



## Optimizing feed utilization and reducing deterioration of African catfish feed with sodium propionate supplementation

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### ABSTRACT

A 56-day, two-phased experiment was conducted to investigate the effect of sodium propionate (NaP) on the growth performance of African catfish and the feed quality. One hundred juveniles with an average body weight of 50.47 g ( $\pm$  4.60) were procured and used for the study's first phase. Fish were randomly assigned to five groups (A, control group), B, C, D, and E) and replicated four times with five fish each. Respective groups were fed diets containing NaP at 0, 1.67, 3.33, 5.00, and 6.67 g kg<sup>-1</sup> feed, respectively. For the second trial, the individual diets were analyzed for quality characteristics on 0-, 28- and 56-days of storage, using a mixed-model analysis of variance. Results showed significant differences in most growth performance parameters among groups except for the final body length and condition factor. Group C had lower final body weight (FW), weight gain (WG), average feed intake (AFI), specific growth rate (SGR), and higher feed conversion ratio (FCR) than the control. However, these parameters did not differ in Group D, which also showed lower AFI compared to the control. Increasing NaP decreased the sensory attribute scores of the feed and its crude protein (CP), ether, and ash levels while increasing moldiness, crude fiber, nitrogen-free extract, and thiobarbituric acid reactive substances (TBARs). NaP inclusion in feed resulted in a dose-dependent reduction in the feed deterioration, as seen in the lowered aggregate changes in sensory attributes, CP, moisture, and ash levels, with Group D having an optimal effect. The TBARs level (rancidity) decreased in Group B but increased in Group E during the study. The study concludes that there was a loss of fish feed quality with increasing storage time and that NaP particularly at 5.0 g kg<sup>-1</sup> feed optimally improves feed utilization and effectively lowers feed deterioration for 56 days. Therefore, the use of NaP is recommended for improved aquaculture production.

### 1. Introduction

Fish is an essential source of high-quality nutrition and vital to global food security. Aquaculture is the fastest-growing food production sector, offering economic growth, environmental and ecosystem benefits, and supplementing wild-caught fish options (Troell et al., 2023; Stead, 2019; Stevens et al., 2018). According to earlier reports, world aquaculture production (live weight) more than tripled between 1997 and 2017 (Naylor et al., 2021). Sustaining this growth trajectory can help achieve various United Nations Sustainable Development Goals (SDGs) by providing a means to satisfy the global demand for fish and high-quality

nutrition in a sustainable and climate-friendly manner (Naylor et al., 2021; National Oceanic and Atmospheric Administration, NOAA Fisheries, 2020; Stead, 2019). However, the aquaculture sector faces various challenges. In many developing countries, such as Nigeria, overreliance on costly foreign feeds inflates production expenses, reducing farm profitability (Onuche et al., 2020; Naylor et al., 2000). This has necessitated increased fish feed production using common low-cost ingredients (Couture et al., 2019; Gabriel et al., 2007). Fish's health and final quality largely depend on the fish feed quality (Aleström and Winther-Larsen, 2016). Consequently, in line with the high cost associated with farm feed procurements, feed management, and biosecurity

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are critical for ensuring aquaculture production development and sustainability.

Fish feeds are susceptible to spoilage due to their ingredients' characteristic nature. The grade of stored feed is negatively impacted by various factors, including insect and microbial infestations from poor handling; and physicochemical alterations resulting from varying temperature and humidity conditions (Nyong and Olubunmi, 2014; Sancho-Madriz, 2003). Many common fungi species (such as the molds and *Aspergillus* spp.) are known to attack stored feed products and produce aflatoxins, patulins, and trichothecens (Mohamed et al., 2017; Santacroce et al., 2008). These substances result in feed spoilages, reduced growth, and disease incidences in fish and can also cause liver damage, cancers, and mutations in human consumers (Ciceron et al., 2008; Russo and Yanong, 2006). Also, aquaculture feeds contain appreciable quantities of fish products (oil and meal) which contain high amounts of polyunsaturated fatty acids (abbreviated as PUFAs) (Hua et al., 2019). These PUFAs are susceptible to oxidation reactions during storage and can turn rancid at high storage temperatures, accompanied by foul smells, rendering the feed unusable (Desai et al., 2014). Malondialdehyde (MDA) is a metabolite of PUFA oxidation used as an important indicator of lipid peroxidation processes and obtained through the thiobarbituric acid reactive substances (abbreviated as TBARS) test (De Lima Júnior et al., 2013; Min and Ahn, 2005). Due to its simplicity and correlation with the sensory properties of feed, the TBARS value is a reliable parameter for assessing lipid oxidation levels in food products (Ayala et al., 2014).

The prevailing high temperature and humidity conditions in many tropical regions favor microbial proliferation, lipid oxidation, and hydrolytic rancidity processes (Adaga, 2014; Effiong and Alatise, 2009; Mohapatra et al., 2017; Solomon et al., 2016). These vagaries adversely affect these feeds' attractiveness, flavor, taste, and nutrient contents, possibly producing harmful toxicants. Therefore, appropriate measures are considered during the processing and storage of these feeds in order to limit feed contaminations and deteriorations for enhanced shelf life. Sodium propionate ( $\text{Na}[\text{C}_2\text{H}_5\text{COO}]$ , abbreviated as NaP) is a white, deliquescent, crystal-like, and compact product from propionic acid ( $\text{C}_2\text{H}_5\text{COOH}$ ) reacting with sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (Aba et al., 2018). Their widespread use as preservatives in feed production is mainly due to their ability to suppress microbial growth in feedstuff, limiting their contamination and recontamination in feed (Bobadilla-Carrillo et al., 2020). There are more than a few studies previously on the impacts of storage methods and durations on the quality of stored fish feeds and feedstuffs (Aanyu and Ondhoro, 2016; Fagbohun and Lawal, 2011; Hossen et al., 2011). Also, attempts have been made to evaluate microbial growth and spoilage in feed ingredients and fodder materials preserved using different concentrations of propionic and formic acids or their salts (Cherrington et al., 1990; Lv et al., 2020; Malicki et al., 2004; Shin et al., 2002). However, information about using organic acids or their salts as preservatives in aquaculture feeds is scarce.

Further, organic acids and their salts, such as NaP, have been found to enhance gut health and functionality, growth performance, and general well-being in various aquatic species, including European sea-bass (*Dicentrarchus labrax*) (Wassef et al., 2020), Beluga (*Huso huso*) (Ahmadifar et al., 2022), Caspian white fish (*Rutilus kutum*) (Hoseinifar et al., 2016), and common carp (*Cyprinus carpio*) (Liu et al., 2014; Safari et al., 2021). However, despite the potential benefits of NaP as a feed additive, there is a paucity of information on its effect on the growth performance of African catfish (*Clarias gariepinus*). The African catfish is a significant species in Nigeria's aquaculture industry due to its fast growth rate, high fecundity, omnivorous feeding habit, and high tolerance to disease and poor rearing conditions (Obwanga et al., 2018; Ozigbo et al., 2014). Its economic importance lies in its value as a source of food and income, as well as its potential for export. In a previous study, we reported on the impact of NaP on the liver histomorphological changes and oxidative stress status of African catfish (Aba et al., 2018).

The present study aims to investigate the effects of storage time and NaP treatment on the sensory quality and proximate compositions of African catfish feed. Furthermore, we investigated the impact of dietary NaP supplementation on the growth performance of African catfish to optimize aquaculture production.

## 2. Materials and methods

### 2.1. Study location and duration

The trial was conducted for two months (between 16th April and 11th June 2017) at the Nutrition and Biochemistry Laboratory of the Department of Animal Science, University of Nigeria, Nsukka (UNN), Nigeria. Nsukka is an area in Southeastern Nigeria, stuck between latitude  $6^{\circ}43' - 7^{\circ}00'$  and longitude  $7^{\circ}13' - 7^{\circ}35'$  (Ozor et al., 2015). It has characteristic wet and dry seasons typical of the West African region. While its rainy period spans between April and October, the dry period is witnessed between November and March (Onyishi et al., 2018). As reported, the maximum and minimum temperature variables are  $37.4^{\circ}\text{C}$  (in March) and  $17.9^{\circ}\text{C}$  (in December), respectively (Yusuf et al., 2017), with a mean annual temperature, rainfall, and relative humidity values of  $24.9^{\circ}\text{C}$ , 1579 mm, and 75%, respectively (<http://www.climate-data.org>). The temperature and relative humidity values of the study area during the experimentation are shown in the timeline chart (Fig. 1). The mean ( $\pm$  SD) temperature and relative humidity values for the various months were April ( $32.27 \pm 0.98^{\circ}\text{C}$  and  $65.03 \pm 4.11\%$ ), May ( $30.45 \pm 1.74^{\circ}\text{C}$  and  $72.09 \pm 5.28\%$ ), and June ( $29.03 \pm 1.15^{\circ}\text{C}$  and  $77.97 \pm 3.36\%$ ).

