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Growth, hematology and liver health of African catfish (*Clarias gariepinus*, Burchell 1822) fed varying amounts of *Bacillus subtilis*

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ABSTRACT

The study assessed the effects of Bacillus subtilis (Bs) on the growth, hematology and liver health of Clarias gariepinus. The experiment was conducted by supplementing a commercial fish feed with different doses of Bs thus 0 g/kg Bs, 10 g/kg Bs, 20 g/kg Bs and 30 g/kg Bs. Fish of average weight 13.0 ± 0.22 g were put into four groups in triplicates and feed the prepared diets for 8 weeks. At the end of the experimental period, growth parameters were evaluated and blood samples examined for effects of treatments on hematological parameters and liver toxicity. The results showed that in terms of growth fish groups fed the Bacillus diets had significantly higher weight gain, lower feed conversion ratio, higher viscerosomatic index and hepatosomatic index, and condition factor compared to the control group. In the Bacillus group, 10 g/kg Bs showed a significant increase in hematological parameters compared with the other groups. Lower amounts of aspartate aminotransferase and alanine transaminase were observed in the Bacillus fed fish compared to the control group. The results suggest that dietary administration of Bacillus subtilis at 10 g/kg can effectively improve the growth and health Clarias gariepinus.

Keywords: *Catfish, Probiotic, Feed additive, Bacteria, Blood, Liver*

INTRODUCTION

Probiotics have emerged as a feasible and effective option for enhancing animal health and promoting growth in aquaculture (Tachibana et al., 2020). Referred to as "life promoters," these beneficial bacteria have shown superior benefits in improving fish development and overall well-being, making them a prominent area of research in fish culture (Soto-Dávila et al., 2024). Probiotic application has been linked to growth enhancement in fish, attributed to increased nutrient intake and nutrient delivery (Wuertz et al., 2021) Additionally, probiotics have been found to positively influence important hematological parameters such as white blood cell count, red blood cell count, packed cell volume, and hemoglobin content in fish, indicating improved health (Feliatra et al., 2018). Moreover, probiotic *Bacillus* spp., such as *Bacillus licheniformis* and *Bacillus subtilis*, have demonstrated the ability to modulate enzymes such as AST and ALT, which are essential for detecting tissue damage induced by toxicants in fish (Adorian et al., 2018).

Bacillus spp. are highly promising as feed additives among various probiotic bacteria due to their positive effects on growth, immunological response, and disease resistance in farmed fish. *Bacillus subtilis, Bacillus amyloliquefaciens* and *Bacillus licheniformis* have demonstrated encouraging benefits on growth parameters, serum and mucosal immunology, and disease resilience in fish. Their successful utilization is attributed to their ability to produce spores that can withstand heat during feed pelletization, survive passage through fish stomachs, and establish themselves in the intestines, where they can multiply and contribute to the synthesis of various digestive enzymes (amylase, protease, and lipase) (Soto-Dávila et al., 2024;Tachibana et al., 2020;Wuertz et al., 2021).

Aquaculture technology development worldwide aims to increase productivity, with a focus on fish feed management due to its significant contribution (70 – 80%) to total production costs (Munguti, 2021). *Clarias gariepinus*, commonly known as African catfish, is an important cultivated fish species in aquaculture. It is widely farmed in many countries, especially in Africa and parts of Asia. *Clarias gariepinus*, a vital cultivated fish species, is gaining popularity in fish farming for its income generation, job creation, and contribution to food security in Africa (Dalsgaard et al., 2012). However, there is limited knowledge about the effects of probiotic addition in the diet of *Clarias gariepinus*, despite the potential benefits of probiotics in enhancing growth and improving feed utilization and digestibility (Merrifield et al., 2010; Miranda et al., 2009; Reda & Selim, 2015). This study aimed to investigate the effect of *Bacillus*-fed feed on the growth, hematological parameters, plasma chemistry, and liver health of *Clarias gariepienus.*

MATERIALS AND METHODS

Diet preparation

A commercial fish feed known as Raanan Fish Feed (composition: protein, 42% ash, 10.0%; moisture, 9.0%; fiber, 3.0%; vitamin A, 900 IU/kg; and vitamin C, 300 mg/kg) shown in Plate 1, was purchased in the open market at the Tamale central market, Ghana, and the probiotic *Bacillus subtilis* (Bs) in the powered form was also purchased from ECOT company in China (Plate 2) for use in the preparation of the experimental feed. One kilogram of each of the commercial feeds was measured into four separate plastic bowls. Two grams of starch was dissolved in one hundred milliliters of distilled water and added to one of the four bowls containing a kilogram of feed and dried at ambient temperature to serve as a control diet (0 g/kg Bs). To the remaining three measured portions of feed, probiotic *Bacillus subtilis* levels of 10 g/kg Bs, 20 g/kg Bs and 30 g/kg Bs were each added and mixed thoroughly by adding 100 ml of distilled water containing 2 grams of dissolved starch as adhesive. The diets were dried at room temperature and, for the purpose of the experiment, were stored in insect-proof bags. Table 1 shows the various codes that were used.

