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Supplement Effect of Freeze Dried *Tribulus terrestris* Seeds Extract in Feed of Nile Tilapia Fries *O. niloticus*, on Growth Performance and Feed Efficiencies

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Abstract

The freeze-dried dietary extract of *Tribulus terrestris* seeds were incorporated into the feed of *O. niloticus* fries. Four experimental diets were prepared using commercial feed containing 0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg of the extract. These diets were referred to as control feed, feed1, feed2, and feed3, respectively, and were tested in triplicate. Fries (0.012 ± 0.002 g) were randomly distributed into aquaria at a density of 180 fries per aquarium. Experimental feeds were provided to the fries immediately after they completely absorbed their yolk sacs. The fry was fed four times a day (at 7:00 am, 11:00 am, 4:00 pm, and 7:00 pm) for 70 days. The experiment was conducted indoors to prevent the growth of algae in the aquaria, and the water conditions were monitored regularly. The growth performance and feed efficiency parameters were calculated. The effects of dietary treatments on different parameters were analyzed using one-way analysis of variance (ANOVA). Significant differences were found, and Tukey's HSD/Duncan's test multiple comparison tests were performed to determine the mean differences. Statistical analysis was conducted using SPSS version 16.0, and the data are presented as mean ± standard deviation (SD). The weight gain (WG) of the fish group fed with feed3 (3.00 g/kg) was significantly the highest at 0.086 ± 0.024 g, and the highest absolute growth rate (AGR) was also recorded for this group. Conversely, the lowest growth performance (WG and AGR) was observed in the fish group fed with feed2 (2.00 g/kg), although they exhibited the best feed conversion ratio (FCR). These findings indicate that incorporating dietary extract of *Tribulus terrestris* seeds into the feed improved the growth and feed efficiency of *O. niloticus* fries, with the improvements correlating with increased extract dosage. The extracted product is cost-effective and can potentially substitute other expensive fish additives used in fish feed formulations.

Keywords: **Tribulus terrestris*, seed extraction, fish fry feed, growth performance, feed efficiency.*

الملخص

تم دمج المستخلص الغذائي المجفف بالتجميد لبذور *Tribulus terrestris* في علف زريعة *O. niloticus*. وقد تم إعداد أربع حميات تجريبية باستخدام علف تجاري يحتوي على 0.00 جم/كجم، و2.00 جم/كجم، و2.50 جم/كجم، و3.00 جم/كجم من المستخلص، وقد أُشير إلى هذه الحميات بالعلف الضابط، والعلف1، والعلف2، والعلف3 على التوالي، وتم اختبارها على ثلاث مكررات. وزعت الزريعة (0.012 ± 0.002 جم) عشوائياً في أحواض الأسماك بكثافة 180 زريعة لكل حوض.

وقدمت الأعلاف التجريبية للزريعة فور امتصاصها الكامل لأكياس المح. تم تغذية الزريعة أربع مرات يومياً (في الساعة 7:00 صباحاً، و11:00 صباحاً، و4:00 مساءً، و7:00 مساءً) لمدة 70 يوماً. أُجريت التجربة داخلياً لمنع نمو الطحالب في الأحواض، وتم رصد ظروف المياه بانتظام. حُسبت معاملات الأداء النمائي وكفاءة العلف. وحُللت تأثيرات المعاملات الغذائية على المعاملات المختلفة باستخدام تحليل التباين أحادي الاتجاه (ANOVA). وقد وُجدت فروقات معنوية، وأُجريت اختبارات المقارنات المتعددة باستخدام اختبار Tukey's HSD/Duncan's لتحديد الفروقات بين المتوسطات. وأُجري التحليل الإحصائي باستخدام برنامج SPSS الإصدار 16.0، وعُرضت البيانات كمتوسط ± الانحراف المعياري (SD). وكانت الزيادة الوزنية (WG) لمجموعة الأسماك المغذاة بالعلف3 (3.00 جم/كجم) الأعلى معنوياً عند 0.086 ± 0.024 جم، كما سُجل أعلى معدل نمو مطلق (AGR) لهذه المجموعة. على العكس من ذلك، لوحظ أدنى أداء نمائي (WG وAGR) في مجموعة الأسماك المغذاة بالعلف2 (2.00 جم/كجم)، ورغم أنها أظهرت أفضل معامل تحويل غذائي (FCR). تشير هذه النتائج إلى أن دمج المستخلص الغذائي لبذور *Tribulus terrestris* في العلف حسّن من النمو وكفاءة العلف لزريعة *O. niloticus*، حيث ارتبطت التحسينات بزيادة جرعة المستخلص، والمنتج المستخلص فعّال من حيث التكلفة ويمكن أن يحل محل إضافات الأسماك الأخرى باهظة الثمن المستخدمة في تركيبات أعلاف الأسماك.