### 2.2. Ethics statement

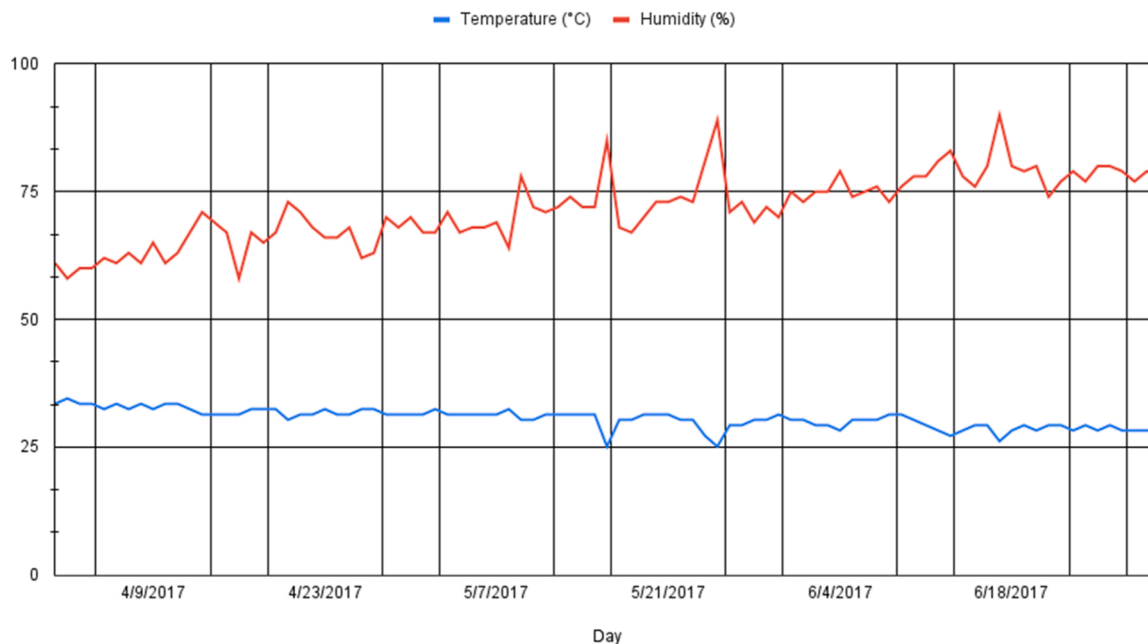
All experimental procedures involving animals were carried out with the approval of the Animal Welfare and Ethics Committee (DAS-UNN-IAUCC-2017-017) and following ethical standards for biomedical research on both human and animal subjects, as outlined in the UNN Research Policy Document (Research Ethics Committee Recommendations, 2013).

### 2.3. Experimental fish and diets

For the growth performance study, one hundred mixed-sex juveniles of African catfish with similar body weight ( $50.47 \pm 4.60$  g) and standard length ( $15.60 \pm 0.78$  cm) were procured from the same African catfish breeding stock and hatchery Unit of the Department of Zoology, UNN. The fish were initially acclimatized for one week during which they were fed a similar basal diet. The fish were randomly assigned to five experimental groups of A, B, C, D, and E in a completely randomized design and replicated four times with five fish each and kept in individual rectangular plastic bowls (20 L capacity). For the experiment, a basal fish diet was formulated to contain 42% crude protein and 12 KJ  $\text{g}^{-1}$  of Metabolizable energy (Table 1). The respective treatment groups were fed basal diet supplemented with NaP (Sigma-Aldrich, St. Louis, MO, USA; Assay:  $\geq 99.0\%$ ) at the levels of 0, 1.67, 3.33, 5.00, and 6.67 g  $\text{kg}^{-1}$  feed (as elaborated later). Before the commencement of the study, the body weight and standard length of the fish were measured and recorded, respectively, and afterward, the fish were introduced to the experimental diets. The feeding trial lasted 8 weeks and was done at 5% of the gross body weight given twice daily (08:00 a.m.; 03:00 p.m.). The remaining uneaten feed pellets were collected 1 h post-feeding and used for calculating feed intake.

### 2.4. Growth performance

The fish were first fasted for 24 h to evaluate the growth performance. Afterward, the body weight and total length measurements of each replicate group were taken under anaesthetics ( $0.1$  g  $\text{L}^{-1}$  Tricaine



**Fig. 1.** Daily temperature and relative humidity values between 1st April and 30th June 2017 in Nsukka, Enugu State, Nigeria (Source: (Weather and Climate, 2017). Blue indicates the temperature in °C, and orange indicates the relative humidity in percentage (%).

**Table 1**  
Basic composition of experimental feed.

Ingredients	Compositions (%)
Fish meal	35
Soybean meal	43
Wheat offal	6
White corn	10
Multi-vitamins and amino acid supplements (Vitalyte)	3
Dicalcium phosphate (D.C.P.)	3

mesylate: MS-222). Measurements were made at the onset and the end of the study. The determination of growth performance and calculation of feed utilization were made using the following formulae:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight(g)}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln \text{ Final weight (g)} - \ln \text{ Initial weight(g)}}{\text{time (duration of the experiment)(day)}} \times 100$$

Where *ln* represents the natural logarithm

$$\text{Feed conversion rate (FCR)} = \frac{\text{Total feed Intake(g)}}{\text{Weight gain(g)}}$$

Where Total feed intake represents the sum of the feed offered to the fish throughout the study.

$$\text{Condition factor(K)} = \frac{\text{Weight of the fish(g)}}{\text{Fish Length(L)}^3(\text{cm})} \times 100$$

**2.5. Fish Feed Processing, Preservation, and Storage**

For the second experiment, the basal diet was also divided into five separate groups of similar NaP inclusions: 0, 1.67, 3.33, 5.00, and

6.67 g kg<sup>-1</sup> feed, which was also assigned, respectively, to Groups -A, -B, -C, -D, and -E. To process the feed, after the addition of NaP, diets in the various treatment groups were thoroughly mixed to get an even blend. Afterward, distilled water was added to the mix at the proportion of 3:7 before further mixing for 15 min. The formulated feeds were cooked for 15 min for easy palletization and then sundried for about 24 h before being taken to the laboratory for oven drying to a minimum moisture level. After that, feed samples in the respective groups were placed in different plastic containers, sealed, and stored immediately under room conditions until used for evaluation and analysis.

**2.6. Sensory evaluation and proximate analysis of the feed**

Descriptive sensory evaluations were carried out on the feed immediately after formulation (day 0) and subsequently, on days 28 and 56 of storage, respectively, of the study to assess the changes in the physical

characteristics of the feed. For this purpose, a sensory panel of carefully selected individuals (n = 6) engaged in several training sessions to correctly identify, describe, and characterize the various feed attributes. The characterization of the feed attributes was based on the indices earlier described by (Hossen et al., 2011), with score ratings assigned quantitatively on a 7-point scale according to references and scales developed during training as adapted from (Chumngoen and Tan, 2015). To evaluate for quality changes, the physical characteristics of color, odor, texture, mold infestations, and general quality remarks were considered, and individual scores were assigned to the feed samples, given the intensities of the attributes. Initially, the formulated feed samples in the various groups were observed to be shiny, hard, and dark brown, with a characteristic odor of soybean and fish meal blend. The assessors evaluated the intensities of the feed attributes and scored a 7-point rating with 1 = low and 7 = high (representing the original feed

characterization) for color, flavor, texture, and general remark. While the original feed characteristics represented the reference point or highest scores, the low ratings were considered according to their degrees of discolorations (color), crumbliness (texture), staleness or off odor (flavor), and unacceptability (general remarks) with 1 representing marked levels. The scoring was inversely done for feed moldiness and representative of the degree of mold development on the feed. The determinations of the feed proximate compositions of crude protein (abbreviated to CP), crude fiber (abbreviated to CF), ether extract (abbreviated to EE), ash, moisture, and nitrogen-free extract (abbreviated to NFE) were made according to standard methods of the Association of Official Analytical Chemists (AOAC, 2012).

### 2.7. Determination of thiobarbituric acid (TBA) reactive substances

To measure lipid peroxidation levels in the stored feed samples, determinations of thiobarbituric acid (abbreviated to TBA) reactive substances levels were made in triplicates for the respective groups on days 28 and 56 of the study, using the distillation procedure described by (Tarladgis et al., 1960). The determinations were made spectrophotometrically with the absorbance of the test samples taken at 530 nm. The TBARS values were calculated and presented as mg malondialdehyde per kg feed sample ( $\text{mg MDA kg}^{-1}$ ), with 1,1,3,3-Tetraethoxypropane used to construct the TBARS standard curve.

### 2.8. Statistical analysis

Data on the growth performance of the fish and the physical characteristics of the feed samples were subjected to one-way analysis of variance (ANOVA) using the Agricolae Statistical package (de Mendiburu and Yaseen, 2020) in R Studio (RStudio Team, 2022). As a post hoc test, Tukey's test was used to assess the significance of differences between pairs of group means. Data on the sensory evaluations, proximate compositions and the TBARS values were analyzed using Mixed-model ANOVA with the rstatix package (Kassambara, 2021) in R Studio. NaP treatment was considered the between-subject factor, while storage time was the within-subject factor. Differences in means were determined with Bonferroni corrections for multiple comparisons and accepted at a 0.05 probability level. Also, a One-way ANOVA was further carried out to assess corresponding changes in the proximate compositions of the preserved feed in each group over the various storage times. The normality of data was tested by the Shapiro-Wilk test. The homogeneity of variances was tested with the Levene's test.