Plate 1: Raanan fish feed

Plate 2: Probiotic, *Bacillus subtilis* 200

Table 1: Dietary codes used in this study

Dietary Codes	Dietary combination
0 g/kg Bs	A commercial pellet diet that is void of Bs
10 g/kg Bs	A commercial pellet diet supplemented with 10 g Bs w/w
20 g/kg Bs	A commercial pellet diet supplemented with 20 g Bs w/w
30 g/kg Bs	A commercial pellet diet supplemented with 30 g Bs w/w

*where Bs, *Bacillus subtilis*, w/w, weight by weight

Experimental setup and design

Two hundred and twenty-eight (228) African Catfish (*Clarias gariepinus*) fingerlings without symptoms of disease (i.e., no abdominal distension, ragged fins, lethargic or hemorrhage) with an average body weight of 13.0 ± 0.22 g (mean ± SE) were obtained from Pilot Aquaculture Centre, Kumasi, Ghana. Fish were distributed at random into concrete tanks with a capacity of 80 L containing 50 L of water and a circumference of 3 ft with a height of 2 ft. Experimental fish were allowed to acclimatize for a week, during which time they were fed twice with the control diet on a daily basis of their body mass at 2% in two equal rations at 08:30 GMT and 16:00 GMT. The tanks were then randomly assigned to groups of 0 g/kg Bs, 10 g/kg Bs, 20 g/kg Bs and 30 g/kg Bs in triplicate after acclimatization and sustained over the course of the eight-week trial. During the experiment, fish in the 0 g/kg Bs group were fed diets without Bs supplementation, while those in the 10 g/kg Bs-, 20 g/kg Bs- and 30 g/kg Bs-supplemented groups were fed a control diet supplemented with Bs twice daily at 2% of the body weight.

Growth performance parameters and analysis

Fish samples were weighed biweekly using an electronic scale to the nearest 0.01 g over an 8-week experimental period. At the end of the fourth and eighth weeks, six fish specimens were taken from each treatment and dissected to visualize visceral mass and for measurement of the viscerosomatic index (VSI) and hepatosomatic index (HSI). Fish were killed by breaking the backbone near the head with a scissors. At the end of eight weeks of feeding, indices of growth, feed utilization, and condition of fish were calculated using the formulars below:

Mean weight gain (MWG) = $Final\ mean\ weight\ (g) - Initial\ mean\ weight\ (g)$

Feed conversion ratio = $\frac{Total\ feed\ given\ to\ fish\ (g)}{total\ weight\ gained\ by\ fish\ (g) + dead\ body\ weight - initial\ body\ weight\ (g)}\ x\ 100$

Viscerosomatic index (VSI) =100 x ($\frac{Versra\ weight\ (g)}{total\ body\ weight\ (g)}$)

Hepatosomatic index (HSI) = 100 x ($\frac{liver\ weight\ (g)}{total\ body\ weight\ (g)}$)

Falcon condition factor (Kc) = 100 x ($\frac{W}{L^3}$)

Blood sample collection and analysis

At the fourth and eighth week, 0.5 ml of blood samples were taken from each sampled fish fed diets treated with Bs and the control group (i.e., pooled into 9 EDTA per treatment) to analyze the impact of giving *Clarias gariepinus* a diet supplemented with *Bacillus subtilis* at various doses. Blood samples were taken from the caudal vein of fish using a 2 ml disposable syringe and transferred to heparinized tubes as described by (Adorian et al., 2018) and taken to the Tamale Teaching Hospital laboratory for analys of hematological parameters (red blood cells, white blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC)), plasma chemistry (total protein (TP), albumin (ALB), total bilirubin (T-BIL) and gamma-glutamyltransferase (y-GT)). Liver health was assessed by analyzing the levels aspartate transaminase (AST), alkaline phosphatase (ALP) and alanine transaminase (ALT) as indices of toxicity.

Statistical analyses

One-way analysis of variance was carried out using the IBM Statistical package for social sciences (SPSS version 21.0) to determine differences ($P < 0.05$) in growth, hematological, and toxic parameters. In cases where treatments showed a significant difference in means, the Tukey HSD test was further used to determine which means were different ($P < 0.05$).

