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Effect of implementation levels of biosecurity measures and genetic type on the epidemio-zootechnical characteristics of farmed *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Fish diseases and inbreeding are among the factors limiting fish production. The present study aimed to assess the effect of biosecurity measures implementation levels (BMIL) and genetic type on epidemiological and zootechnical characteristics in hatcheries, in order to contribute to healthy and sustainable fish farming. Crosses (Beninese males x Cameroonian females and Cameroonian males x Cameroonian females) were carried out between two strains of domestic broodstock. The epidemiozootechnical characteristics evaluated (mortality rate, prevalence of pathologies, fertilization rate, hatching rate, productivity and economic profitability) were subjected to descriptive statistics, Student's t-test and two-factor analysis of variance. The resulting larvae were distributed in duplicate to three hatcheries, in which biosecurity measures were selectively applied to obtain the desired BMILs (low, medium and high) for each hatchery. As results, the highest mortality rate (100%) was recorded in the hatchery where the BMIL was low, then decreased significantly in crossbreds for both medium and high BMILs. Apart from fish deformities, the prevalence of other pathologies (anorexia and abnormal swimming) decreased significantly with BMIL. The prevalence of pathologies was about two times significantly higher for the pure strain than for the crossbreds. Fertilization and hatching rates dropped significantly with BMIL, with hybrid cross recording the highest values. Productivity and economic profitability increased significantly with BMIL. Values were 16 and 2 times higher in crossbreds than in the pure strain for medium and high BMIL respectively. Overall, the increase in BMIL and the use of genetic types have improved the epidemio-zootechnical characteristics of *Clarias gariepinus* in hatcheries. Fish farmers need to focus on biosecurity measures and use crossbreds from the Cameroon x Benin cross for optimum production.

Introduction

In Cameroon, the annual demand for table fish is close to 500,000 tonnes, whereas the national supply is estimated at only 335,000 tonnes, with almost 5% coming from fish farming. To bridge this production gap, around 200,000 tons of additional fish, sometimes of doubtful quality, are imported annually, at a cost of over US$0.23 billion (Nack *et al*., 2022). In fact, the fish farming sector, which is likely to offset the production deficit, faces numerous limiting factors, including a lack of fry, the high cost and scarcity of manufactured feed, and difficulty accessing credit from commercial banks and microfinance institutions (Kenfack *et al*., 2019; Tiogue *et al*., 2020). In addition to these constraints, there is the lack of implementation of a fish genetic improvement program, the key to developing high-performance fish farming (Lazard, 2009), inbreeding among broodstock and fish diseases (Fonkwa *et al*., 2022). Fonkwa *et al*. (2022) therefore recommended that fish diseases be taken into account for efficient, healthy, profitable and sustainable fish farming. The same authors noted that diseases were the cause of morbidity, massive fish mortality, reduced productivity and considerable economic losses. In addition, between April and May 2021, they recorded an epizootic of Yersiniosis (*Yersinia* sp) in farmed Oreochromis niloticus and *Cyprinus carpio* causing losses of around US$420.50 in the Centre Region of Cameroon. These diseases are generally attributable to fish inbreeding, poor disease risk management or failure by fish farmers to comply with biosecurity or hygiene measures (Racicot and Vaillancourt, 2009; Fonkwa *et al*., 2023b). Once the disease appears, its treatment becomes technically and financially constraining, hence the need to focus on prevention through rigorous compliance with biosecurity measures (Fonkwa *et al*., 2023b). FAO (2007) reported that biosecurity is as an integrated strategic approach encompassing policy, regulatory frameworks and activities for risk analysis and management. Farmers in developing countries do not yet perceive the benefits of implementing biosecurity measures on their farms. They regard the costs associated with biosecurity practices as fortuitous, since they have no effect on the livestock's zootechnical performance, hence the need for such a study (Kone *et al*., 2012).

Data on the effect of biosecurity implementation levels and fish genetic type on epidemiological and zootechnical characteristics in hatcheries are non-existent. For this reason, Kpoumie *et al*. (2023) recommended work on the disease resistance of genetic strains of fish. This study addresses issues relating to aquaculture biosecurity, farmed fish pathologies, genetic improvement and zootechnical performance. It aimed to assess the effect of biosecurity measures implementation levels and genetic type on the epidemiological (mortality rate, prevalence of pathologies) and zootechnical (fertilization rate, hatching rate and productivity) characteristics and economic profitability of *Clarias gariepinus* in hatcheries, as a contribution to efficient, healthy, profitable and sustainable fish farming.