الكلمات المفتاحية: *Tribulus terrestris*، *استخلاص البذور، علف زريعة الأسماك، الأداء النمائي، كفاءة العلف.*

Introduction

Medicinal herbs have garnered considerable attention for their ability to stimulate digestion, particularly their effects on bile secretion and pancreatic enzyme activity. Essentially, olfactory feed ingredients serve as powerful feeding enhancers, encouraging fish to consume more feed than they typically would under normal conditions (Adams 2005). As the aquaculture industry shifts away from its reliance on synthetic drugs, medicinal herbs are gaining acceptance as viable alternatives to antibiotic growth promoters in fish farming (Adedeji *et al.*, 2008). Among the array of available phytoproducts, the extract of the herb *Tribulus terrestris* has emerged as one of the most prominent. Rich in furostanol-type saponins, particularly a compound known as protodioscin, this herb has captured the interest of researchers and aquaculturists alike. Recent studies involving various farm animals, including rats, pigs, cocks, rabbits, and fish, have documented the remarkable benefits of *Tribulus terrestris* extract on reproductive parameters, such as sperm quality, sexual behavior, and fertilization ability. Despite these promising findings, there remains a notable scarcity of literature addressing the influence of *Tribulus terrestris* extract on the growth performance and feed efficiency of fish, especially when compared with the conventional use of antibiotics and synthetic chemicals. Therefore, exploring dietary supplements and ingredients that bolster fish health, enhance growth rates, improve feed efficiency, and ensure the production of safe, high-quality aquaculture products has become increasingly crucial. Over the past two decades, an upsurge in research has yielded conclusions affirming that *Tribulus terrestris* can serve as a suitable alternative to conventional antibiotics, chemotherapeutics, and synthetic hormones in aquaculture (El-Sayed et al., 2004). Furthermore, the integration of plant-based additives into fish diets is an established strategy for improving weight gain and feed efficiency in cultivated fish species and for producing good fish seeds, fry, and fingerlings. However, studies specifically focusing on the effects of *Tribulus terrestris* seed extract on fish performance and feed utilization during the early growth stages are remarkably limited. Consequently, there is a pressing need for information regarding the growth performance and feed utilization of Nile Tilapia, *O. niloticus*. The importance of quality ingredients and artificial feeds, even for omnivorous species such as tilapia, makes perfect sense at critical stages (juveniles or brood stock) when fish are maintained under intensive clear-water farming conditions and are entirely dependent on nutritionally complete diets (Tacon, 1988). Global research for the identification of cost-effective substitutes for conventional materials continues (Ayoola, 2010; Elsayed, 2004; El-Sayed and Tacon, 1997; Hasan et al., 2007; Karalazos, 2007; Obirikorang et al., 2015). There are many feed additives available to improve fish growth performance. Some of these additives used in feed mills are chemical products, especially hormones and antibiotics, which may cause unfavorable side effects. The use of antibiotic growth promoters (AGPs) as feed additives in the aquaculture industry has been criticized by government policies and consumers because of the possible development of microbial resistance to these products and their potentially harmful effects on human health (Baruah et al., 2008). This study aimed to evaluate the effects of varying supplementation levels of *Tribulus terrestris* on the growth performance and feed efficiency of *O. niloticus* fry. However, the specific objectives of this study were twofold: first, to prepare fish feed that incorporates different levels of freeze-dried extract from freeze-dried *Tribulus terrestris* seeds; and second, to assess the impacts of these inclusion levels on the growth performance and feed efficiency of *O. niloticus* fry.

1. Materials and Methods

1.1 Experimental sites

The extraction of Tribulus terrestris was performed at the Institution of Aromatic and Medicinal Plants (IAMP) of the Ministry of Agriculture and Animal Resources, Khartoum, Sudan. The experiment on growth performance was conducted at the Department of Fisheries Science Hatchery, College of Natural Resources and Environmental Studies, University of Bahri, Khartoum, Sudan.