RESULTS

Effects of treatment on the growth performance of *Clarias gariepinus*

The growth pattern of catfish (*Clarias gariepinus*) fed diets supplemented with different levels of *Bacillus subtilis* is shown in Figure 1. A significant increase was observed in all Bs-supplemented diets compared to the control (0 g/kg Bs), with 10 g/kg Bs being significantly higher among the supplemented diets at the end of week 8. The mean final weights of the fish samples at week 8 were 44.65 ± 0.41 g, 77.97 ± 0.89, 54.18 ± 0.95, and 55.92 ± 0.30 for 0 g/kg Bs, 10 g/kg Bs, 20 g/kg Bs and 30 g/kg Bs, respectively. The development of *Clarias gariepinus* when given various doses of *Bacillus subtilis* is shown in Table 2. A significant increase in final weight was observed in the fish fed Bs-supplemented diets in comparison to the control diet; however, the results showed that 10 g/kg Bs was significantly higher among the treated groups ($P < 0.05$). The *Bacillus*-fed groups also showed a significant increase in weight gain in comparison to the control group, with 10 g/kg Bs showing superior results ($P < 0.05$). A significant decrease in FCR values was observed in the supplemented diet groups (10 g/kg Bs, 20 g/kg Bs, 30/kg Bs) compared to the control (0 g/kg Bs). The FCR of fish fed the supplemented diets did not differ significantly ($P > 0.05$). Significant differences were observed in the HSI and VSI for the *Bacillus*-fed groups (10 g/kg Bs, 20 g/kg Bs, 30 g/kg Bs) in comparison to 0 g/kg Bs ($P < 0.05$). The 10 g/kg Bs group demonstrated better results among the treated diet groups ($P < 0.05$). For Kc, a significant increase was observed for 0 g/kg in comparison to the *Bacillus*-fed groups (10 g/kg Bs, 20 g/kg Bs, 30 g/kg Bs). However, there was no significant difference in K_c among the treated diet groups ($P > 0.05$).

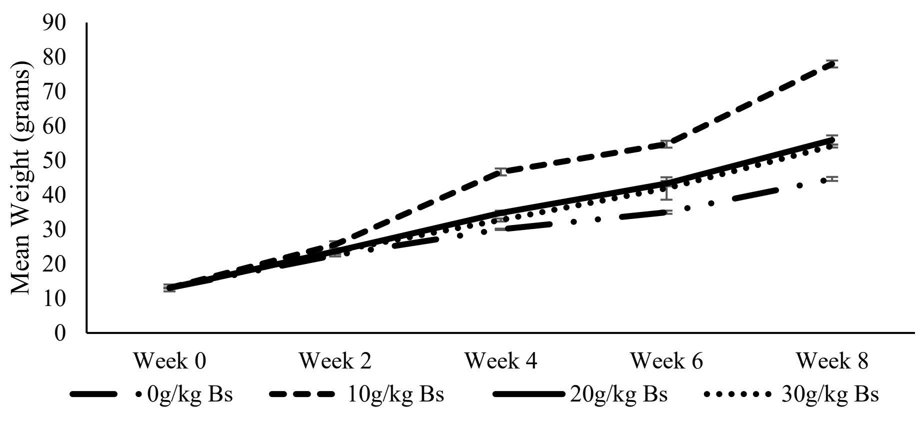


Figure 1: **Growth pattern of *Clarias gariepinus* fed Bs supplemented diets for eight weeks**

Table 2: Growth parameters of *Clarias gariepinus* fed the test diets

Growth parameter	Dietary Treatments 0 g/kg Bs	10 g/kg Bs	20 g/kg Bs	30 g/kg Bs	P-value
Wi (g)	13.07 ± **1.22**^{a}	13.05 ± 2.44^{a}	13.06 ± **1.03**^{a}	13.07 ± **0.87**^{a}	0.78
Wf (g)	44.65 ± **2.71**^{c}	77.97 ± **2.11**^{a}	55.92 ± **1.55**^{b}	54.18 ± **2.05**^{b}	0.03
WG (g)	31.07 ± 0.71 ^{c}	64.92 ± 1.70 ^{a}	42.6 ± 0.95 ^{b}	41.11 ± 3.01 ^{b}	0.01
FCR (%)	2.72 ± 0.90 ^{a}	1.28 ± 0.05 ^{b}	1.25 ± 0.19 ^{b}	1.32 ± 0.24 ^{b}	0.00
VSI (%)	5.42 ± 0.62 ^{c}	8.24 ± 0.36 ^{a}	7.43 ± 0.15 ^{b}	7.21 ± 0.29 ^{b}	0.00
HS1 (%)	0.09 ± 0.01 ^{c}	0.87 ± 0.67 ^{a}	0.57 ± 0.54 ^{b}	0.54 ± 0.54 ^{b}	0.03
K_c	1.78 ± 0.04 ^{b}	2.52 ± 0.03 ^{a}	2.65 ± 0.03 ^{a}	2.58 ± 0.01 ^{a}	0.03

Note: The means in rows with the similar superscript do not differ significantly
where Wi=initial weight, Wf=final weight, WG=weight gain, FCR=feed conversion ratio, VSI=viscerosomatic index, HSI=hepatosomatic index and Kc=Fulton condition factor.