Materials and methods

Study area

The study took place from May to July 2022 in an experimental fish farm located in the Littoral Region of Cameroon, Mungo Division and Mbanga District (4°22'15 "N; 9°32'39 "E). The climate is of the equatorial type, with a long rainy season from mid-March to mid-November and a short dry season from mid-November to mid-March. The mean annual temperature is 29°C, while rainfall is around 2400mm (Molua, 2006).

Broodstock origin and handling and conduct of the experiment

The domestic strains (Cameroon and Benin) of *Clarias gariepinus*

(Burchell, 1822) used for intraspecific crosses included broodstock, descended from the crossing of a single male and a single female, thus highly inbred, and then their offspring, by artificial reproductions at the Koupa-Matapit fish farming research station of IRAD (Agriculture Research Institute for Development) in Foumban (LN: 5° 21′- 5° 58′; LE: 10°17′- 11° 02′) in the West region of Cameroon. Fish from each strain were reared in an earthen pond until sexual maturity. They were fed once a day with local compound feed containing 40% crude proteins at 1% of body weight until the start of the trial.

The completely randomized experimental design consisted of 3 hatcheries, each containing two treatments (Beninese males x Cameroonian females and Cameroonian males x Cameroonian females), and each consisting of 4 x 20l basins of borehole water, mounted in duplicate and in parallel.

Broodstock selection and hormone injection

Broodstock selection and hormonal injection were carried out according to Tiogue *et al*. (2008), with 4 males broodstock selected on the basis of age (≥1 year), vigor, weight (≥ 300g) and a well-developed genital papilla. As for the females, 4 were selected for their swollen and soft abdomen, a protuberant, reddish or pinkish genital papilla, and whose oocytes with a diameter greater than 1 mm and a greenish color were easily obtained by light manual pressure of the abdomen, and their weight between 300 and 500g. Individually, the selected broodstock were placed in concrete tanks in the hatchery for 24-hour stabulation. Female broodstock were weighed and then injected in the dorsal muscle to stimulate oocytes maturation and ovulation at a dose of 0.5mL HCG hormone/kg body weight. Each treated female was returned to her tank. Water temperature and time of injection were recorded. Males were not injected.

Harvesting of gametes

Milt was collected using the partial gonadectomy technique described by Nguenga *et al*. (2000) for males. Oocytes obtained by manual pressure of the female abdomen were then collected in dry plastic bowls and weighed to the nearest gram on an electronic balance (Sartorius brand, Germany, 10mg error margin.) Oocytes from females of each strain were mixed with milt from males of the same strain.

Cross-breeding, fertilization and egg incubation

To produce pure genetic types (Cameroonian males x Cameroonian females) and crossbreds (Beninese males x Cameroonian females), milt was collected with a syringe and mixed with oocytes collected with a stainless steel spatula. Fertilization was performed by adding a few ml of physiological liquid (0.5g of salt in 1L of water) to the mixture to activate and mobilize the milt, followed by moderate agitation for 1 min. Fertilized eggs were then rinsed with water to remove excess milt. The time of water addition to the eggs was recorded as the time of fertilization. The homogenized mixture was spread on the monolayer trays and incubated at 27°C. Each hatchery was incubated with 2g of eggs resulting from crosses between the two strains of domestic *Clarias gariepinus* broodstock (Cameroon and Benin). The larvae obtained were distributed as summarized in Table 1, then fed from day 3 at a frequency of 5 times a day with Coppens brand feed of diameter 0.2mm. In addition, biosecurity measures were selectively applied to obtain the desired compliance rate values for each hatchery (Table 2).