1.2 Materials

1.2.1 Chemicals and apparatus used

Solutions: Ethanol, Formalin were used in the extraction method of Tribulus terrestris, and nitrogen gas was used in the extraction process. Freeze-dryers were used to dry the liquid extract of the Tribulus terrestris seeds. Aquaria were used for the acclimatization of hatched O. niloticus eggs and fry. Dragonstar grinder (Development Industrial Ltd, Hong Kong, China), rotary evaporator (Scientific Industrial and Trade Ltd, Greatwall, China), incubator (Nanchang Huibing Electronics Co., Ltd., China), and Sensitive Balance (Super Accomplish Health Technical Co. Ltd, China), centrifuge (Changsha Yingtai Instrument Co., Ltd., China) Glass wares, Refrigerator and Petri dish using in extraction method, oxygen meter, pH meter, ammonia test kits, thermometer and where apparatus were used during growth experiment. A freeze dryer (Changzhou Yang Chuan Precision Machinery Co., Ltd., China) was used to dry the extracted substances.

1.3 Tribulus trrestris seed collection and preparation

The wild herbal plant Tribulus terrestris seeds were collected from the Alkadro area of the North East University of Bahri campus, washed with distilled water, air-dried under an open-air shaded area, and then ground using a Dragonstar grinder. The finely ground seed powder was kept in a clean, dry box for extraction

1.4 Extraction method of Tribulus terrestris

The finely ground seed powder sample was extracted at the Institution of Aromatic and Medicinal Plants (IAMP) in Khartoum Bahri. A 250 g sub-sample was soaked in alcohol (ethanol, 70%) for five days, followed by daily filtration and evaporation using (Soxhlet apparatus. The extracted substance was freeze-dried (Image 1)

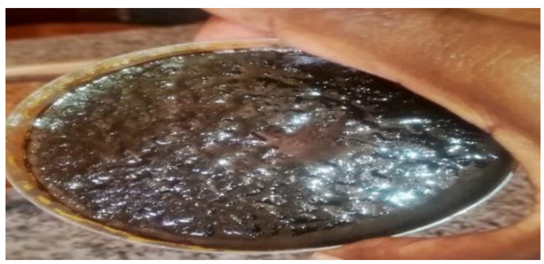

Figure1 : the resultant extract of Tribulus terrestris seeds

1.5 Experimental feeds preparation using tilapia commercial feed

A local commercial tilapia feed (powdered basal) purchased from Altkhasosi Fish Feeds Factor in Khartoum Bahri, with a proximate chemical composition as shown in Table 1, was used. Four experimental feeds were prepared, each containing different levels of Tribulus terrestris seed extract. Four feeds were prepared to include 0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg of the Tribulus terrestris seed extract, which were referred to as control, feed1, feed2, and feed3, respectively. Two kilograms of each feed were prepared. Using Guerrero (1975), the extracts were dissolved in 100 ml of ethanol 95% and added to the feed in spray form, then left for 24 hours to dry in an open shaded place (Image 2).

Table 1: proximate composition of the commercial feed (powdered basal) used in preparation of the four experimental feeds

Nutrient	%
Moisture	4,39
Crude Protein	41
Crude Fat	17,85
Crude Fiber	5,76
Ash	12,03
NFE	81,.3

1.6 **Experimental Fish**

A total of 4000 fertilized eggs of Nile tilapia, O. niloticus, were obtained from the Arab Organization for Agricultural Development (AOAD). The fertilized eggs were brought to the hatchery and allowed to hatch artificially in a three-liter incubator. The hatched fries were fed with commercial feed after total absorption of the yolk sac and when fries started searching for feed. The fry was acclimatized for one week prior to the start of the experiment.

1.**7 Experimental design**

A completely randomized design was used to conduct the experiment. One hundred eighty O. niloticus fry with an average body weight of 0.012 g were randomly distributed into twelves plastic Aquaria with triplicates and fed with experimental feeds of different dosages of 0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg of the extract. A total of 2700 fries of and total length 10 mm were equally distributed in 12 aquaria (225 fries) in each aquarium (41 × 32 × 30 cm) with a 26 cm water column fed with experimental feed for 70 days.

1.8 **Water Quality Mentoring**

The water quality parameters, such as dissolved oxygen, pH, ammonia concentration, and water temperature, were regularly monitored throughout the experimental period using an oxygen meter, pH meter, ammonia test kit, and thermometer, respectively.