**Table 2**  
Growth performance of African catfish fed varying levels of NaP.

Parameters	Groups (Dietary levels of NaP in $\text{g kg}^{-1}$ diet)					SEM	P-Value
	A (control) 0	B 1.67	C 3.33	D 5.00	E 6.67		
IW (g)	50.28	50.81	51.00	49.00	51.25	1.03	0.97 <sup>NS</sup>
FW (g)	138.82 <sup>a</sup>	133.70 <sup>a</sup>	110.17 <sup>b</sup>	144.82 <sup>a</sup>	125.78 <sup>ab</sup>	3.94	< 0.05*
WG (g)	88.5 <sup>ab</sup>	82.75 <sup>ab</sup>	59.00 <sup>c</sup>	95.75 <sup>a</sup>	74.5 <sup>bc</sup>	3.70	< 0.05*
IL (cm)	15.62	15.47	15.78	15.31	15.85	0.18	0.93 <sup>NS</sup>
FL (cm)	22.64	22.21	21.29	23.00	20.88	0.184	0.07 <sup>NS</sup>
LG (cm)	7.02 <sup>ab</sup>	6.74 <sup>ab</sup>	5.51 <sup>bc</sup>	7.69 <sup>a</sup>	5.03 <sup>c</sup>	0.29	< 0.05*
AFI (g)	212.07 <sup>b</sup>	198.59 <sup>bc</sup>	193.12 <sup>c</sup>	192.64 <sup>c</sup>	259.28 <sup>a</sup>	6.17	< 0.01*
FCR	2.49 <sup>b</sup>	2.40 <sup>b</sup>	3.30 <sup>a</sup>	2.03 <sup>b</sup>	3.48 <sup>a</sup>	0.14	< 0.001*
K	1.19	1.23	1.15	1.19	1.45	0.05	0.847 <sup>NS</sup>
SGR	1.80 <sup>ab</sup>	1.73 <sup>ab</sup>	1.38 <sup>c</sup>	1.93 <sup>a</sup>	1.61 <sup>b</sup>	0.05	< 0.001*

\* : row means with different superscripts (a,b,c) are significantly different ( $p < 0.05$ ); <sup>NS</sup>: Non-Significant; IW: Initial Body Weight; FW: Final Body Weight; WG: Weight Gain; IL: Initial Standard Length; FL: Final Standard Length; LG: Length Gain; AFI: Average Feed Intake; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; K: Condition factor.

## 3. Results

### 3.1. Experiment I: growth performance of fish

The results of the growth performance of the African catfish juveniles fed varying levels of NaP are presented in Table 2. Table 2 shows the results of the growth performance study too. The analysis revealed no significant differences ( $p \geq 0.05$ ) in the initial body weight, initial standard length, final standard length, and condition factor of fish among the treatment groups. However, there were significant differences ( $p < 0.05$ ) in the growth performance parameters of final body weight (FW), weight gain (WG), length gain (LG), average feed intake (AFI), feed conversion ratio (FCR), and specific growth rate (SGR). Group D (5 g NaP/kg diet) had the highest mean values of FW (144.82 g), WG (95.75 g), LG (7.69 g), and SGR (1.93), which were similar ( $p \geq 0.05$ ) to the control and Group B but different ( $p < 0.05$ ) from Group C, which had the lowest values.

Concerning AFI, Group D had the lowest values comparable to those of Groups B and C but differed from those of other groups. Similarly, Group D showed the lowest FCR value, similar to those of the control and Group B but differed from those of Groups C and E, which had the highest FCR values. For SGR values, the highest mean values were observed in Group D, similar to those of the control and Group B but different from those of the other groups. Overall, Groups C had the least values of AFI, FCR, and SGR in the study.

### 3.2. Experiment II: physical characteristics of stored fish feed

Table 3 summarizes the mean sensory attributes and physical characteristics of fish feed stored using NaP. The results of the mixed model ANOVA revealed a significant main effect of storage time (ST) on all the feed indices considered, including color ( $F_{2, 50} = 78.6$ ,  $p < 0.001$ ,  $\eta^2 p = 0.76$ ), odor ( $F_{2, 50} = 84$ ,  $p < 0.001$ ,  $\eta^2 p = 0.77$ ), texture ( $F_{2, 50} = 44.9$ ,  $p < 0.001$ ,  $\eta^2 p = 0.64$ ), moldiness ( $F_{1.9, 21.7} = 47.8$ ,  $p < 0.001$ ,  $\eta^2 p = 0.66$ ), and general remark ( $F_{2, 50} = 116$ ,  $p < 0.001$ ,  $\eta^2 p = 0.82$ ). As the storage time increased from day 0 to day 28 and subsequently from day 28 to day 56 of the study, there was a marked progressive decrease ( $p < 0.001$ ) in the original feed color, odor, texture, and general remark scores, as well as an increase ( $p < 0.001$ ) in the degree of moldiness.

A significant main effect of NaP treatment was also observed in feed color ( $F_{4, 25} = 35.7$ ,  $p < 0.001$ ,  $\eta^2 p = 0.85$ ), odor ( $F_{4, 25} = 34.1$ ,  $p < 0.001$ ,  $\eta^2 p = 0.85$ ), texture ( $F_{4, 25} = 21.7$ ,  $p < 0.001$ ,  $\eta^2 p = 0.78$ ), moldiness ( $F_{4, 25} = 30.7$ ,  $p < 0.001$ ,  $\eta^2 p = 0.83$ ), and general remark ( $F_{4, 25} = 48.7$ ,  $p < 0.001$ ,  $\eta^2 p = 0.89$ ). The control group showed the least mean scores in color, odor, texture, general remark, and the highest degree of moldiness, which differed ( $p < 0.05$ ) from the other groups. The feed samples in groups C, D, and E had the highest mean color, odor,

**Table 3**  
Physical attributes of fish feed containing levels of NaP and stored for 56 days.

Source	Color	Odor	Texture	Moldiness	General remark
<b>Storage Time</b>					
0 Day	6.667 <sup>a</sup>	6.700 <sup>a</sup>	6.733 <sup>a</sup>	1.267 <sup>a</sup>	6.733 <sup>a</sup>
28 Days	5.933 <sup>b</sup>	5.700 <sup>b</sup>	5.900 <sup>b</sup>	1.800 <sup>b</sup>	5.767 <sup>b</sup>
56 Days	4.800 <sup>c</sup>	4.500 <sup>c</sup>	4.700 <sup>c</sup>	2.900 <sup>c</sup>	4.133 <sup>c</sup>
SEM	0.414	0.378	0.347	0.306	0.342
<b>Treatments</b>					
Control	4.056 <sup>c</sup>	4.167 <sup>c</sup>	4.611 <sup>c</sup>	3.222 <sup>a</sup>	3.778 <sup>c</sup>
Group B	5.333 <sup>b</sup>	5.056 <sup>b</sup>	5.556 <sup>b</sup>	2.222 <sup>b</sup>	5.333 <sup>b</sup>
Group C	6.444 <sup>a</sup>	6.222 <sup>a</sup>	6.278 <sup>a</sup>	1.500 <sup>c</sup>	6.000 <sup>a</sup>
Group D	6.611 <sup>a</sup>	6.389 <sup>a</sup>	6.333 <sup>a</sup>	1.500 <sup>c</sup>	6.222 <sup>a</sup>
Group E	6.556 <sup>a</sup>	6.333 <sup>a</sup>	6.111 <sup>ab</sup>	1.500 <sup>c</sup>	6.389 <sup>a</sup>
SEM	0.207	0.214	0.247	0.2	0.209
<b>Interaction</b>					
Control x Day 0	6.67 <sup>a</sup>	6.83 <sup>a</sup>	6.83 <sup>a</sup>	1.17 <sup>a</sup>	6.67 <sup>a</sup>
Group B x Day 0	6.67 <sup>a</sup>	6.50 <sup>a</sup>	6.67 <sup>a</sup>	1.33 <sup>a</sup>	6.83 <sup>a</sup>
Group C x Day 0	6.67 <sup>a</sup>	6.83 <sup>a</sup>	6.67 <sup>a</sup>	1.17 <sup>a</sup>	6.67 <sup>a</sup>
Group D x Day 0	6.67 <sup>a</sup>	6.67 <sup>a</sup>	6.83 <sup>a</sup>	1.33 <sup>a</sup>	6.83 <sup>a</sup>
Group E x Day 0	6.67 <sup>a</sup>	6.67 <sup>a</sup>	6.67 <sup>a</sup>	1.33 <sup>a</sup>	6.67 <sup>a</sup>
Control x Day 28	3.67 <sup>b</sup>	3.83 <sup>b</sup>	4.33 <sup>b</sup>	3.33 <sup>b</sup>	3.17 <sup>b</sup>
Group B x Day 28	6.17 <sup>a</sup>	5.33 <sup>b</sup>	5.83 <sup>a</sup>	1.67 <sup>a</sup>	6.17 <sup>a</sup>
Group C x day 28	6.50 <sup>a</sup>	6.33 <sup>ab</sup>	6.33 <sup>a</sup>	1.33 <sup>a</sup>	6.17 <sup>ab</sup>
Group D x Day 28	6.67 <sup>a</sup>	6.67 <sup>a</sup>	6.67 <sup>ab</sup>	1.33 <sup>a</sup>	6.67 <sup>a</sup>
Group E x Day 28	6.67 <sup>a</sup>	6.33 <sup>a</sup>	6.33 <sup>ab</sup>	1.33 <sup>a</sup>	6.67 <sup>a</sup>
Control x Day 56	1.83 <sup>c</sup>	1.83 <sup>c</sup>	2.67 <sup>c</sup>	5.17 <sup>c</sup>	1.50 <sup>c</sup>
Group B x Day 56	3.17 <sup>b</sup>	3.33 <sup>c</sup>	4.17 <sup>b</sup>	3.67 <sup>b</sup>	3.00 <sup>b</sup>
Group C x Day 56	6.17 <sup>a</sup>	5.50 <sup>b</sup>	5.83 <sup>a</sup>	2.00 <sup>a</sup>	5.17 <sup>b</sup>
Group D x day 56	6.50 <sup>a</sup>	5.83 <sup>a</sup>	5.50 <sup>b</sup>	1.83 <sup>a</sup>	5.17 <sup>b</sup>
Group E x day 56	6.33 <sup>a</sup>	6.00 <sup>a</sup>	5.33 <sup>b</sup>	1.83 <sup>a</sup>	5.83 <sup>a</sup>
SEM	0.459	0.413	0.35	0.341	0.435
<b>P-values</b>					
Storage Time	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Interaction	< 0.001	< 0.001	0.001	< 0.001	< 0.001

<sup>a,b,c</sup> The values with different letters in the same column for each factor and the interaction are significantly different ( $p < 0.05$ ); SEM: standard error of mean; control – 0 g kg<sup>-1</sup> NaP; Group B – 1.67 g kg<sup>-1</sup> NaP; Group C – 3.33 g kg<sup>-1</sup> NaP; Group D – 5.00 g kg<sup>-1</sup> NaP; Group E – 6.67 g kg<sup>-1</sup> NaP

texture, and general remark scores and the lowest degree of moldiness, which were similar but differed from those of the control and Group B.