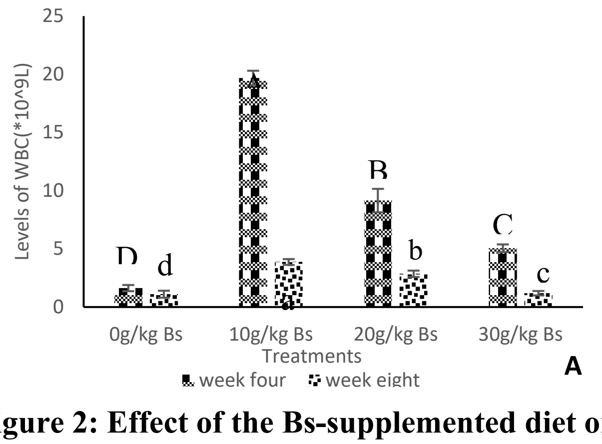
Influence of *B. subtilis* on the hematology of *Clarias gariepinus*

White blood cells (WBCs)

As shown in Figure 2 A, the WBCs were significantly higher in fish fed Bs supplement (10 g/kg Bs, 20 g/kg Bs and 30 g/kg Bs) diets than in those fed a 0 g/kg Bs diet at the end of four weeks ($P < 0.05$). However, among the supplemented diet groups, 10 g/kg Bs showed significantly higher values ($P < 0.05$). Similarly, after eight weeks, fish fed Bs meals demonstrated significantly higher ($P < 0.05$) WBC levels in comparison to the control, with 10 g/kg Bs showing significantly higher levels among the supplemented diet groups. In general, as the experiment progressed from four to eight weeks, the WBCs in the Bs-supplemented diets dropped but were in optimal ranges. Additionally, although the results showed that *Bacillus subtilis* supplementation has the potential to significantly increase ($P < 0.05$) the WBCs in comparison to the control, there appears to be a decreasing trend with increasing doses of *Bacillus subtilis* in the diets.

Red Blood Cell (RBC)

The effects of dietary supplemented diets on the level of RBCs in *Clarias gariepinus* for four and eight weeks are shown in Figure 2 B. The treated diet groups exhibited a considerable significant rise in comparison to the control at the end of four weeks ($P < 0.05$). The 10 g/kg Bs was significantly higher among the supplemented diets with 20 g/kg Bs and 30 g/kg Bs, showing similarities ($P > 0.05$). At the end of the eight-week period, the results showed a significant increase between *Bs*-fed groups and the control, with a significant increase for 10 g/kg Bs among the supplemented diet groups ($P < 0.05$).



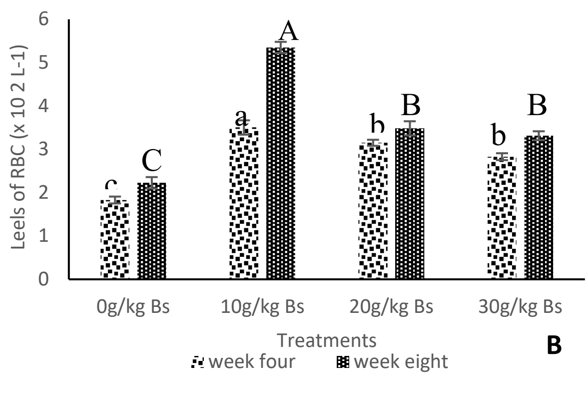


Figure 2: Effect of the Bs-supplemented diet on WBCs (A) and RBCs (B) after four and eight weeks

Note: Bars per sampling point with the same letter are not significantly different

Differential WBCs

The effects of the Bs diets and control diet on the differential WBCs, including neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS) and basophils (BASO), of *Clarias gariepinus* fed *Bacillus subtilis*-supplemented diets are shown in Table 3. Inclusion of *Bs* resulted in significantly higher differential WBCs ($P < 0.05$) in comparison to the control. However, higher levels of Bs supplementation showed a downward trend among the treated fish at both sampling times.

Table 3: Differential WBCs of *Clarias gariepinus* fed treated diets

Dietary Treatments	LYM	MON	NEU	EOSF	BASO
0 g/kg Bs @Week 4	40.73 ± 0.40^c	16.47 ± 2.50^b	39.15 ± 0.42^b	0.68 ± 0.60^b	0.73 ± 0.41^b
10 g/kg Bs @Week 4	73.63 ± 0.64^a	21.07 ± 1.03^a	48.70 ± 0.16^a	2.77 ± 1.26^a	1.63 ± 1.24^a
20 g/kg Bs @Week 4	45.23 ± 0.30^b	19.44 ± 2.41^a	46.66 ± 0.53^a	2.44 ± 1.25^a	0.83 ± 0.84^a
30 g/kg Bs @Week 4	41.68 ± 0.37^c	20.19 ± 2.30^a	48.59 ± 0.62^a	2.01 ± 1.41^a	0.79 ± 0.66^a
P-value	0.00	0.01	0.00	0.00	0.00
0 g/kg Bs @Week 8	34.46 ± 0.93c	25.27±1.03c	18.28±0.81^d	1.46 ± 0.33^c	0.21 ± 0.11^d
10 g/kg Bs @Week 8	41.13 ± 0.68^a	36.76±0.54a	41.89 ± 0.26^a	8.04 ± 0.20^a	1.84 ± 1.67^a
20 g/kg Bs @Week 8	37.59 ± 0.95^b	30.58±0.73^b	30.63 ± 1.03^b	4.13 ± 0.39^b	0.56 ± 0.05^b
30 g/kg Bs @Week 8	37.55 ± 0.72^b	25.19± 0.92^c	25.59 ± 0.74^c	4.45 ± 0.28^b	0.32 ± 0.17^c
P-value	0.00	0.00	0.03	0.00	0.03