1.9 **Growth Performance and feed conversion ratio determination**

The growth performance was evaluated by applying the following parameters: the Weight Gain determination of fish was calculated by formula: (WG) = 100 × [(final fish weight – initial fish weight) / initial fish weight]. Absolute growth rate determination was calculated by formula: (AGR) =100× [(final fish weight –initial fish weight/ period of time. Survival Rate determination was calculated by formula (SR) = [(total number of fish at the end of trial / total number of fish at the start of trial) × 100]. The feed conversion ratio (FCR) was calculated using the following formula: FCR = feed intake/weight gain.

1.10 **Statistical Analysis**

The data were subjected to statistical analysis, including analysis of variance (one-way ANOVA), using 17.0 (SPSS Inc.,) software program. The significance levels between the means of the groups were assessed using Tukey's honest significance difference (HSD), with a significance level of $P < 0.05$. The data are presented as mean± standard deviation (mean ± S.D, n =3). Graphs were plotted using Microsoft Excel software (2010 version).

Results

2. Results

2.1 Growth performance determination

2.1.1 Total weight gain of fish

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The results shown in Figure 2 below show a significant difference ($p<0.05$). The highest weight gain (0.086±0.024a) was recorded for the experimental fish group fed diets with 3 g/kg of Tribulus terrestris extract, and (0.079±0.010b) for the experimental fish group fed diets with 2.5 g/kg of Tribulus terrestris extract. However, there were no significant differences in the weight gain of the experimental fish group fed diets with treatment (2 g/kg), (0.062±0.008c) compared to the control groups fed with 0.0 g/kg of Tribulus terrestris extract (0.063 ± 0.006 c).

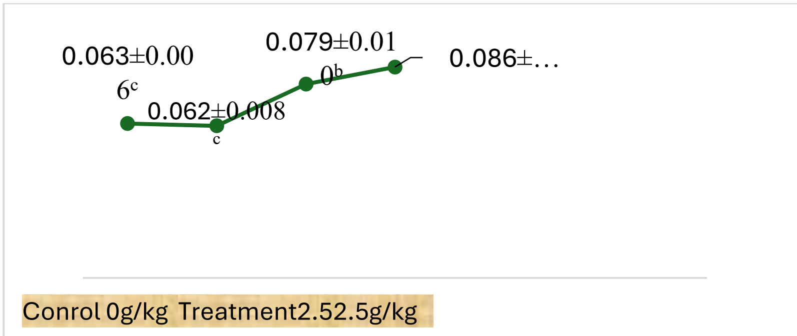

Figure2: Total weight gain for the four groups of the experimental *Nile tilapia* fries with different dosage of *Tribulus terrestris* extract (0.00 g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg)

2.1.2 Absolute growth rate of fish fries

Figure 3 shows the absolute growth rate results. shows significant difference ($p<0.05$) in the (AGR) (0.123±0.034a) was recorded in experimental fish group fed with 3.00 g/kg and (0.114±0.014b) of experimental fish groups fed diets with 2.50 g/kg, Tribulus terrestris extract, there are no significant difference in experimental fish group fed diets with treatment (2.00 g/kg), (0.058±0.041^{c}) comparing with control groups (0.00 g/kg). (0.090±0.023) *of Tribulus terrestris*

Figure 3: Absolute growth rate of the four groups of experimental Nile tilapia fries fed with different doses of *Tribulus terrestris* extract (0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg).

2.1.3 Survival rate

The Highest survival rate percentage was recorded for the fish group fed with feed2 (2.50 g/kg) (43.13±6.31a), while the lowest survival rate was observed for the fish group treatment3 (3.00 g/kg) (28.67±8.32^{c}). The control fish group fed with 0.00g\kg with and treatment1 (2.00g\kg) were given (31.23±3.37^{c}) and (34.97±6.2^{b}), Figure 4 shows the survival rates of the four *O. niloticus* fry groups fed different dosages of *Tribulus terrestris* extracts (0.00, 2.00, 2.50, and 3.00 g/kg).