Furthermore, there was a significant interaction effect of storage time and NaP treatment on feed color ( $F_{8, 50} = 23.2$ ,  $p < 0.001$ ,  $\eta^2 p = 0.78$ ), odor ( $F_{8, 50} = 12.6$ ,  $p < 0.001$ ,  $\eta^2 p = 0.67$ ), texture ( $F_{8, 50} = 4.3$ ,  $p = 0.001$ ,  $\eta^2 p = 0.41$ ), moldiness ( $F_{7.6, 21.8} = 8.6$ ,  $p < 0.001$ ,  $\eta^2 p = 0.58$ ), and general remark ( $F_{8, 50} = 14$ ,  $p < 0.001$ ,  $\eta^2 p = 0.69$ ). Bonferroni-corrected post hoc tests showed marked deteriorations in mean feed color, odor, texture, moldiness, and general remark scores in the control group as storage time increased from day 0 (6.67, 6.83, 6.83, 1.17, and 6.67, respectively) to day 28 (3.67, 3.83, 4.33, 3.33, and 3.17, respectively), and from day 28 to day 56 (1.83, 1.83, 2.67, 5.17, and 1.50, respectively) of the study. Similar deteriorations were observed in the mean odor score of feed in Group B as ST was extended from day 0 to days 28 and 56, respectively, while these changes were observed to be significant for Group C only by day 56 of the study. The mean odor score for Groups D and E were observed to be similar and relatively unchanged throughout the study duration. Feed samples in Groups D and E had slight negative changes in their mean texture scores as storage proceeded from day 0 (6.83 and 6.67, respectively) to day 56 (5.50 and 5.33, respectively), whereas those of Group C remained relatively unchanged during the trial. While a marked ( $p < 0.001$ ) decrease in the feed color and increase in the moldiness score of feed samples in Group B were observed only by day 56 of the storage, those of Groups C, D, and E remained similar for the 56 days with no physical evidence of mold infestation (Fig. 2). Similarly, there were marked progressive negative changes in the general remark scores of the feed in the control group as storage time increased. Slight decreases ( $p < 0.001$ ) in the general remark scores of Groups B, C, and D were observed after day 56 of the study, whereas that of Group E remained relatively unchanged ( $p \geq 0.05$ ) during the study. Overall, there were deteriorative changes in

the sensory attributes of the feed samples as storage time increased in the study. Adding NaP to the feed slowed down these deteriorations.

### 3.3. Effect of storage time and NaP on chemical compositions of fish feed

A mixed-model ANOVA was conducted to evaluate the impact of storage time (ST) and NaP on the proximate compositions and TBARs levels of the feed. Table 4 presents the results which indicate that the main effect of storage time (ST) had a significant impact on all the measured proximate characteristics, including CP ( $F_{2, 20} = 10.96$ ,  $p < 0.01$ ,  $\eta^2 p = 0.523$ ), moisture ( $F_{2, 20} = 13.63$ ,  $p < 0.001$ ,  $\eta^2 p = 0.577$ ), ether ( $F_{1.26, 12.57} = 7.844$ ,  $p < 0.05$ ,  $\eta^2 p = 0.44$ ), ash ( $F_{2, 20} = 222.9$ ,  $p < 0.001$ ,  $\eta^2 p = 0.957$ ), CF ( $F_{2, 20} = 6.25$ ,  $p < 0.01$ ,  $\eta^2 p = 0.385$ ), NFE ( $F_{2, 20} = 55.0$ ,  $p < 0.001$ ,  $\eta^2 p = 0.846$ ), and TBARs values ( $F_{1, 10} = 6.46$ ,  $p < 0.05$ ,  $\eta^2 p = 0.392$ ). The results (Table 4) indicate that the percentage of CP levels decreased significantly ( $p < 0.05$ ) as storage time increased from day 0 to day 28 (27.04% and 25.24%, respectively), while no significant difference was observed when ST was extended from day 28 to day 56 (24.74%,  $p \geq 0.05$ ). The percentage of moisture levels in the feed initially decreased ( $p = 0.003$ ) as ST increased from day 0 to day 28. However, as storage proceeded from day 28 to day 56, a reversed increase ( $p < 0.01$ ) was observed in moisture to a level similar ( $p \geq 0.05$ ) to the original value in the stored feed. There was a gradual decrease in the ether level of the feed as ST increased, with a marked difference ( $p < 0.001$ ) observed only by day 56 of storage. The percentage of ash level in the feed progressively decreased with increasing storage time from day 0 to day 28 ( $p < 0.001$ ) and day 28 to day 56 ( $p < 0.01$ ), respectively. Conversely, there were significant increases in the percentage of CF ( $p = 0.03$ ) and NFE ( $p < 0.001$ ) levels of the feed as ST increased to day 28, beyond which (on day 56) no variations ( $p \geq 0.05$ ) were seen in the feed. Similarly, the TBARs values of the feed

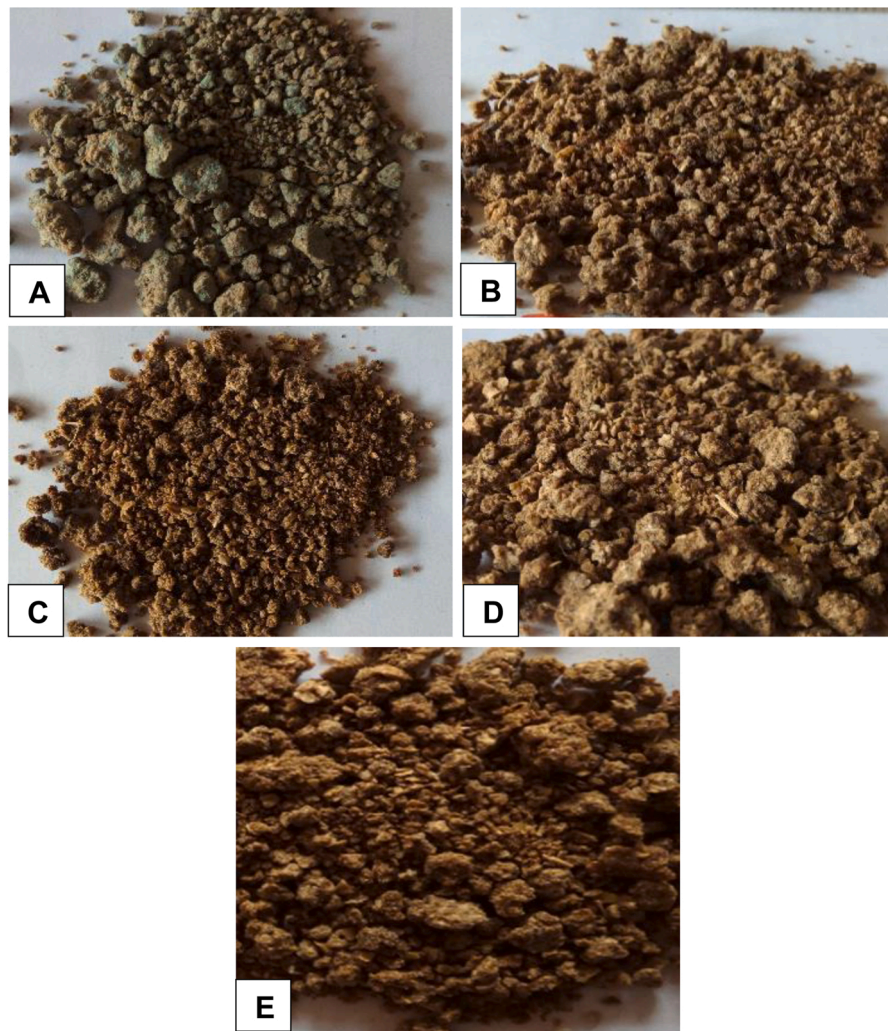


Fig. 2. Photograph of the fish feed containing varying levels of NaP after 56 days of storage. Picture A) control/0 – 0 g kg<sup>-1</sup> NaP; Picture B) Group B – 1.67 g kg<sup>-1</sup> NaP; Picture C) Group C – 3.33 g kg<sup>-1</sup> NaP; Picture D) Group D – 5.00 g kg<sup>-1</sup> NaP; Picture E) Group E – 6.67 g kg<sup>-1</sup> NaP.