The linear scoring of the biosecurity measures was empirically determined as previously described (Can and Altug, 2014; Gelaude *et al*., 2014; Maduka *et al*. 2016). Thus, the values 1 and 0 were assigned to the implemented or not implemented biosecurity measured respectively. The final score of a hatchery was the sum of all the values recorded. The compliance rate of biosecurity measures was defined after Racicot and Vaillancourt (2009). The biosecurity measures were weighted equally because the main interest was how implementing biosecurity measures influence the health of reared fish and not the level of risk caused by the lack of implementing a biosecurity measure as it is the case in disease transmission pathways. The typology of hatcheries and implementation levels according to the compliance rate of biosecurity measures (Table 3) was that used by Fonkwa *et al*. (2023b).

Fish behavior was observed regularly and inspections fishing were carried out to determine the epidemio-zootechnical characteristics studied. To this effect, 100% of fry from each treatment were collected using a sieve and 3 x 15l buckets. Weights were measured to the nearest gram using an electronic balance. Fertilization, hatching and mortality rates were determined as per the technic used by Tiogue *et al*. (2008). From an epidemiological point of view, fish were examined following the standard parasitological procedure. Indeed, each fish specimen was examined with the naked eye and then with a hand-held magnifying glass to diagnose clinical signs of disease. Values of 1 and 0 were assigned to affected and unaffected fish respectively. Water physico-chemical characteristics were assessed twice daily (morning and evening) using an analysis kit. Values were expressed as mean ± standard deviation (Temperature= 27.96±0.58°C ; pH =7.4±0.2).

Epidemiozootechnical characteristics evaluated and statistical analysis

The epidemiozootechnical characteristics were defined as follows:
Mortality rate: percentage of fish dying during a given period compared with the initial total number.
Prevalence of pathology: percentage of fish showing clinical signs of pathology compared with the total number of fish examined.
Fertilization rate: percentage of fertilized eggs in relation to the total number of incubated eggs. Hatching rate: percentage of larvae hatched out of the total number of eggs incubated.
Productivity: total weight (g) of individuals obtained divided by the required volume (m3) of water times the production period (days).
Economic profitability: ratio between productivity and cost of selling a fry.

Table 1. Characteristics of treatments during the experimentation.

Hactcheries	Treatments				
	Names	Characteristics			
		Number of larvae	Genetic strains	Compliance rate (%)	Implementation levels of biosecurity measures
E1	T1	150	Cameroon x Cameroon	12.5	Low
	T2	150	Cameroon x Benin		
E2	T1	150	Cameroon x Cameroon	33.33	Intermediate/medium
	T2	150	Cameroon x Benin		
E3	T1	150	Cameroon x Cameroun	79.17	High
	T2	150	Cameroon x Benin		

Table 2. Scores and compliance rates of biosecurity measures in the hatcheries.

		Hatcheries		
		E1	E2	E3
N°	Biosecurity component in relation to isolation			
1	Farm is fenced	0	0	0
2	Other animals' species are absent on the farm	1	1	1
3	New fish are quarantined before rearing	0	0	1
4	Absence of bushes and trees around farms	0	0	0
5	Space for visitors	0	0	0
6	Water flow is continuous	1	1	1
7	Breeding infrastructures are layout in derivation	1	1	1
	Biosecurity component in relation to traffic control			
8	Visitors not allowed to have contact with water	0	1	1
9	No exchange of breeding tools between farms	0	0	1
10	Water supply tracks protected to trap debris and unwanted aquatic animals	0	1	1
	Biosecurity component in relation to sanitation			
11	Use of footbaths	0	0	1
12	Veterinary intervention	0	0	1
13	Well preserved feed	0	0	1
14	Incineration of dead fish	0	0	0
15	Special outfit (clean coverall and boots) for staff	0	0	1
16	Special outfit for visitors	0	0	1
17	Analysis of water quality	0	1	1
18	Diagnosis of fish diseases	0	0	1
19	Sanitary lock	0	1	1
20	Disinfection of hands at the entrance and exit of the hatcheries	0	0	1
21	Disinfection of breeding tools before use	0	1	1
22	Disinfection of breeding tools after use	0	0	1
23	Treatment of fish disesases	0	0	1
24	Captured fish not put back into water	0	0	0
	Overall score	3	8	19
	Compliance rate (%)	12.5	33.33	79.17

Table 3. Typology of hatcheries and implementation levels according to the compliance rate of biosecurity measures.

