0.16±0.00^{a}	13.33±0.01^{a}	1.94±0.01^{a}	371.29±0.84^{a}	0.02±0.00^{a}
Probiotics	2.52±0.03^{a}	18.14±0.01^{c}	15.60±0.01^{c}	0.16±0.01^{ab}	19.84±0.02^{d}	2.95±0.005^{b}	607.84±0.60^{d}	0.01±0.00^{a}
Symbiotics	2.31±0.06^{a}	16.88±0.01^{b}	14.55±0.03^{b}	0.17±0.01^{b}	17.95±0.00^{c}	3.17±0.05^{b}	470.36±0.67^{c}	0.05±0.00^{a}

Values were expressed as mean ± SEM. Columns with different superscripts were significantly different ($P < 0.05$).

where, W_f refers to mean final weight, W_i refers to mean initial weight, T is the feeding trial period in day and ln is natural log base.

Immune parameters (antioxidant enzymes activities)

Antioxidant enzymes activities catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione s-transferase (GST) of fish were determined using UV-3100PC spectrophotometer according to modified methods of Beers and Sizer (1952), Misra and Fridovich (1972), Mauk et al. (1998) and Habig et al. (1977), respectively.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA). Results are presented as mean ± SEM of three replicate determinations. *P*-values of <0.05 were considered significant when compared by Turkey's test. All statistical analyses were carried out using statistical software package (SPSS v.21).

RESULTS

Results for feed efficiency (FI, FCR, PI and PER), growth parameters (WG and SGR) and antioxidant enzymes activities monitored during the trial (CAT, SOD, GPx and GST) on the skin and liver of fish are represented in Table 1 to 3, respectively.

Table 1 reveals the growth parameters and feed efficiency of *C. gariepinus* fed formulated fish diet supplemented with *S. muticum, P. biglobosa* and a combination of the two. There was no significant differences noted in the initial weight, final weight significantly ($p<0.05$) differed with probiotic having the highest and the lowest was recorded in fish fed prebiotic diet. WG significantly ($p<0.05$) differed in the supplemented diets relative to the control, with the highest observed in fish fed probiotic diet and lowest in fish fed prebiotic diet. SGR was similar in fish fed supplemented diets relative to the control. FI, FCR, PI were all significantly ($p<0.05$) different relative to the control, but PER was similar across the diets.

CAT activity on the skin of fish fed supplemented diet was observed to be low relative to the control. There were significant ($p<0.05$) differences in the activity of CAT on the skin of fish fed supplemented diets relative to control.

Catalase activity was noted more in the liver of fish fed prebiotic diet relative to fish fed control diet. Fish fed probiotic and symbiotic diet were similar but recorded more activity relative to the control. CAT activity significantly ($p<0.05$) differed in the liver of fish fed supplemented diets relative to control.

Activity of SOD was similar on the skin of fish fed prebiotic and probiotic diets, more activity was noted relative to the control. However, SOD activity on the skin of fish fed symbiotic diet was similar to the control. SOD activity significantly ($p<0.05$) differed on the skin of fish fed prebiotic and probiotic diets relative to fish fed control diet. No significant difference was noted in SOD activity in fish fed symbiotic diet relative to control.

Activity of superoxide dismutase was noted to be more in the liver of fish fed probiotic diet relative to the liver of fish fed control diet. Prebiotic and symbiotic which were similar recorded more activity relative to the control. There were significant ($p<0.05$) differences in the activity of SOD in the liver of fish fed supplemented diets relative to the liver of fish fed control diet.

GPx activity was noted more on the skin of fish fed supplemented diets relative to the control. There were significant ($p<0.05$) differences in activity of GPx on the skin of fish fed supplemented diets relative to fish fed control diet.

Glutathione peroxidase was similar in the liver of fish fed prebiotic and probiotic diets, more activity was noted relative to the control. However, fish fed symbiotic diet was observed to have less activity relative to control. There were significant ($p<0.05$) differences in activity of CAT in the liver of fish fed supplemented diets relative to fish fed control diet.

On the other hand, activity of GST on the skin of fish fed supplemented diets were similar but, recorded more activity relative to fish fed control diet. There were significant ($p<0.05$) differences in GST activity on the skin of fish fed supplemented diets relative to fish fed control diet.