**Table 4**  
Effects of NaP concentrations and storage time on feed quality attributes.

Source	CP (%)	Moisture (%)	Ether (%)	Ash (%)	CF (%)	NFE (%)	TBARS (mg MDA kg <sup>-1</sup> )
<b>Storage Time</b>							
0 Day	27.04 <sup>a</sup>	14.14 <sup>a</sup>	5.88 <sup>a</sup>	11.49 <sup>a</sup>	4.73 <sup>a</sup>	36.71 <sup>a</sup>	ND
28 Days	25.24 <sup>b</sup>	12.40 <sup>b</sup>	4.90 <sup>ab</sup>	6.70 <sup>b</sup>	5.70 <sup>b</sup>	45.46 <sup>b</sup>	3.13 <sup>a</sup>
56 Days	24.74 <sup>b</sup>	14.27 <sup>a</sup>	4.47 <sup>b</sup>	4.97 <sup>c</sup>	5.57 <sup>ab</sup>	45.95 <sup>b</sup>	3.65 <sup>b</sup>
SEM	0.654	0.477	0.476	0.463	0.389	1.791	0.864
<b>Treatments</b>							
Control	26.11 <sup>a</sup>	13.95 <sup>a</sup>	4.97 <sup>a</sup>	7.11 <sup>a</sup>	5.06	42.81	2.77 <sup>b</sup>
Group B	24.89 <sup>a</sup>	13.18 <sup>a</sup>	5.67 <sup>a</sup>	7.00 <sup>a</sup>	5.00	44.27	0.54 <sup>c</sup>
Group C	26.09 <sup>a</sup>	13.33 <sup>a</sup>	5.50 <sup>a</sup>	8.13 <sup>a</sup>	5.39	41.50	4.27 <sup>ab</sup>
Group D	25.61 <sup>a</sup>	13.44 <sup>a</sup>	4.50 <sup>a</sup>	8.19 <sup>a</sup>	5.72	42.43	4.14 <sup>ab</sup>
Group E	25.68 <sup>a</sup>	14.12 <sup>a</sup>	4.78 <sup>a</sup>	8.18	5.50	42.52	5.24 <sup>a</sup>
SEM	1.158	0.749	0.881	0.964	0.727	1.791	0.283
<b>P-values</b>							
Storage Time	0.001	< 0.001	0.012	< 0.001	0.008	< 0.001	0.029
Treatment	0.488	0.285	0.262	0.170	0.511	0.258	< 0.001
Interaction	0.296	0.979	0.670	0.056	0.306	0.167	0.979

<sup>a,b,c</sup> The values with different letters in the same column for each factor group are significantly different ( $p < 0.05$ ); SEM: standard error of mean; control – 0 g kg<sup>-1</sup> NaP; Group B – 1.67 g kg<sup>-1</sup> NaP; Group C – 3.33 g kg<sup>-1</sup> NaP; Group D – 5.00 g kg<sup>-1</sup> NaP; Group E – 6.67 g kg<sup>-1</sup> NaP

showed a marked increase as ST increased from day 28 to day 56 of the study ( $p < 0.05$ ).

The study found that the treatment with NaP did not have a significant main effect on the determined proximate characteristics of the

feed, including percentage CP ( $F_{4, 10} = 0.923, p \geq 0.05, \eta^2p = 0.27$ ), moisture ( $F_{4, 10} = 1.461, p \geq 0.05, \eta^2p = 0.37$ ), ether ( $F_{4, 10} = 1.547, p \geq 0.05, \eta^2p = 0.38$ ), ash ( $F_{4, 10} = 2.005, p \geq 0.05, \eta^2p = 0.45$ ), CF ( $F_{4, 10} = 0.877, p \geq 0.05, \eta^2p = 0.26$ ), and NFE ( $F_{4, 10} = 1.56, p \geq 0.05, \eta^2p = 0.26$ ).

**Table 5**  
Influence of NaP treatment on the feed quality changes over time.

Parameters	Day	Groups (Dietary levels of NaP in g kg <sup>-1</sup> diet)				
		A (control) 0	B 1.67	C 3.33	D 5.00	E 6.67
Crude protein	0	28.87 <sup>a</sup>	25.78 <sup>a</sup>	27.88 <sup>a</sup>	25.89 <sup>a</sup>	26.79 <sup>a</sup>
	28	24.65 <sup>b</sup>	24.80 <sup>a</sup>	25.26 <sup>a</sup>	25.39 <sup>a</sup>	26.12 <sup>a</sup>
	56	24.80 <sup>b</sup>	24.08 <sup>a</sup>	25.15 <sup>a</sup>	25.53 <sup>a</sup>	24.15 <sup>a</sup>
	SEM	0.777	0.315	0.726	0.260	0.703
	P-value	0.009	0.057	0.67	0.771	0.061
Moisture	0	14.65 <sup>a</sup>	13.88 <sup>a</sup>	13.84 <sup>a</sup>	13.67 <sup>a</sup>	14.68 <sup>a</sup>
	28	12.83 <sup>b</sup>	11.83 <sup>b</sup>	12.50 <sup>a</sup>	12.17 <sup>a</sup>	12.67 <sup>a</sup>
	56	14.37 <sup>a</sup>	13.83 <sup>a</sup>	13.67 <sup>a</sup>	14.50 <sup>a</sup>	15.00 <sup>a</sup>
	SEM	0.344	0.402	0.408	0.517	0.499
	P-value	0.035	0.026	0.397	0.179	0.08
Ether	0	5.73 <sup>a</sup>	6.17 <sup>a</sup>	6.50 <sup>a</sup>	4.83 <sup>a</sup>	6.17 <sup>a</sup>
	28	4.50 <sup>a</sup>	5.33 <sup>a</sup>	5.50 <sup>a</sup>	4.83 <sup>a</sup>	4.33 <sup>b</sup>
	56	4.67 <sup>a</sup>	5.50 <sup>a</sup>	4.50 <sup>a</sup>	3.83 <sup>a</sup>	3.83 <sup>b</sup>
	SEM	0.398	0.333	0.471	0.276	0.442
	P-value	0.446	0.623	0.244	0.258	0.045
Ash	0	10.99 <sup>a</sup>	11.17 <sup>a</sup>	12.05 <sup>a</sup>	10.60 <sup>a</sup>	12.67 <sup>a</sup>
	28	6.00 <sup>b</sup>	6.33 <sup>b</sup>	6.67 <sup>b</sup>	7.97 <sup>b</sup>	6.53 <sup>b</sup>
	56	4.33 <sup>c</sup>	3.50 <sup>c</sup>	5.67 <sup>b</sup>	6.00 <sup>c</sup>	5.33 <sup>b</sup>
	SEM	1.015	1.155	1.041	0.721	1.202
	P-value	0	0	0.001	0.003	0.001
Crude fiber	0	4.50 <sup>a</sup>	5.00 <sup>a</sup>	5.00 <sup>a</sup>	4.67 <sup>a</sup>	4.50 <sup>a</sup>
	28	5.50 <sup>a</sup>	5.33 <sup>a</sup>	5.00 <sup>a</sup>	6.50 <sup>a</sup>	6.17 <sup>a</sup>
	56	5.17 <sup>a</sup>	4.67 <sup>a</sup>	6.17 <sup>a</sup>	6.00 <sup>a</sup>	5.83 <sup>a</sup>
	SEM	0.212	0.167	0.274	0.472	0.391
	P-value	0.138	0.296	0.121	0.293	0.191
NFE	0	35.27 <sup>b</sup>	38.01 <sup>b</sup>	34.74 <sup>b</sup>	40.34 <sup>a</sup>	35.20 <sup>b</sup>
	28	46.51 <sup>a</sup>	46.37 <sup>a</sup>	45.08 <sup>a</sup>	42.81 <sup>a</sup>	46.51 <sup>a</sup>
	56	46.67 <sup>a</sup>	48.42 <sup>a</sup>	44.68 <sup>a</sup>	44.13 <sup>a</sup>	45.85 <sup>a</sup>
	SEM	1.977	1.680	1.971	0.810	2.052
	P-value	0.001	0.001	0.018	0.148	0.008

<sup>a,b,c</sup> The values with different letters in the same column for each factor group are significantly different ( $p < 0.05$ ); SEM: standard error of mean

= 0.38). However, there was a significant main effect of NaP on the levels of TBARs in the stored feed ( $F_{4, 10} = 22.237, p < 0.001, \eta^2p = 0.90$ ). When the feed was treated with a lower level of NaP (Group B- 1.67 g kg<sup>-1</sup> diet), the TBARs levels were significantly reduced compared to the control. However, as the NaP levels in the feed increased beyond this level ( $\geq 3.33$  g kg<sup>-1</sup> diet), there was a progressive increase

( $p < 0.05$ ) in the TBARs values, with Group E (6.67 g kg<sup>-1</sup> diet) showing the highest TBARs values, which were significantly different ( $p < 0.05$ ) from the control. Groups C and D had similar TBARs values to the control and Group E.