CR	Implementation levels	Biosecurity practice	Risk ranking	Types of hatcheries
[0-25]	Low	Poor	Major	A
]25-75]	Intermediate	Intermediate/medium	Moderate	B
]75-100]	high	Good	Minor	C

CR: compliance rate

Statistical Analysis

The epidemiozootechnical characteristics were subjected to descriptive statistics. Student's t-test and two-factor analysis of variance (F) were used to compare these characteristics according to the level of implementation of biosecurity measures and the genetic type of fish. In the case of a significant difference, i.e. a probability of error $p < 0.05$, the means were separated using Tukey's multiple comparison test (q). Graph pad version 8.0 was used for this purpose.

Results

Effect of biosecurity measures implementation levels (BMILs) and genetic type on the fish mortality rate

The effect of biosecurity measures implementation levels and genetic type on fish mortality rate was illustrated in Fig. 1. It reveals that, irrespective of genetic type, mortality rate was the highest (100%) in the hatchery where the BMIL was low, then decreased significantly (F= 86000; p = 0.001) with the MBIL.

Comparing mortality rates between genetic types shows maximum (100%) and similar values between pure strain (T1) and crossbreds/hybrids (T2) at low BMIL. However, mortality rates were significantly higher in the pure strain than in the crossbreds for medium (t = 12; p = 0.01) and high (t = 24; p = 0.01) BMILs.

When the effect of the BMILs on the mortality rates of the genetic types is compared, it appears that mortality rates dropped significantly by about 70% and 83% respectively in the pure strain (F= 16250; p = 0.001) and the crossbreds (F= 34120; p = 0.001) when the BMILs varied from low to high.

Effect of biosecurity measures implementation levels and genetic type on the prevalence of pathologies

The effect of BMILs and genetic type on the prevalence of pathologies (Fig. 2) shows three types of pathologies, namely anorexia, abnormal swimming and deformities. Irrespective of genetic type, and excluding deformities, the prevalence of pathologies decreased significantly with

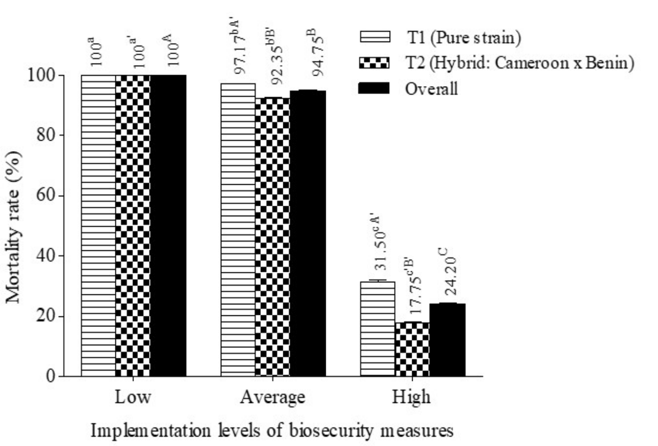

Fig. 1. Effect of biosecurity measures implementation levels and genetic type on the fish mortality rate. T1: Pure strain (Cameroon x Cameroon); T2: Crossbreds or hybrids (Cameroon x Benin); A,B, C: on the same band (Overall), values assigned different letters differ significantly (p <0.05) between levels of implementation of biosecurity measures; A',B', C': for the same level of implementation of biosecurity measures, bands (T1 and T2) assigned different letters differ significantly (p <0.05); a,b,c: on band T1, the values assigned to the different letters differ significantly (p <0.05) between the levels of implementation of biosecurity measures; a',b',c': on band T2, the values assigned to the different letters differ significantly (p <0.05) between the levels of implementation of biosecurity measures.