Note: The means in columns with the same superscript do not differ significantly ($p > 0.05$) **where** LYM=Lymphocyte, MON=Monocyte, BASO=Basophiles, NEU=Neutrophiles, EOSF=Eosinophiles,

Hemoglobin (Hb) and hematocrit (Hct)

As shown in Figure 4A, there were significant differences in Hb among the *Bs*-fed groups and the control ($P < 0.05$). At four weeks, all *Bs*-supplemented groups had significantly higher Hb levels than the control group ($P < 0.05$). Fish fed 10 g/kg Bs showed the best increasing effects on Hb compared to all other groups in the period of study ($P < 0.05$). Similar trends were observed in Hct (Figure 4B).

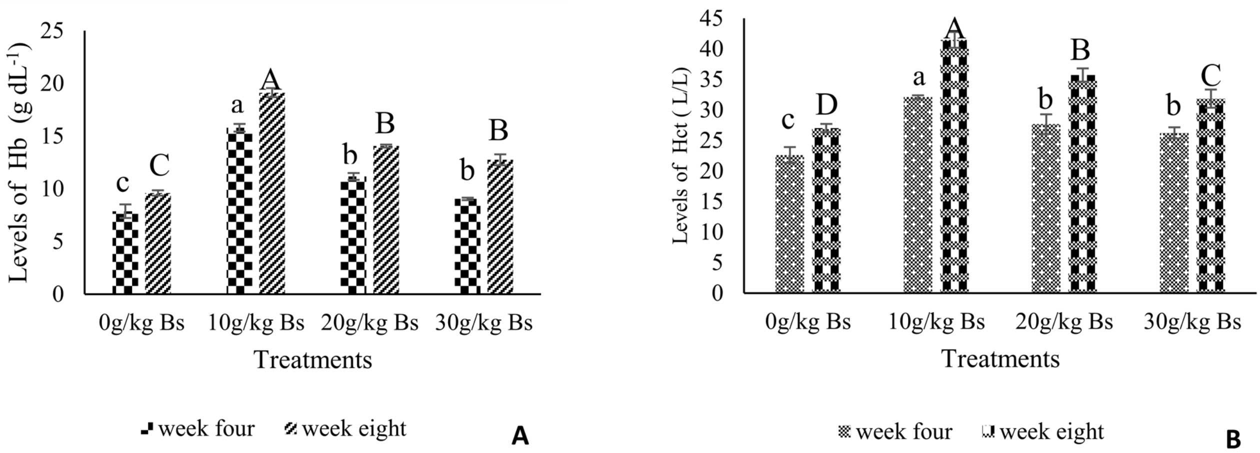


Figure 3: **Effect of the Bs-supplemented diet on Hb (4A) and Hct (4B) after four and eight weeks**
Note: Bars per sampling point with the same letter are not significantly different

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of *Clarias gariepienus* fed Bs

Table 4 shows the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of the test fish. At four and eight weeks, MCV and MCH were significantly higher in Bs-fed fish than in control-fed fish. Fish fed 10 g/kg Bs seemed to show higher levels of these parameters than their counterparts. Additionally, MCHC was found to be significantly higher in 10 g/kg Bs-fed fish than in the other groups but only at eight weeks ($P < 0.05$).

Table 4: **MCV, MCH, and MCHC of *Clarias gariepinus* fed Bs supplemented diets**

Dietary Treatments	MCV	MCH	MCHC
0 g/kg Bs @Week 4	115.65 ± 2.75^c	40.80 ± 0.30^b	33.43 ± 3.52^a
10 g/kg Bs @Week 4	132.60 ± 0.65^a	47.06 ± 0.41^a	35.83 ± 1.02^a
20 g/kg Bs @Week 4	123.36 ± 1.60^b	46.96 ± 3.02^a	34.86 ± 0.75^a
30 g/kg Bs @Week 4	122.16 ± 1.14^b	45.06 ± 1.81^a	34.46 ± 0.26^a
P-value	0.00	0.03	0.09
0 g/kg Bs @Week 8	130.01 ± 3.96^c	51.19 ± 1.11^c	37.75 ± 0.45^b
10 g/kg Bs @Week 8	146.49 ± 0.63^a	63.03 ± 0.44^a	46.24 ± 0.46^a
20 g/kg Bs @Week 8	137.65 ± 1.92^b	59.74 ± 1.14^b	38.93 ± 0.91^b
30 g/kg Bs @Week 8	136.43 ± 1.94^b	57.35 ± 0.94^b	39.73 ± 0.45^b
P-value	0.00	0.03	0.01

Note: The means in columns with the same superscript do not differ significantly ($p > 0.05$) where MCV= mean corpuscular volume, MCH= mean corpuscular hemaglobin, MCHC= mean corpuscular hemaglobin concentration.