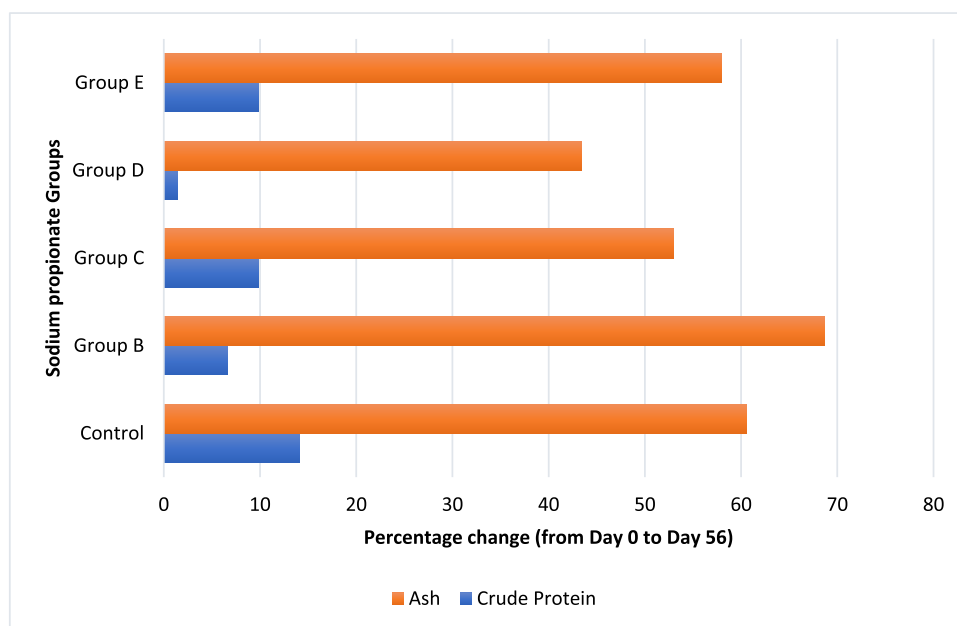
Furthermore, the study found no significant interaction effect of NaP and ST on all the measured feed quality characteristics of CP ( $F_{8, 20} = 1.307, p \geq 0.05, \eta^2p = 0.34$ ), moisture ( $F_{4, 20} = 2.36, p \geq 0.05, \eta^2p = 0.09$ ), ether ( $F_{5,03, 12,57} = 0.65, p \geq 0.05, \eta^2p = 0.21$ ), ash ( $F_{8, 20} = 2.379, p \geq 0.05, \eta^2p = 0.49$ ), CF ( $F_{8, 20} = 1.285, p \geq 0.05, \eta^2p = 0.34$ ), and NFE ( $F_{8, 20} = 1.672, p \geq 0.05, \eta^2p = 0.40$ ), and the TBARs values ( $F_{4, 10} = 0.102, p \geq 0.05, \eta^2p = 0.04$ ).

### 3.4. Changes in the chemical composition of the feed over time

A one-way ANOVA was conducted to analyze the data to assess the effects of NaP treatment on changes in proximate characteristics of stored feed over time. The results showed that the CP level of feed in the control group significantly decreased ( $p < 0.05$ ) as storage time progressed from day 0 to day 28 (28.87–24.65%) (Table 5). However, no significant changes in CP levels were observed in the NaP-treated groups as storage time increased. The control group had the highest total loss in CP level for 56 days of storage (14.1%), while Group D had the minimum loss (1.39%). Similar percentage changes were observed in Groups C and E (9.79% and 9.85%, respectively), while Group B had a percentage loss of 6.59%.

Feed moisture levels in Groups C, D, and E remained unchanged ( $p \geq 0.05$ ) as storage time progressed from day 0 to day 56. However, significant variations ( $p < 0.05$ ) in moisture levels of feed in control and Group B were observed as storage time increased in the study. Ether levels decreased in Group E as storage time increased from day 0 to day 28, while no significant changes were observed in the ether levels of the control Groups B, C, and D ( $p > 0.05$ ).

The percentage ash level of the feed progressively decreased ( $p < 0.05$ ) in the control, Group B, and D as storage time progressed from day 0 to day 28 and day 56, respectively. In contrast, ash levels initially decreased ( $p < 0.05$ ) from day 0–28 in Groups C and E but remained unchanged ( $p > 0.05$ ) with a further extension of storage to day 56. Group B showed the highest percentage change in ash level (68.67%), with other groups (control, Groups C and E) having more than



**Fig. 3.** Percentage Changes in the feed ash and crude protein levels over the 56 days of storage; control – 0 g kg<sup>-1</sup> NaP; Group B – 1.67 g kg<sup>-1</sup> NaP; Group C – 3.33 g kg<sup>-1</sup> NaP; Group D – 5.00 g kg<sup>-1</sup> NaP; Group E – 6.67 g kg<sup>-1</sup> NaP.

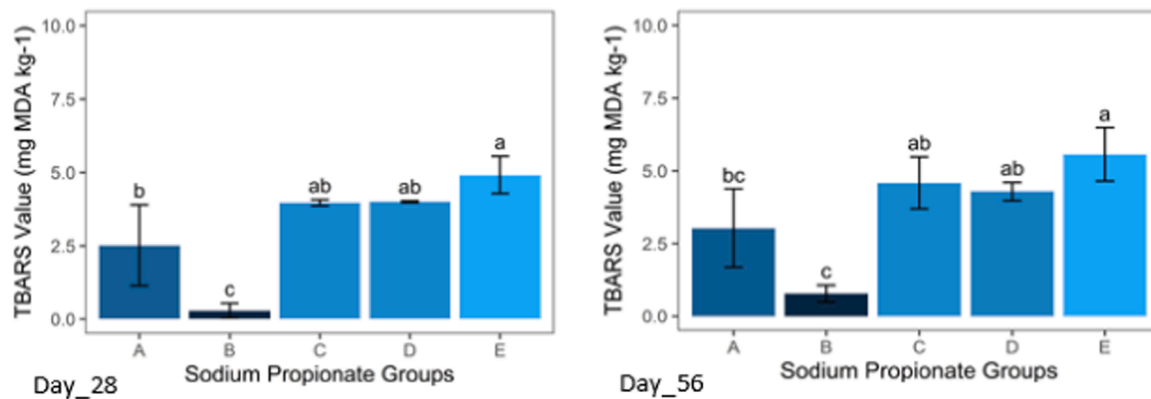


Fig. 4. Effect of NaP on the TBARS levels of feed stored for 28 days and 56 days; control – 0 g kg<sup>-1</sup> NaP; Group B – 1.67 g kg<sup>-1</sup> NaP; Group C – 3.33 g kg<sup>-1</sup> NaP; Group D – 5.00 g kg<sup>-1</sup> NaP; Group E – 6.67 g kg<sup>-1</sup> NaP.

50% loss in value (60.6%, 52.95%, and 57.93%, respectively). Group D feed had the least percentage change in ash level, with about a 43.4% reduction. No significant changes were observed in the CF levels in the various groups as storage time increased in the study.

Except for Group D, which showed no differences ( $p < 0.05$ ) in the NFE levels of feed at the various storage time levels, the NFE of feed in the control and groups B, C, and E initially increased as storage time increased to day 28 while remaining unchanged with further increase in storage time.

Overall, the results suggest that NaP treatment can help preserve the quality of stored feed, as evidenced by the more minor changes in CP, moisture, ether, ash, and NFE levels observed in the treated groups compared to the control group. Group D, with a 5.0 g of NaP per kg diet, was considered more effective, as evidenced by the minimal changes observed in the chemical characteristics of the feed.

Fig. 4 illustrates the mean ( $\pm$  SEM) TBARS values of fish feed samples with varying levels of NaP inclusion measured on days 28 and 56 of the study. The results of the one-way ANOVA showed significant differences ( $p < 0.05$ ) in mean TBARS values of the stored feed at the two storage times. The results indicated that lower levels of NaP inclusion (Group B – 1.67 g kg<sup>-1</sup> diet) markedly decreased ( $p < 0.05$ ) lipid oxidation process (TBARS) by 88.5% and 74.2%, respectively, at 28 and 56 days of storage, respectively. However, higher doses of NaP inclusion (Groups C and D) stimulated some increases in the TBARS values (Group C: 57.7% and 51.6% and Group D: 58.9% and 41.7% respectively, at days 28 and 56), which became significantly different ( $p < 0.05$ ) at the highest level of NaP inclusion (Group E: 95.2% and 84% at days 28 and 56, respectively). Overall, the study found a similar trend in the effect of NaP treatment on the TBARS values of the feed in the two different storage times. The results indicated that NaP lowered the TBARS value in the feed at a lower inclusion level (1.67 g kg<sup>-1</sup> feed in Group B), but beyond this level, the TBARS levels increased with increasing NaP dietary levels, becoming markedly different at 6.67 g kg<sup>-1</sup> feed of NaP (Group E).

## 4. Discussion

### 4.1. Growth performance of fish

As the fish farming industry seeks to increase cultured fish production worldwide, various challenges arise, such as regulating water quality, controlling disease outbreaks, and optimizing fish growth and feed utilization. Synthetic chemical treatments have received heavy criticism due to their residual and negative effects on human health and the environment (Dawood and Koshio, 2018). As a result, several organic feed additives, including pre- and pro-biotics and various organic acids, have been explored to improve fish health and

productivity and limit antibiotic use in aquaculture operations. NaP has been used as an acidifier in aquafeed due to its beneficial effects on gut health, digestive capabilities, and the general health of animals (Hoseinifar et al., 2017; Ng and Koh, 2017). In this light, an eight-week study was conducted to assess the effect of dietary supplementation of NaP on fish growth performance to identify the optimal level of inclusion to support fish growth. The findings revealed that the dietary inclusion of NaP did not significantly improve African catfish's overall growth performance traits in the study. However, fish fed NaP at a 5.0 g kg<sup>-1</sup> diet (Group D) showed slightly higher (numerically) WG, LG, and SGR, with markedly lower ( $p < 0.005$ ) AFI. Surprisingly, Group C (3.33 g of NaPkg<sup>-1</sup> diet) exhibited adverse growth performance, with lower ( $p < 0.05$ ) mean values of final weight, WG, AFI, SGR, and higher ( $p < 0.05$ ) FCR compared to the control. Available evidence suggests inconsistencies in the reported benefits of NaP in different aquaculture species. While several studies have noted improved growth performance in Caspian white fish (Hoseinifar et al., 2016), zebrafish (Safari et al., 2016), sturgeon (Ahmadifar et al., 2022), goldfish (Sheikhzadeh et al., 2021), and yellowfin seabream (Sangari et al., 2021) fed NaP supplemented diets, other studies documented no significant effects of mixed short-chain fatty acids (formic, acetic and propionic acid) treatments in *C. gariepinus* (Asriqah et al., 2018). Short-chain fatty acids (SCFAs) are known to activate the secretion of digestive enzymes (Castillo et al., 2014), modulate Na<sup>+</sup>/K<sup>+</sup> balance in the gut cellular surface (Morken et al., 2012), and improve gut microbiome and functions (Hao et al., 2017; Piazzon et al., 2017), thereby stimulating enhanced nutrient absorption, food utilization and subsequently fish growth. Also, NaP is easily absorbed in the gastrointestinal tract and has been shown to up-regulate the growth hormone-releasing hormone and insulin-like growth factor, which are involved in regulating growth performance in fish (Ahmadifar et al., 2022; Hoseinifar et al., 2017). This may explain the lower AFI ( $p < 0.05$ ) and numerically higher FCR observed in Group D compared to the control. The lowered growth performance traits observed at lower levels of NaP inclusion (3.33 g kg<sup>-1</sup>) may be attributed to the lower AFI in this group. Further increase in dietary levels of NaP was seen to restore AFI and utilization, as seen in Groups E and D, respectively. The findings were consistent with the reports of previous studies (Lim et al., 2010), which noted that lower levels of propionic acid might suppress feeding in fish, whereas, at higher levels, feeding was enhanced. Similar reports of adverse growth effects of feeding varying levels of organic acids, such as 1.5–2 g kg<sup>-1</sup> fumaric acid (Omosowone et al., 2015) and 0.05% butyric acid (Asriqah et al., 2018) have been made in *C. gariepinus* fish.