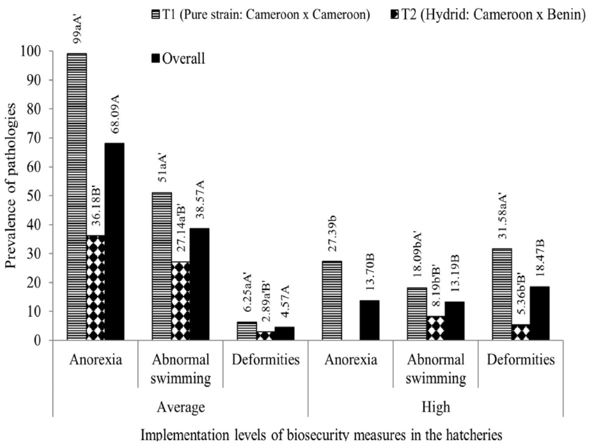

Fig. 2. Effect of biosecurity measures implementation levels and genetic type on the prevalence of pathologies. T1: Pure strain (Cameroon x Cameroon); T2: Crossbreds or hybrids (Cameroon x Benin); A,B, C: on the same band (Overall), values assigned different letters differ significantly (p <0.05) between levels of implementation of biosecurity measures; A',B', C': for the same level of implementation of biosecurity measures, the bands (T1 and T2) assigned different letters differ significantly (p <0.05); a,b,c: on band T1, the values assigned different letters differ significantly (p <0.05) between levels of implementation of biosecurity measures.

the BMIL Whatever the genetic type and for the medium BMIL, anorexia recorded the highest prevalence (p = 0.01), followed by abnormal swimming and deformities. However, the same trend was not noted for the high BMIL. Whatever the BMIL, the prevalence of pathologies was approximately two times significantly (p = 0.01) higher for the pure strain (T1) than for the crossbreds (T2).

Effect of biosecurity measures implementation levels and genetic type on zootechnical characteristics and economic profitability of Clarias gariepinus hatchery farming

The effect of BMILs and genetic type on the zootechnical characteristics and economic profitability of *Clarias gariepinus* hatchery farming was summarized in Table 4. It highlights that, whatever the genetic type, fertilization and hatching rates decreased significantly (p = 0.01) with the BMIL. A comparison of fertilization and hatching rates between genetic types reveals significantly higher values for hybrid cross. As for productivity and economic profitability, values increased significantly with the BMIL and independently of genetic type. Furthermore, values were null

Table 4. Effect of biosecurity measures implementation levels and genetic type on zootechnical characteristics and economic profitability of *Clarias gariepinus* hatchery farming.

Zootechnical characteristics and economic profitability	Implementation levels of biosecurity measures in the hatcheries								
	Low			Intermediate			High		
	T1	T2	Overall	T1	T2	Overall	T1	T2	Overall
Fertilization rate (%)	$59.50^{a}\pm0.71$	$74.50^{a'}\pm0.69$	$67^{A}\pm0.70$	$53.50^{b}\pm0.60$	$70.50^{b'}\pm0.59$	$62^{B}\pm0.65$	$44^{c}\pm1.41$	$72.50^{c'}\pm0.71$	$58.25^{C}\pm1.06$
t (p)	21(0.01*)			24(0.01*)			25(0.01*)		
Hatching rate (%)	$54.50^{a}\pm0.70$	$71.75a'\pm0.35$	$63.13^{A}\pm0.53$	$50.25^{b}\pm0.37$	$67.25^{b'}\pm0.36$	$58.75^{B}\pm0.30$	$45^{c}\pm1.42$	$70.25^{c'}\pm0.35$	$57.75^{B}\pm1.06$
t (p)	30 (0.01*)			48 (0.01*)			24 (0.01*)		
Productivity (g/day/m^3)	0^{a}	$0^{a'}$	0^{A}	$1.67^{a}\pm0.01$	$27.55^{b'}\pm0.07$	$14.61^{B}\pm0.04$	$171^{b}\pm1.41$	$326.50^{c'}\pm2.12$	$248.80^{C}\pm0.35$
t (p)	-	-	-	507(0.001*)			86.26 (0.001*)		
Economic profitability (g/day/m^3/USD	0^{a}	$0^{a'}$	0^{A}	$0.03^{b}\pm0.00$	$0.48^{b'}\pm0.01$	$0.26^{B}\pm0.00$	$3.10^{c}\pm0.00$	$5.95^{c'}\pm0.01$	$4.53^{C}\pm0.00$
t (p)	-	-	-	39(0.001*)			255(0.00*)		

T1: Pure strain (Cameroon x Cameroon); T2: Crossbreds or Hybrids (Cameroon x Benin); A,B, C: for the same row, the values assigned to the different letters differ significantly (p <0.05) between the levels of implementation of the biosecurity measures; a,b,c: for the same row and T1 treatment, the values assigned to the different letters differ significantly (p <0.05) between the levels of implementation of the biosecurity measures; a',b',c': for the same row and T2 treatment, the values assigned to the different letters differ significantly (p <0.05) between the levels of implementation of biosecurity measures; t: student's t test; p: probability of error; USD: United States Dollars; -: no value; *: Significant.

for a low BMIL and increased by around 17 times when the BMIL rose from medium to high. The comparison of productivity and economic profitability between genetic types showed values around 16 and 2 times higher (p = 0.01) in crossbreds than pure strain respectively for medium and high BMILS.

