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Effects of *Kigelia africana*, *Beta vulgaris* and *Ricinodendron heudelotti* as feed additive on growth performance, survival and whole-body composition of Nile tilapia (*Oreochromis niloticus*) Linnaeus, 1758) fry

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Abstract
The effect of three African medicinal herbs used as feed additive on the growth and survival of Nile tilapia (*Oreochromis niloticus*) was studied. Four isoproteins (32%) and isolipid (13%) diets, namely RC (control diet or 0% plant), 3% *B. vulgaris*, 3% *K. africana* and 3% *R. heudelotti*, were manufactured. One hundred and eighty (180) Nile tilapia fry with an initial average weight of 0.15 ± 0.01 g were randomly distributed in 12 plastic tanks (50 L / tank) at a density of 15 fish per tank and fed three times (8.00 h am, 1.00 h pm and 5.00 h pm) per day for 90 days. For the parameters studied (average weight gain (AWG), feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR)) no significant difference was observed between diets at the end of the experiment. Furthermore, the addition of 3% *K. africana* or *B. vulgaris* or *R. heudelotti* had no impact on the dry matter, ash, crude protein and whole-body fat content of Nile tilapia fry. This study showed that 3% *B. vulgaris* or *K. africana* or *R. heudelotti* has no adverse effect on the growth, survival and whole-body composition of Nile tilapia fry.

Keywords: *Kigelia africana, Beta vulgaris, Ricinodendron heudelotti*, additives, growth, whole-body composition

Introduction
The Nile tilapia (*Oreochromis niloticus* L., 1758), a species of the family Cichlidae, is one of the major freshwater fish species produced in the world [1]. The intensification of aquaculture systems has triggered the occurrence of many diseases. On the other hand, antibiotics use has caused many issues such spread of drug-resistant pathogens, suppression of aquatic animal immunity, and adverse environmental effects [2]. In this respect, measures to reduce and develop alternatives are becoming a priority for international organizations (FAO, WHO, OIE, EFSA). For these reasons, the aquaculture industry's interest in plant resources is increasing day by day [3]. Plant products stimulate growth rate and immunity due to active compounds such as alkaloids, terpenoids, saponins and flavonoid components [4]. The World Health Organization also encourages the use of herbs and medicinal plants to replace or minimize the use of chemicals. Herbal additives can be used for phytotherapy, immunostimulant effect and growth promoter in aquaculture [5]. *R. heudelotti* is a tree of the Euphorbiaceae family that can reach a height of 50 m and a circumference of 2.7 m on average. The seed powder of *R. heudelotti* contains 31.4% protein and 44.7% lipids, of which 73% are polyunsaturated [6]. It is one of the most widely used plants in therapeutics since ancient times. In Africa, it is used for its anti-inflammatory, antimicrobial, and anti-aging skin effects. Studies by Olasehinde *et al.* [7] showed that *R. heudelotti* nuclei's essential oil has phytochemical compounds and metals of medicinal importance. Beet (*Beta vulgaris* L.) is a herbaceous plant belonging to the Chenopodiaceae family. It is famous for its juice value and medicinal properties [8]. The chemical and mineral composition shows that the moisture content was 87.4%, fat 0.3%, protein 1.35%, carbohydrates 7.59%, crude fiber 1.9% and ash 1.4% [9]. The beet could be incorporated up to 300g/kg without changing the growth and feed efficiency parameters of Tilapia Nile (*Oreochromis niloticus*) [10].

It is an excellent dietary supplement rich in minerals, nutrients and vitamins. It has unique phytoconstituents, which have several medicinal properties. *Kigelia africana* (Lam Benth) is a plant from tropical Africa widely grown and distributed throughout central, southern and western Africa. It belongs to the family Bignoniaceae. In Malawi, the seeds are roasted for human consumption [11]. These seeds are potentially rich in certain essential fatty acids such as linolenic and linoleic acids [12]. *K. africana* is an antioxidant, and these phytochemicals, nutritional and mineral constituents make *K. africana* an interesting plant that can be used to cure many diseases and as a food additive [13]. The objective of this study was to evaluate the impact of *K. africana*, *B. vulgaris* and *R. heudelotti* as feed additive on the growth performance, survival and whole-body composition of Nile tilapia (*O. niloticus*) fry.