Glutathione s-transferase in liver significantly ($p<0.05$) differed among treatments. Fish fed prebiotic and symbiotic diets recorded more enzyme activity relative to fish fed control diet. However, no significant difference noted in fish fed probiotic diet relative to the control.

Table 2. Activities of antioxidant enzymes on the skin of African catfish fed supplemented diets.

Treatment	Catalase (µ/l)	Superoxide dismutase (µ/l)	Glutathione peroxidase (µ/l)	Glutathione s-transferase (µmole/min/mg protein)
Control	11.19±0.67^{b}	2.27±0.16^{a}	564.00±5.67^{a}	0.14±0.00
Prebiotic	9.90±0.22^{ab}	3.11±0.20^{b}	761.00±6.32^{c}	0.17±0.02^{b}
Probiotic	7.78±0.36^{a}	3.13±0.19^{b}	837.94±7.65^{d}	0.19±0.01^{b}
Symbiotic	7.99±0.43^{a}	2.35±0.21^{a}	627.44±5.67^{b}	0.18±0.02^{b}

Values were expressed as mean ± SEM of 3 determinations. Columns with different superscripts were significantly different ($P < 0.05$).

Table 3. Activities of antioxidant enzymes in the liver of African catfish fed supplemented diets.

Treatment	Catalase (µ/l)	Superoxide dismutase (µ/l)	Glutathione peroxidase (µ/l)	Glutathione s-transferase (µmole/min/mg protein)
Control	11.55±0.55^{a}	1.56±0.22^{a}	807.10±7.89^{b}	0.18±0.02^{a}
Prebiotic	19.26±0.56^{b}	2.49±0.27^{b}	874.14±8.90^{c}	0.20±0.01^{b}
Probiotic	13.27±0.57^{a}	4.78±0.35^{c}	885.75±11.34^{c}	0.17±0.01^{a}
Symbiotic	15.36±0.61^{ab}	2.98±0.26^{b}	658.74±7.89^{a}	0.20±0.01^{b}

Values were expressed as mean ± SEM of 3 determinations. Columns with different superscripts were significantly different ($P < 0.05$).

DISCUSSION

Growth of an animal is described as a change in the animal, either through size (weight and length), tissues, internal chemical compositions or even reproductive abilities. Different factors are involved to achieve maximum growth potential. Dietary input with sufficient, high quality digestible nutrients and environmental input especially through oxygen and water are vital to drive the growth rate (Ahmad and Ibrahim, 2016; Bureau et al., 2000).

C. gariepinus has been described to be an omnivorous scavenger. Based on this, it should be expected to have the potential to efficiently utilize a wide range of feed ingredients of both plant and animal origin (Udo and Umorem, 2011; Clay, 1979). This contention was supported by the high numerical values of feed intake (FI) and protein intake (PI) in study of Udo and Umorem, (2011) which have shown catfish to consume more protein-rich diets.

The trial revealed no significant difference was noted in the initial weight of fish in this study. The final weight was significantly ($p<0.05$) different relative to the control, the significant different could be attributed to the inclusion of prebiotic, probiotic and symbiotic in the diets of the fish.

Weight gain significantly ($p<0.05$) differed, with the highest recorded in fish fed probiotic diet and lowest in fish fed prebiotic diet, however, fish fed symbiotic diet was similar to fish fed control. All the feeds performed well but probiotic diet performed better.

In this regards, the performance was an indication of positive contribution to growth. Orire and Muhammed, (2014) reported the best weight gain in fish fed diet supplemented with 100% *P. biglobosa* as protein source, the literature of Orire and Sadiku (2014) observed no significant differences in fish fed three levels of carbohydrate and protein. Nwanna et al. (2017) also reported fish fed with diets supplemented with probiotic had the better weight gain.

Specific growth rate in this trial differed insignificantly, in other words, they were similar. Specific growth rate expresses the growth over a certain period of time, and is the more popularly used formula (Strand et al., 2011). Oso et al. (2011) reported no significant differences in the specific growth rate of fish fed supplemented diets, insignificant differences was also reported in the literature of Orire and Sadiku (2014).