In our previous study (Aba et al., 2018), it was found that African catfish juveniles fed diets containing higher levels of NaP (5.0–6.67 g kg<sup>-1</sup> diet) exhibited improved antioxidant status and maintained hepatic cell architecture. The current study builds on these

findings and represents one of the earliest reports of the growth-enhancing potential of NaP in African catfish. These results support using NaP (at or above 5.0 g kg<sup>-1</sup> diet) as a potential tool for improving feed utilization and general productivity of African catfish aquaculture.

#### 4.2. Physical characteristics

Due to the recent increase in the cost of imported fish feed in Nigeria, farmers have started producing and storing local feeds on their farms. This has made it necessary to use appropriate preservatives to maintain the nutritive values of the feeds (Njobeh et al., 2004). This study investigated the effects of storage time and NaP treatment on the physical quality and sensory attributes, proximate compositions, and lipid peroxidation levels of locally produced fish feed. Our physical evaluations revealed evidence of deteriorative changes in the physical characteristics of the feed after 28 days of storage, which worsened as storage time increased to 56 days. We observed decreased mean texture scores and increased development of off-odors and greenish discolorations in the control group, making the feed unacceptable by day 56 of storage (Fig. 2). These changes could be attributed to increased mold growth and proliferation, causing moldiness in the stored feed. This is consistent with reports that mold infestation in stored bio-products causes discolorations and produces enzymes that stimulate microbial rancidity, resulting in undesirable odors and flavors (Jarvis, 2001; Motalebi et al., 2008). Mold infestation is expected in the tropics, where high temperature and moisture conditions support their growth and proliferation (Aanyu and Ondhoro, 2016; Mohapatra et al., 2017). Spores of different fungi species can contaminate stored products during production or handling processes. Although many of these fungi are killed during processing, their heat-resistant sexual spores (ascospores) remain to establish themselves when conditions are right (Tournas, 1994). These results are consistent with those of Hossen et al. (2011), who observed a marked deterioration in the odor and texture of three different commercial fish feeds following 60 days of storage at both rooms (25–30 °C) and low (5–8 °C) temperature conditions. Additionally, Alam et al. (2014) reported a significant increase in the total fungi count of broiler finisher feed as storage time increased.

Also, we observed that including NaP in the feed slowed down the deterioration of the physical and sensory attributes (color, odor, texture, moldiness, and general remark) during the 56 days. This may be attributed to the fungicidal or fungistatic activity of NaP in the feed. According to Cegielska-Radziejewska et al. (2013), the proliferation of fungi, such as molds, can adversely affect animal feed quality, including its sensory and chemical characteristics. Propionic acids (PA) and their salts have been documented as weak acid preservatives generally considered fungicidal and effectively limit mold growth in stored food products (Dijksterhuis et al., 2019; Yun and Lee, 2016). The mechanism of fungi inhibitory action of PA includes both fungicidal and fungistatic pathways, and they have been noted to generally induce fungi death through mitochondrial-mediated apoptotic processes (Yun and Lee, 2016). Furthermore, sodium salts are known to decrease the water activity of food and bio-materials, thereby reducing the quantity of water available for microbial growth (Miller, 1996; Vandegrift et al., 1975).

Our observations suggest a dose-dependent mold inhibitory activity of NaP, as higher levels in the diet were associated with greater efficacy. This could explain the higher mold infestations seen in group B (1.67 g NaP kg<sup>-1</sup> feed) compared to groups C, D, and E (3.33, 5.0, and 6.67 g NaP levels kg<sup>-1</sup> feed) after 56 days of storage. These findings provide some evidential support to previous reports of dose-dependent activities of propionic acid on the selective inhibition of fungi growth in preserved hay (Lord et al., 1981) and maize (Marín et al., 2000; Vandegrift et al., 1975), with lower dosages active against fewer species. Alam et al. (2014) reported that calcium propionate inclusion (at 5.00 g kg<sup>-1</sup> diet) markedly reduced the total viable fungi count of broiler finisher feed stored for 28 days. Generally, although there were perceived spoilage

initiations in the control and group B feeds (after days 28 and 56 of storage, respectively), they were still considered good and acceptable for use. However, when storage was extended to 56 days without NaP inclusions (as in the control group), the feed became bad and was considered unacceptable (Mean General remark Score: 1.50). Also, regardless of the slight deteriorative changes in the feed samples of groups C, D, and E, they were still observed to maintain a relatively high sensory attributes/physical characteristics score throughout the study duration, suggesting that higher levels of NaP ( $\geq 3.33$  g kg<sup>-1</sup> feed) were effective in maintaining the physical quality of fish feed for at least 56 days in storage as was considered in this study.

#### 4.3. Chemical compositions

The results of the proximate compositions revealed a significant progressive decrease in crude CP, EE, and ash levels of the feed with increasing storage time ( $p < 0.05$ ). This finding is consistent with previous reports (Aanyu and Ondhoro, 2016; Nyong and Olubunmi, 2014; Solomon et al., 2016) that showed a declining trend in CP, lipid, and ash levels of fish feed with increasing storage time. The decrease in the percentage of CP over time may be due to the protein aging effect, as earlier postulated (Shyong et al., 1998). Additionally, the reduction in CP, EE, and ash contents could be attributed to degradations caused by microbial proliferation and/or their metabolic activities during storage, as reported (Fagbohun and Lawal, 2011; National Research Council (U. S.), 2011). Mold growth and proliferation on feedstuffs are supported by their enzymatic breakdown and utilization of the host's nutrients, resulting in a reduction in amino acids (mainly lysine and arginine), lipids, minerals, and vitamins levels in the feedstuffs, as noted by (Greco et al., 2014; Smits et al., 1996). This supports the observations of (Fagbohun and Lawal, 2011), who noted decreasing mineral (Na, Ca, Mg, Fe, Mn, and Cd) levels in soybean preserved over time.

Further, fish feed typically contains fish meal and oils high in PUFAs. These PUFAs are easily susceptible to lipid oxidation at ambient and sub-ambient temperatures, as reported (Pezzuto and Park, 2002; Siddhuraju and Becker, 2003). The marked decrease in the percentage of EE observed in feed stored for 56 days in our study could be attributed to increased lipid oxidation reactions stimulated by the high ambient temperature conditions of the study location (Fig. 1). In contrast to the results of this study, (Bhilave, 2018) reported no significant changes in the proximate compositions of commercial fish feed stored for a year. The disparities in these studies could be due to variations in feed compositions and prevailing environmental conditions in the storage locations. Commercial feeds are usually produced with appropriate preservatives and dried to moisture levels that reduce fungal growth, making them suitable for longer storage times and far distributions (Ono et al., 2002; Sahar et al., 2015). This may explain the extended feed storage capacity reported by Bhilave (2018).

We observed a considerable decrease in the nutritional compositions of the fish feed used in the study, which may be attributed to the poor quality of locally available fish feed ingredients. Previous reports have noted the poor quality and high variability of locally available fish meal and feed ingredients as a significant challenge facing the aquaculture industry in Nigeria (Adéyemi et al., 2020). To investigate the possible impact of storage time on the nutritional compositions of fish feed treated with NaP, we conducted a One-Way ANOVA to compare the different storage periods in each group. Overall, we observed marked changes in the proximate compositions of the control feed, as evidenced by their significant decline ( $p < 0.05$ ) in percentage CP levels, as well as the higher aggregate losses in CP and ash values when compared to other groups. In contrast, the groups treated with NaP showed relatively steady CP levels and lower percentage aggregate loss in ash levels as the storage time increased. These findings can be attributed to the fungicidal and mold-inhibitory properties of NaP, which limit protein and nutrient breakdown and their utilization by fungi in these groups (Dijksterhuis et al., 2019). Adding salts to food can result in osmotic shock in the cells

of microorganisms, leading to cellular water loss and eventually reduced growth or death (Davidson, 2001). We observed no physical signs of mold infestations in the NaP-treated groups during the study period (Fig. 1), which may be due to the mold-inhibitory properties of NaP. The degree of nutrient reduction in mold-infested bio-products reportedly depends on the level of mold growth (Vieira, 2003). Our findings of a non-significant ( $p \geq 0.05$ ) change in the CP (in the NaP-treated groups) and lowered percentage loss in CP and ash levels (in higher NaP-treated groups) of feeds with increasing storage time may provide additional evidence of the dose-dependent effect of NaP on mold inhibition and nutrient preservation in stored feed, as reported by (Marín et al., 2000).