Discussion

Mortality rates decreased significantly as the BMIL increased. Thus, in a fish farm, the higher the hygiene conditions, the lower the mortality rate. This is the expected result, as the compliance with the biosecurity measures reduces the risk of transmission of pathogens to fish, hence the high mortality rates (100%) in the hatchery where the BMIL was low. The beneficial effect of biosecurity practices on fish mortality rates has been reported in Ivory Coast by Kone *et al*. (2012). The latter recorded lower mortality rates in pond-reared Oreochromis niloticus than in the present study, i.e. 40%, 25% and 5% respectively for low (5%), medium (55%) and high (85%) BMILs. The discrepancy in experimental conditions (culture facilities, fish species and development stages, biosecurity measures applied, etc.) is thought to account for this observation. In particular, the fact that the present study was carried out in a hatchery. Indeed, the probability of disease occurrence and therefore mortality is higher in hatcheries due to the fragility of the immune system of larvae and fry compared to other developmental stages. Fish acquire adaptive immunity with age (Fonkwa *et al*., 2023a). A mortality rate of 7.74% caused by Yersiniosis (*Yersinia* sp) was observed by Fonkwa *et al*. (2022) in Oreochromis niloticus and *Cyprinus carpio* farmed (ponds) in Cameroon, where the BMIL was medium (37.50%). Mortality rates were significantly higher in pure strains than in crossbreds/hybrids, highlighting hybrid vigor or heterosis. Gene mixing in crossbreds is thought to be the source of disease resistance induced by improved immune response. In particular, the fact that the present study was carried out in a hatchery. Indeed, the probability of disease occurrence and therefore mortality is higher in hatcheries due to the fragility of the immune system of larvae and fry compared to other developmental stages. Fish acquire adaptive immunity with age (Fonkwa *et al*., 2023a).

The prevalence of pathologies decreased significantly with BMIL. Thus, the implementation of biosecurity measures in hatcheries has resulted in limiting the penetration and transmission of pathogens responsible for the anatomical, ethological and physiological abnormalities observed in fish. Gene mixing in hybrids is thought to be the cause of the prevalence of pathologies being around two times significantly higher in the pure strain than in the hybrids. In other words, the hybrids were more resistant to pathologies.

Both fertilization and hatching rates were higher in treatment T2 (female Cameroon x male Benin), irrespective of BMIL. Gametes from the two brood stocks strain would have physiological affinities likely to increase fertilization rates. The genetic material would have improved during fertilization, hence the higher hatching rate of eggs in treatment T2 compared with the pure strain (T1), in which the hatching rate decreased with BMIL. Crossbreds' eggs are apparently more resistant to disease. The values of the fertilization and hatching rates were lower than those obtained by Nguenga *et al*. (2000) and higher than those reported by Tiogue *et al*. (2008). The characteristics of the broodstock (origin, weight, age) and therefore the quality of the gametes, as well as variations in the geoclimatic characteristics of the study areas could explain this disparity in the findings.

The productivity and economic profitability increased with BMIL. They were null for the low BMIL and approximately doubled for the hybrids (T2). The application of biosecurity measures would have limited the spread of pathologies, thereby limiting fish death and the spread of clinical signs of pathologies, which are the main factors in the decline in productivity and profitability of the farm.

Conclusion

At the end of this study, it was clear that compliance with biosecurity measures and genetic types improved the epidemiological and zootechnical characteristics of *Clarias gariepinus* in hatcheries. Farmers are advised to emphasize biosecurity practices and use Cameroon x Benin hybrids for efficient, healthy, profitable and sustainable fish farming.

Conflict of interest

The authors declare that they have no conflicts of interest.

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