Material and Methods

Medicinal plants

The medicinal plants such as *K. africana*, *B. vulgaris* and *R. heudelotti* were selected on the basis of scientific literature. The seed powders *K. africana* and *R. heudelotti* and the roots of *B. vulgaris* were purchased from the local market. The roots of *B. vulgaris* was washed, pressed, dried and processed into powder in the IUPA experimental unit. The powders were stored in glass jars until their use.

Experimental diets

The diets were formulated to be isonitrogenic (32% CP) and isolipidic (13% CL). One control diet and three test diets were manufactured. The control diet and the three test diets, i.e. 3% *B. vulgaris*, 3% *K. africana* and 3% *R. heudelotti*, were manufactured to obtain 1 kg of feed. The composition of the different diets is recorded in (Table 1).

After pre-treatment of the ingredients (removing certain impurities), they were weighed according to the diet composition (Table 1) and mixed. 30% of water was added to the mixture to obtain malleable dough. This dough was transformed by a grinder (Moulinex) into filaments like spaghetti. The diets were dried in an artisanal dryer for 1 h at 36 °C. These dried filaments were grounded to powder using a mortar before being packaged in glass jars for fry feeding.

Study environment and fish feeding

The study was conducted in the University Institute of Fisheries and Aquaculture (IUPA) experimental unit at the Cheikh Anta Diop University of Dakar (UCAD). One hundred and eighty (180) Nile tilapia fry with an initial mean weight of 0.15 ± 0.01 g were randomly assigned to 12 plastic tanks (50 L / tank) at the density of 15 fish per tank in an isolated system. At the beginning of the test, the weight of each fish was taken. The fish fasted 24 h before weighing. Each tank was equipped with an air pump for the oxygenation of the water. The tanks were cleaned daily to remove fecal matter and any leftover feed.

The experimental fish, divided into four batches, were fed with the four diets. They were fed manually during 90 days of experimentation at 15% of their biomass three times a day (8.00 h am, 1.00 h pm, and 5.00 h pm). The quantities of feed distributed to the fish were readjusted after each sampling according to the biomass's evolution.

Growth parameters, survival and feed efficiency

Sampling was carried out every 15 days by weighing each fish. At the end of the experiment, all fish were individually weighed using a scale to determine average weight gain (AWG (g)), specific growth rate (SGR (% /day)), feed conversion ratio (FCR) and survival rate (SR (%)). The equations used to calculate the results for each parameter were as follows:

- AWG (g) = Wf - Wi;
- SGR (% / day) = 100 * (lnWf - lnWi) / T;
- FCR = Total dry weight of diet fed (g) / weight gain (g);
- SR = (number of final fish / number of initial fish) * 100.

where Wf and Wi are respectively the final average weight and the initial average weight and T = time in days.

Chemical analysis of experimental diets

Samples of *K. africana* and *R. heudelotti* seed powder, *B. vulgaris* roots powder and the experimental diets were sent to the laboratory for biochemical analysis to determine their composition (dry matter, crude protein, ash, and fat). After the feeding trial, the whole body of the fish fed the different diets was freeze-dried and analyzed for dry matter, crude protein, ash and fat content.

The analysis was based on the Association of Official Analytical Chemists (AOAC) procedure. Samples of fish feed and fish muscle were dried to a constant weight at 103 °C for 24 h to determine the moisture content and consequently the dry matter values were determined. Crude protein (total nitrogen x 6.25) was determined using the Kjeldahl method. Fat was determined by the Randall method (extraction of fat with petroleum ether) with prior hydrolysis. The ash was determined by incineration of samples in a muffle furnace at 550 °C for 4 h.

Statistical Analysis

Microsoft Excel was used to enter and calculate the data. The analysis of these data was carried out with the Statistical Analysis System software (SAS-PC) [14] subjected to an analysis of variance (ANOVA). The results were presented as means of the three replicates. The Duncan test was used to compare significant differences between treatments. The difference was considered significant at P-values < 0.05.