Effects of treatments on the biochemistry of *Clarias gariepinus*

Albumin (ALB) and total protein (TP)

Figure 5A represents the effects of *Bacillus subtilis* supplementation on albumin in *Clarias gariepinus*. At the end of four and eight weeks, the 10 g/kg Bs-supplemented diet group exhibited higher albumin levels than the groups receiving higher *Bacillus subtilis* addition (20 g/kg Bs and 30 g/kg Bs). The control group fed 0 g/kg Bs probiotic presented lower albumin values than the supplemented diet groups ($P < 0.05$). There were statistical similarities between 20 g/kg Bs and 30 g/kg Bs. At the end of the experiment, supplementing *Clarias gariepinus* with 10 g/kg Bs – 30 g/kg Bs enhanced the level of albumin. The results of the total protein analysis are summarized in Figure 5B. *Clarias gariepinus* fed diets containing probiotics had higher total protein than the control group at four and eight weeks. Among the different *Bacillus*-supplemented diets, 10 g/kg Bs was significantly higher within the Bs-treated group, with a declining tendency as the supplemented quantity of Bs increased ($P < 0.05$).

γ-GT and T-BIL levels

Table 5 shows the subprotein levels (γ-GT and T-BIL) of fish fed a control or Bs-enhanced diet at four and eight weeks. At weeks four and eight, adding Bs to the diet of *Clarias gariepinus* enabled the experimental fish to have significantly higher levels of γ-GT than the control group ($P < 0.05$). At the end of four weeks, γ-GT levels were enhanced significantly in all Bs-treated groups in comparison to the control group ($P < 0.05$). Statistically no significant difference was observed within the supplemented diet groups at the end of week eight ($P > 0.05$). When compared to the control group, Bs-supplemented groups had significantly higher **γ**-GT levels, with 10 g/kg Bs increasing significantly ($P < 0.05$). *Clarias gariepinus* fed the Bs-supplemented diet significantly improved the level of T-BIL in fish compared to the control diet ($P < 0.05$). However, 20 g/kg Bs and 30 g/kg Bs showed no significant difference at the end of four and eight weeks whiles fish fed 10 g/kg Bs ($P < 0.05$) demonstrated there was a significant increament in T-BIL.

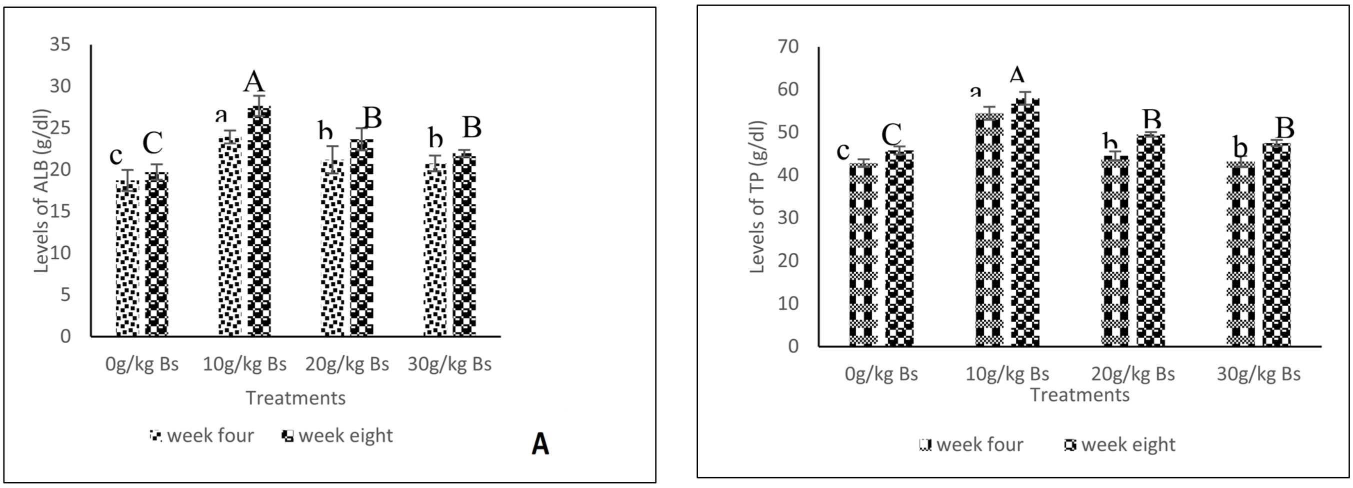


Figure 4: Total albumin (A) and total protein (B) levels of *Clarias gariepinus* for four and eight weeks. Note: Bars per sampling point with the same letter are not significantly different

Table 5: γ -GT and T-BIL of *Clarias ga inus* fed control and Bs supplemented diets