High moisture content in feedstuffs is a significant problem affecting feed storage as it favors insect infestations and microbial growth. Our results showed significant fluctuations ( $p < 0.05$ ) in the moisture levels of the feed as storage time progressed from day 0 to Day 56. Moisture levels initially decreased ( $p < 0.05$ ) after day 28 of storage and subsequently increased ( $p < 0.05$ ) on day 56. This may be due to possible moisture absorption and loss in the feed within the storage conditions of high temperature and relative humidity. In the tropics, the prevailing environmental conditions often exceed the recommended storage conditions of less than 32.2 °C and 75% relative humidity for feeds (Aanyu and Ondhoro, 2016). Regardless of their initial levels in stored feedstuffs, the moisture content is known to increase steadily until it reaches an equilibrium with the prevailing relative humidity of the storage environment (Atter et al., 2017; Volenik et al., 2006). Similar findings have been reported in studies (Aanyu and Ondhoro, 2016; Hossen et al., 2011; Nyong and Olubunmi, 2014) with storage durations of up to 2 months.

Similarly, marked fluctuations in the moisture levels of feed were observed in the control and group B of the study. The non-significant variations in the moisture levels observed in the high inclusion levels of NaP (C, D, and E) when compared to the control may be attributed to the lowering effect of NaP on water activity in the stored feed, especially in the prevailing high relative humidity and temperature conditions of the study location (Fig. 1). Therefore, storage time, processing, packaging, and environmental conditions all influence the rate of increase in the moisture content of the stored feed, as noted by Hossen et al. (2011). Additionally, NaP may have lowered the rate of moisture changes by limiting microbial activities in the stored feed. This is supported by earlier reports of the impact of mold growth on moisture increments in stored feedstuff (Atter et al., 2017; Chow, 1980) and our observation that NaP-treated feeds relatively maintained their texture, with no associated mold infestation during storage. Our findings further support the dose-dependent mold inhibitory action of NaP and suggest that NaP may preserve stored feed quality by slowing microbial proliferation and the rate of moisture increase with 5.0 gkg<sup>-1</sup> diet having an optimal effect.

The changes in percentage fiber and NFE levels of stored feed may be attributed to changes in other proximate indices, such as CP, moisture, ash, and ether. No trend was observed in fiber levels as storage time increased. This study's findings align with previous reports of no consistent change in the CF and a marked increase in NFE levels of fish feed with storage time (Nyong and Olubunmi, 2014).

Aquaculture feeds typically contain more than 5% dietary lipids, crucial for animal productivity, immune status, and product quality (Glodde et al., 2018). Lipid oxidation is a critical issue affecting the quality of dried fish and feed products during storage (Qiu et al., 2019). Therefore, preserving the nutritional value of stored feeds and limiting oxidative processes are equally important (Hu et al., 2020). TBARS are a reliable indicator of secondary lipid peroxidation and deterioration in animal feeds (Farhoosh, 2018; Zipser et al., 1964). In this study, we aimed to investigate the extent of oxidative changes in stored feed by measuring the TBARS levels on days 28 and 56 of the study. Our findings showed a 16.38% increase ( $p < 0.05$ ) in TBARS levels in the feed on day 56 compared to day 28, indicating an increase in lipid oxidation processes in the fish feed with longer storage duration. Since the feed

samples were stored in dark, enclosed spaces, the high content of fish meal and oil in the formulated feed may have contributed to increased peroxidation levels in the feed, as evidenced by the higher ( $p < 0.05$ ) TBARS values observed with longer storage duration. Furthermore, these processes may have been enhanced by the prevailing elevated temperature conditions of tropical environments, where the study was conducted (Fig. 1). These results are consistent with previous reports of progressive increases ( $p < 0.05$ ) in TBARS levels in packed fish oil encapsulate (Jeyakumari et al., 2014), fish oil, ground pork, cat and pig feeds (Hu et al., 2020), beef burgers (Sedlacek-Bassani et al., 2020) and beef sausage (Ojangba et al., 2022) with increasing storage time. Furthermore, comparing the TBA-test results alongside organoleptic evaluations or other suitable tests is crucial to ensure validity (Patton, 1974). In the present study, we observed that the marked increase in TBARS level of the feed with increasing storage time was positively associated with a marked increase in the development of off-flavors. This findings supports earlier reports of Zhao et al. (2020) that lipid oxidation and hydrolysis contribute largely to the development of rancidity and off-flavours in stored products. Overall, our findings suggest an increase in lipid oxidation and decline in feed quality, as indicated by the increase in TBARS levels with increasing storage time (ST).

#### 4.4. Rancidity

Rancidity is the process of lipid decomposition through either chemical or microbial means, resulting in an unpleasant flavor and odor in feed materials. The typical indicators of lipid peroxidation in stored products are MDA/TBARS, but understanding the numerous reactions and pathways involved and the factors that affect the entire process still needs to be completed (Domínguez et al., 2019). The stability of polyphenols and thus, lipid oxidation processes in stored feed components are influenced by various factors, including dietary composition (lipid, unsaturated fats, antioxidants, etc.), high temperature, light, oxygen, pH, salt content and storage methods (Filipe et al., 2023; Kearsley and Rodriguez, 1981; Qiu et al., 2020; Sharma et al., 2016). In this study, the high levels of TBARS in the experimental feed can be attributed to the high content of fish meal and oil, as well as the high-temperature conditions of the study location. The lower TBARS values observed in the lower NaP-treated group B as storage proceeded for 28 and 56 days, suggest a possible antioxidant property of NaP. Similar antioxidant effects have been reported with organic salts such as sodium lactate, which reduced lipid oxidation in fresh ground pork stored under refrigeration and freezing conditions for up to 15 days (Tan and Shelef, 2002).

Surprisingly, however, at higher levels of NaP inclusion ( $\geq 3.33$  gkg<sup>-1</sup> diet), the TBARS increased in the stored feed at both storage times compared to the control becoming markedly higher in Group E (6.67 g NaP kg<sup>-1</sup> diet). Although previous reports on the benefits of short-chain fatty acids (SCFAs) have been inconsistent, several studies have suggested pro-oxidant effects of SCFAs (Piper, 1999) or salts in general (Tan and Shelef, 2002) due to their possible inherent metallic impurities like iron (Bess, 2011). Moreover, high concentrations of salts may interfere with the membrane structures' integrity, exposing substrates to catalytic actions (Amaral et al., 2018). Preservative salts like NaCl may also lower the activities of antioxidant products in stored food products (Min and Ahn, 2005). The findings of this study are consistent with Maca et al. (1997), who reported that NaL inclusion alone caused an increase in the TBARS values of preserved cooked beef. However, in combination with NaP (at 0.1% or 0.2%), there was a marked reduction in lipid oxidation, supporting the antioxidant effects of NaP at lower doses. Further, although the results of this study show comparable lipid peroxidation levels (indicated by TBARS) in the higher NaP-treated groups (C and D) and the control, sensory evaluations did not indicate similar marked development of off-flavors as observed in control. The higher off-flavors perceived in the control and group B may be attributed more to the spoilages introduced by the increased mold proliferations and spoilages

rather than lipid peroxidation processes. Mold growth and activities in stored food products generate metabolites or mycotoxins that produce off-flavors (Garnier et al., 2017). Moreover, mold proliferation in control and Group B may have caused variations in the lipid peroxidation processes and pathways, resulting in different end products and off-flavors.

## 5. Conclusions

In conclusion, the present study revealed that locally produced fish feed stored on-farm is susceptible to mold infestations, moisture level fluctuations, nutrient degradation, and spoilage. These deteriorative processes progressively increase with storage time, rendering the feed unusable after 56 days. While NaP inclusions did not wholly prevent these changes, they notably slowed them down, indicating their mold-inhibiting properties. A dose-dependent action of NaP was also observed in stored fish feed, with 5.00 g kg<sup>-1</sup> dose effective in maintaining optimal feed quality during storage. The findings of this study emphasize the importance of proper storage conditions to minimize the deterioration of feed quality, particularly for small-scale aquaculture farmers who may not have access to commercial feeds with more extended storage capacities.

Additionally, the study demonstrated that lower dietary NaP inclusion (3.33 g kg<sup>-1</sup>) adversely affected fish growth performance, while higher levels (5.00 g kg<sup>-1</sup>) slightly improved performance, with a marked reduction in AFI and a marginally higher FCR and SGR. Therefore, NaP at 5.00 g kg<sup>-1</sup> is recommended as a preservative for on-farm fish feed production and improved fish growth performance. It is important to note that this study only examined NaP's short-term (2 month) effects on fish feed preservation. Further research is necessary to evaluate the longer-term benefits of NaP in fish feed preservation.

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## CRedit authorship contribution statement

**Ifeanyi Emmanuel Uzochukwu:** Conceptualization, Methodology, Investigation, Supervision, Project administration, Formal analysis, Visualization, Writing- Original draft; **Patrick Emeka Aba:** Conceptualization, Methodology, Project administration, Formal analysis; **Nelson Ike Ossai:** Conceptualization, Methodology, Project administration; **Writing- Review & Editing;** **Hillary Chukwuemeka Ugwuoke:** Investigation, Resources; **Krisztián Nyeste:** Formal analysis, Visualization, Writing- Review & Editing; **Ndubuisi Samuel Machebe:** Formal analysis, Visualization; Project administration, Writing- Review & Editing.

## Declaration of Generative Artificial Intelligence (AI) and AI-Assisted Technologies in the Writing Process

During the preparation of this work the author(s) used ChatGPT in order to correct for grammar and improve the readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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