Table 1: Gross composition of experimental diets per kilogram

Ingredients (g)	Experimental diets			
	Control	3% *K. africana*	3% *B. vulgaris*	3% *R. heudelotti*
B. vulgaris	0	30	0	0
K. africana	0	0	30	0
R. heudelotti	0	0	0	30
Fish meal	320	320	320	320
Millet bran meal	200	200	200	200
Rice bran meal	100	100	100	100
Fish oil	50	50	50	50
Corn flour	250	220	220	220
S. cerevisiae	40	40	40	40
Gum *Sterculia*	20	20	20	20
Mineral premix [a]	10	10	10	10
Vitamins premix [b]	10	10	10	10
Total (g)	1000	1000	1000	1000

a = phosphorus 7%; calcium 17%; sodium 1.5%; potassium 4.6%; 7.5% magnesium; manganese 738mg; 3000mg zinc; 4000mg iron; 750mg copper; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine content of the complex 1.5% (approximately), dose 1kg per 100kg of food.

b = lives at 250000 IU; lives D3 250000UI; lives E 5000mg; lives B1 100mg; lives B2 400mg; lives B3 (pp) 1000mg; saw B5 pentode Ca2000mg; lives B6 300mg; lives K3 1000g; saw C 5000mg; H biotin 15mg; choline 100g; special expiant (anti oxidant (BHT), attapulgite crushed and calcined) qs 1000mg.

Table 2: Chemical composition of *K. africana* and *R. heudelotti* seed powder and *B. vulgaris* roots powder

Additives	Dry matter (%)	Ash (%)	Crude protein (%)	Fat (%)
B. vulgaris	82.00	11.09	13.98	0.63
K. africana	90.24	16.51	18.31	3.12
R. heudelotti	90.29	8.52	27.83	3.87

Results

The survival rate was above 98% in all experimental groups and no significant difference between treatments was detected ($P > 0.05$). Water temperature was monitored daily and ranged from 24.4 to 26.5 °C.

At the end of the experiment, parameters such as average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were determined for each treatment. The ANOVA treatment of the different parameters studied didn't show any significant difference ($P > 0.05$) between treatments (Table 3).

Table 3: Growth performance and feed efficiency parameters of Nile tilapia fry fed experimental diets

Experimental diets	Parameters			
	AWG (g)	SGR (%/d)	FCR	SR (%)
Control	2.28±0.51	4.89±0.07	1.43±0.01	100.00
3% *K. africana*	2.21±0.22	4.88±0.19	1.50±0.05	100.00
3% *B. vulgaris*	2.11±0.04	4.77±0.02	1.47±0.04	100.00
3% *R. heudelotti*	2.11±0.09	4.81±0.09	1.49±0.01	98.00

AWG (g): average weight gain; SGR (%/d): specific growth rate; FCR: feed conversion ratio; RS: survival rate

The chemical composition of the different diets, i.e. the control diets, 3% *K. africana*, 3% R. *heudelotti* and 3% *B. vulgaris* is shown in (Table 4). No significant effect was observed following the addition of 3% *K. africana* or *B. vulgaris* or *R. heudelotti* on dry matter and ash contents. A slight increase in protein content and a slight decrease in fat content was observed following the addition of 3% *K. africana* compared to the control diet.

Table 4: Chemical composition of the different experimental diets

Experimental Diets	Dry matter (%)	Ash (%)	Crude protein (%)	Fat (%)
Control	89.93	9.49	30.00	12.30
3% *K. africana*	90.48	9.60	32.15	11.90
3% *B. vulgaris*	91.56	9.96	30.06	12.24
3% *R. heudelotti*	91.17	10.09	31.52	12.44

The results drawn from the whole body chemical analysis of tilapia fry fed the experimental diets are shown in Table 5. The results showed that the levels of dry matter in the whole bodies of the fry fed the diets containing 3% additive, i.e., 3% *K. africana* or 3% *B. vulgaris* or 3% *R. heudelotti* were significantly different from that of the tilapia fry fed the control diet (without additive). The 3% *B. vulgaris* and 3% *R. heudelotti* diets slightly increased the dry matter, crude protein and fat contents and slightly decreased the whole body ash content of tilapia fry compared to the whole body of fry fed the control diet (without additives). The ash content of the whole bodies of the fry fed the diets containing 3% additive, i.e., 3% *R. heudelotti* or 3% *B. vulgaris* were lower that of fish fed the control diet. The Ash content of the whole bodies of the fry fed the diets containing 3% *K. africana* were not significantly different from the fish fed the control diet. The crude protein of the whole bodies of the fry fed with additives increased compared to that of fish fed with the control diet. The whole body crude protein of the fish fed diet containing 3% *K. africana* was higher than that of fish fed 3% *R. heudelotti* or 3% *B. vulgaris* and the control. The crude protein content of the fish fed with 3% *R. heudelotti* was not significantly different from the fish fed with the control diet. The fat content of fish fed with the diets containing 3% *R. heudelotti or* 3% *B. vulgaris* was not different from the fish fed with the control diet. The fat content of fish fed with the diet containing 3% *K. africana* was not different from the fish fed with the control diet.