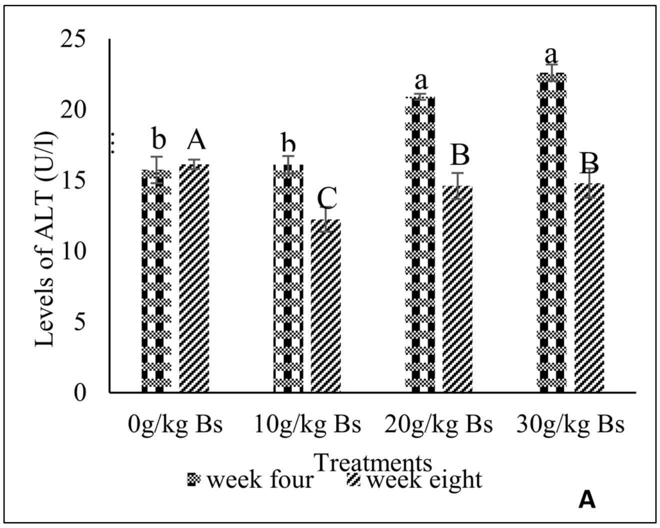
Dietary Treatments	γ -GT (U/l)	T-Bil (mg/dl)
0 g/kg Bs @Week 4	0.76 ± 0.89b	4.60 ± 0.34c
10 g/kg Bs @Week 4	5.89 ± 0.41a	13.53 ± 1.25a
20 g/kg Bs @Week 4	6.83 ± 2.02a	6.85 ± 1.25b
30 g/kg Bs @Week 4	5.70 ± 1.81a	6.16 ± 1.18b
P-value	0.01	0.01
0 g/kg Bs @Week 8	3.51 ± 1.67c	5.19 ± 0.49c
10 g/kg Bs @Week 8	13.61 ± 0.76a	16.28 ± 0.38a
20 g/kg Bs @Week 8	8.86 ± 1.82b	9.92 ± 1.18b
30 g/kg Bs @Week 8	7.02 ± 1.24b	8.35 ± 0.70b
P-value	0	0.01

Note: The means in columns with the same superscript do not differ significantly

Effects of treatments on the liver health of *Clarias gariepinus*

Alanine amino transferase (ALT) and aspartate aminotransferase (AST)

The ALT levels of fish given both control and *Bacillus subtilis*-supplemented diets for four and eight weeks are shown in Figure 6 A. At four weeks, the results showed no significant difference between the control (0 g/kg Bs) and 10 g/kg Bs and between 20 g/kg Bs and 30 g/kg Bs. However, a significant increase in ALT values in the control group (0 g/kg Bs) compared to the *Bacillus*-fed diet groups (10 g/kg Bs, 20 g/kg Bs, 30 g/kg Bs) was observed at the end of eight weeks ($P < 0.05$). Among the treated diet groups, a significant decrease in ALT for 10 g/kg Bs was observed ($P < 0.05$). As shown in Figure 6B, the greatest amount of AST was recorded in the group fed the diet supplemented with 30 g/kg Bs, with the control group showing the lowest values at the end of four weeks. Eight weeks revealed that the control diet, 20 g/kg Bs and 30 g/kg Bs had the greatest AST levels, which were significantly higher ($P < 0.05$) when compared to the 10 g/kg Bs supplemented diet ($P < 0.05$). Generally, in comparison to the control group, AST values in Bs-supplemented meals were lower at eight weeks.



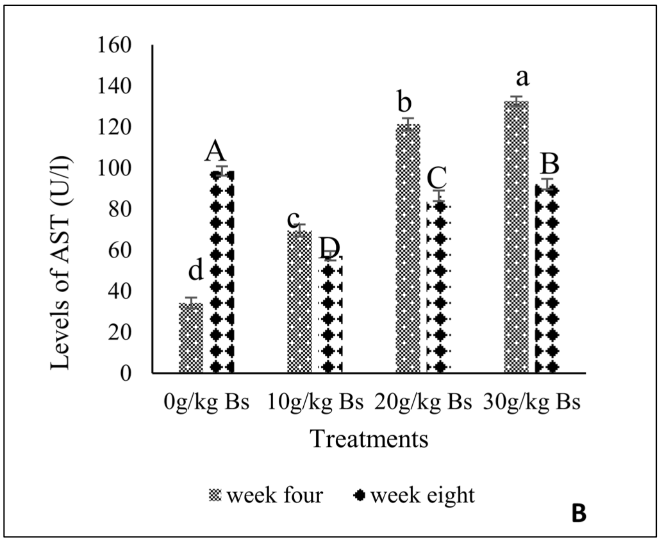


Figure 5: ALT (Figure 6A) and AST (Figure 6B) levels in *Clarias gariepinus* fed control and Bs-supplemented diets at four and eight weeks. **Note: Bars per sampling point with the same letter are not significantly different**

Alkaline Phosphatase (ALP)

ALP levels were lowest in the control group (85.56 U/l) and highest in the 30 g/kg Bs group (164.32 U/l) after four weeks of experimentation. However, after eight weeks of experimentation, the ALP levels in the control group (185.23 U/l) were found to be significantly higher than those in the Bs-treated groups, with scores of 78.15, 121.84 – 126.58 U/l. Among the Bs-treated groups, those fed 10 g/kg Bs were significantly different from the others.