Feed intake significantly ($p<0.05$) differed in fish fed supplemented diets relative to the control. The highest feed intake was noted in probiotic diet and the lowest in prebiotic diet. The highest feed intake was recorded in the probiotic and symbiotic respectively. In this regards, the obtained result could be attributed to the inclusion of *P. biglobosa* in the diets which could lead to proliferation LAB which adhere to the gastrointestinal tract of *C. gariepinus* producing a wide range of relevant digestive enzymes (amylase, lipase and protease) which have the ability to denaturate the indigestible components in the diets, detoxify potentially harmful components of the diets and to produce a lot of essential vitamin B complex members particularly Biotin and vitamin B12, which

enhanced feed utilization and digestibility (Nwanna et al., 2017). The low feed intake in the prebiotic diet could be attributed to the low digestibility of *S. muticum* which could be as a result of high fibre (Orire and Abubakar, 2013).

Feed conversion ratio was significantly ($p<0.05$) different in fish fed supplemented diets relative to fish fed control. The literature of Orire and Sadiku, (2014) reported significant difference in fish, whereas Mahdavi et al. (2013) reported broilers fed with supplemented diet had lower feed conversion ratio but this might be possible because it's a different animal compared to the ones used in the current study and that of Orire and Sadiku (2014). However, this implies that as the fishes grow bigger the rate at which they convert feed to flesh decreases (Orire and Muhammed, 2014).

The trial revealed significant ($p<0.05$) differences in the fish fed the supplemented diets relative to the control. This could be attributed to the feed intake, the protein intake in decreasing order was probiotic, symbiotic, control and prebiotic which was similar with what was noted in the feed intake of the current study. The highest protein intake was observed in probiotic which suggests good growth performance in fish.

Protein efficiency ratio in this study noted symbiotic was significantly ($p<0.05$) different relative to the control but the prebiotic and probiotic were similar to the control. Protein efficiency ratio is an evaluation of the protein quality in the diet which contributes to growth of the fish rather than the overall diet (Kjorsvik et al., 2004). The highest protein efficiency ratio noted in symbiotic suggest fish fed the diet showed great feed efficiency which will equally translate to good growth performance in fish.

The repair enzymes that can regenerate some antioxidants are SOD, GPx, Glutathione Reductase (GR), CAT and the other metalloenzymes. CAT, SOD, and GPx constitute a mutually supportive team of defense against Reactive Oxygen Species (ROS). SOD lowers the steady-state level of oxygen, catalase and peroxidases do the same for peroxidase, hence making them the first line of antioxidant defense mechanism (Ighodaro and Akinloye, 2017; Lushchak, 2012; Wang et al., 2011). Glutathione S-transferases (GSTs), known as ligandins back in the days, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification (Allocati et al.,2009).

In the present trial, fish fed prebiotic, probiotic and symbiotic expressed elevated level of antioxidant enzymes activities relative to the fish fed control diet. The expressed high level could be attributed to probiotic production of proteins in feed containing prebiotic, probiotic and symbiotic inclusion which is a precursor in the production of organic enzymes. Increased level of antioxidant enzymes activities is a sign of improved free radical scavenging in both the liver and skin of the fish.

Oxidative stress is a cellular condition which occurs as a result of physiological imbalance between levels of antioxidants and that of oxidants (ROS or free radicals), in which the imbalance is in favour of free radicals. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them. As a result of this, vital cellular structures and functions are lost and ultimately resulting in various pathological conditions. It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including diabetes, cancer, Alzheimer's disease, strokes, aging process, heart attacks and atherosclerosis (Abd El-rhman et al., 2009; Giustarini et al., 2009).

Hence, the significant increase in CAT, SOD and GPx activity in the liver indicates the first line of immune defence system plays an important role in the total defence mechanism in biological system (Ighodaro and Akinloye, 2017; Lushchak, 2012) where as high level of SOD and GST were noted on the skin, GST detoxifies endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics and may also bind toxins and function as transport proteins (Oakley, 2011; Josephy, 2010).

It may be concluded based on the results obtained in this study that inclusion of prebiotic, probiotic and symbiotic in diets for *C. gariepinus* adversely affect feed efficiency and growth parameters and positively influenced activities of antioxidant enzymes which enhanced resistance against infection and metabolic status.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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