Table 5: Whole body chemical composition of Nile tilapia fry fed experimental diets

Experimental diets	Dry matter (%)	Ash (%)	Crude protein (%)	Fat (%)
Control	90.2	15.9	55.9	19.8
3% *K. africana*	91.5	16.7	60.1	17.3
3% *B. vulgaris*	91.6	14.3	57.4	20.8
3% *R. heudelotti*	91.3	14.1	56.4	21.0

Discussion

The results of the present study showed no significant difference ($P>0.05$) for the growth and feed efficiency parameters, i.e., average weight gain (AWG), specific growth rate (SGR); feed conversion ratio (FCR) and survival rate (SR) for the different treatments. The results of the chemical analysis also showed no significant difference between treatments. The recorded FCRs are in the range (1.4-2.4), which is optimal for farmed tilapia [15]. According to Ahmad and Abdel-Tawwab [16], argued that FCR could measure fish production efficiency. SRs greater than 98.5% suggested that supplementation with 3% *B. vulgaris* or *K. africana* or *R. heudelotti* in the basal diet has no toxic effect on Nile tilapia fry according to Jha *et al*. [17] who argued, based on their results on survival rate between 98.5 and 100%, that 20 g of cumin seed meal as a feed additive per kilogram of feed had no toxic effect on *O. niloticus* fry. This study's results are similar to the findings of Jha *et al*. [18] following the addition of 3% *B. vulgaris* in the diet of *Barilius Bendelisis*. In addition, 3% *B. vulgaris* does not affect growth parameters such as weight gain and specific growth rate of *Schizothorax richardsonii* fry [19]. Also, incorporation of up to 300 g/kg of *B. vulgaris* fodder beets had no negative effect on feed conversion ratio (FCR), specific growth rate (SGR) and weight gain (WG) [20]. *K. africana* seed powder could be incorporated up to 100g/kg wit hout affecting the growth and survival of *Clarias gariepinus* [21]. This study's results are similar to that of Ahmadifar *et al*. [22], who showed that supplementation up to 200mg/kg of *Tetracarpidium conophorum* seed powder as a dietary supplement on the reproductive indices of male *Clarias gariepinus* broodstock has no negative effect on growth and survival. Furthermore, ginger powder supplementation of up to 3% for eight weeks has no negative effect on the growth (RMS and weight gain) and survival of zebrafish (*Danio rerio*) [23]. The addition of 3% Black Garlic Seed and Commercial Biogenes to the diets of Nile Tilapia (*O. niloticus*) also showed no significant difference in growth performance between the different diets implemented [24]. The results of this study are similar to the work of Ekanem *et al*. [25], who added 70 g of *S. jamaicensis* leaf meal and 35 g of *G. kola* + 35 g of *S. jamaicensis* leaf meal in the diet of *Clarias gariepinus*. They showed no significant difference (*P*>0.05) for weight gain, SGR and FCR between treatments. According to El-Dakar [26], most spices and herbs can be considered non-nutritive feeds because of their small amounts used in fish feed. The composition of the diets in this study is similar to that of Hassan *et al*. [27] although the lipid contents are more important in our study. Lipid contents in the present study are in the range of dietary lipid requirements for tilapia (10-15% dry matter) [28-30]. Whole-body chemical analyses of Nile tilapia fry in this study to determine the influence of the experimental diets on dry matter, crude protein, fat, and ash content did not show significant differences between treatments. This study's results are similar to the findings of Mathew *et al*. [31], which showed that the incorporation of 3% *Phoneix dactylifera* seed powder does not affect the body composition (dry matter, protein, ash, and fat) of Nile tilapia.

Conclusion
The results of this study show that supplementation of 3% g of *B. vulgaris* or 3% of *K. africana* or 3% of *R. heudelotti* in one kilogram of basal diet does not alter the parameters of growth (AWG, SGR and FCR), survival and whole-body composition of Nile tilapia fry. However, further research could be implemented to explore the serological and immunological parameters of Nile tilapia fed with a diet containing *B. vulgaris* or *K. africana*, or *R. heudelotti*.

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