DISCUSSION

The documented positive impacts of the probiotic *Bacillus* on the growth performance of fish are well established. It is now widely known that probiotic microorganisms improve growth performance by establishing themselves within the fish's gastrointestinal tract, thereby providing various advantageous effects (Martínez Cruz et al., 2012; Soltani et al., 2019). In this study, it was found that *Clarias gariepinus* fed *Bacillus*-treated diets exhibited significantly higher final weight, weight gain, and feed conversion ratio compared to those fed the control diet. These improvements were more pronounced in the 10 g/kg Bs treatment. The findings in this study corroborate previous reports that indicate that lower doses of probiotic supplementation in diets give the best results in terms of growth performance ((Aly et al., 2008; Cha et al., 2013). In terms of feed utilization, Bs-treated fish exhibited a significantly lower FCR in relation to the control. This is indicative of better feed utilization, as explained previously (Liu et al., 2010; Van Doan et al., 2018). Further support could be drawn from the significantly higher VSI, HSI and K_c values recorded in the present study. Munir et al. (2016) explained that significantly higher values of these body indices in fish relative to those fed a control reflect better feed utilization. Support for these explanations could be drawn from previous reports Abarike et al., 2018; Adorian et al., 2018; Kuebutornye et al., 2019).

Hematological parameters are crucial indicators of the health and functioning of the blood and its components. They provide valuable information about various aspects of an organism's overall health and can aid in diagnosing and monitoring a wide range of medical conditions. Lower levels of these hematological parameters in organisms may indicate poor health, and excessively higher levels may indicate stressful conditions. For instance, lower RBCs may indicate anemia, while excessively higher WBCs may indicate stressed organisms. Dahiya et al. (2012) indicated that treatments that tend to cause an increase in hematological parameters could be an indication of a potential health boost. In the present study, it was found that fish fed Bs-supplemented diets substantially increased the levels of RBCs, WBCs, hematocrit, and hemoglobin in comparison to those fed the control diet. It was obvious that among the Bs treatments, the lowest supplementation of 10 g/kg Bs was best, and the highest supplementation of 30 g/kg Bs was worse but better relative to the control. We are of the opinion that Bs can improve the health of fish, and the best results are obtainable with lower doses, such as 10 g/kg Bs. The conclusion arrived at in this study supports the accessions made in previous studies (Garcia-marengoni et al., 2015; Rajikkannu et al., 2015; Feliatra et al., 2018; Garcia-marengoni et al., 2015). In addition, the levels of the hematological parameters recorded in this study are within the reference ranges reported for healthy catfish (Akinrotimi et al., 2011), suggesting that Bs supplementation up to 30 g/kg might not adversely affect the health of *Claria gariepinus*.

Knowledge of plasma biochemistry is a good measure of the health status of fish. The biochemistry of blood could be monitored as an indicator/marker of change in response to dietary treatments (Eissa et al., 2022). Reduction in values of biochemical parameters may indicate inadequate feed intake and utilization, whereas a rise indicates improved feed intake and utilization (Chowdhury & Roy, 2020). There have been reports of positive effects regarding probiotic use in fish culture on blood biochemistry (Sîrbu et al., 2022), and these have been corroborated by the results in this study, which showed increased levels of the blood parameters TP, ALP, γ-GT and T-Bill in fish fed *Bacillus*-supplemented diets compared to fish fed the control diet.

It is widely known that monitoring the levels of AST, ALT, and ALP enzymes in the blood of fish can help reveal the effects of feed additives, such as probiotics and herbal supplements, on liver health (Abarike et al 2020; Abdollahi-Arpanahi et al., 2018; Babazadeh et al., 2011) . Feed additives that reduce the leaching of these enzymes into the bloodstream could have hepatoprotective properties, while those that promote leaching of enzymes into the bloodstream could be considered toxic (Abarike et al., 2022). Generally, it was observed that fish that consumed Bacillus-supplemented diets could have initially reacted to it as a foreign entity as there was an observed significant increment in AST, ALT and ALP levels in comparison to those fed the control after four weeks of exposure. This seems to be the obviously observed pattern for most studies with feed additives such as probiotics (Sirbu et al., 2022). However, compared to the control, Bacillus-supplemented fed fish had lower levels of AST, ALT, and ALP after eight weeks. This suggested that Bacillus supplementation might help improve the health of the fish's liver after exposure/feeding for eight week by significantly reducing the leaching of liver enzymes into the blood stream. This supports earlier reports of the hepatoprotective properties of probiotics. (Abdollahi-Arpanahi et al., 2018; Adorian et al., 2018),

In conclusion, the present study demonstrates that supplementing the diets of *Clarias gariepinus* with *Bacillus subtilis* 200 might improve its growth performance, feed utilization, hematology and blood biochemistry as well as improving liver health after 8 weeks of feeding. To achieve best performance, a dose of 10 g/kg Bs is recommended. With these findings, this study data suggests that *Bacillus subtilis* 200 could lead potentially to increasing the yield and profitability of the farming of *Clarias gariepinus*. However, as with all scientific research, these findings would likely need to be replicated in further studies to confirm their validity and applicability in different contexts.

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Conflict of interest
The authors declare that they have no conflicts of interest.

Ethical approval
This article does not contain any studies with animals performed by any of the authors.

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