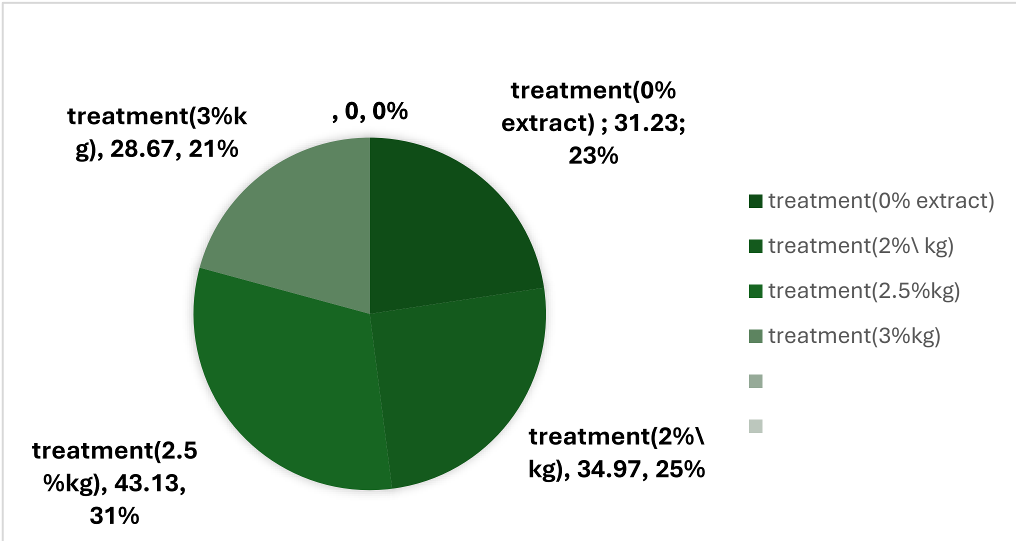

Figure 4.: Survival rate for the four groups of *O. niloticus* fries fed different concentrations of *Tribulus terrestris* extract (0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg).

2.2 Feed conversion ratio by fish fries

The highest feed conversion ratio (FCR) among the four experimental groups was recorded for the fry in treatment1 2g/k (4.02±0.37) and the lowest was in treatment3 3g/k (3.06± 0.98), while the results of treatment 2 and the control group were similar.

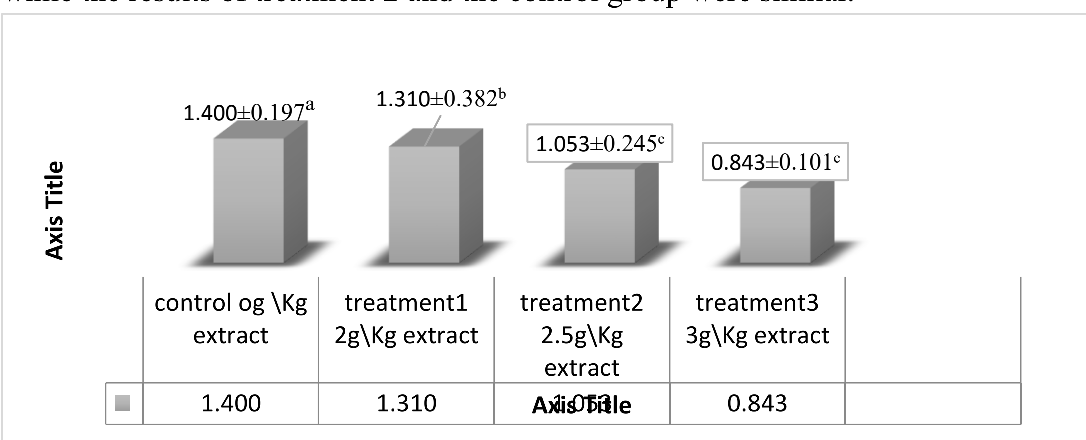

Figure 5: Feed conversion ratio of the four groups of experimental *O. niloticus* fry fed diets with different concentrations of *Tribulus terrestris* extract (0 g/kg, 2 g/kg, 2.5 g/kg, and 3 g/kg).

3. Discussion

3.1 Growth performance of *O. niloticus* fry

The obtained results showed the positive effects of dietary Tribulus terrestris extract on the performance and efficiency of fry feeding when compared with the control feed (0.063±0.006c). The treatment3 which was recorded to give the highest performance and efficiency, as shown in

Figures 2, 3, and 5. Compared to feed treatment2 and treatment1 (0.079±0.010b) is higher, and the control (Treatment1 and treatment2 is significantly different (0.05) effect compared with the control. The performance and feed conversion ratio of *the O. niloticus* fry group fed with treatment1 were not significantly different from those of the control compering with control group of *O. niloticus* fry. The results showed a positive increase in fry weight gain with increased Tribulus terrestris extract inclusion levels, with the inclusion level being 2.50 g/kg. In other words, the 0.20 g/kg of the extract showed insignificant difference in the total weight gain of fry compared to the control fry group. These results agree with those of Gültepe *et al*. (2014) on the effects of dietary extract supplementation on growth, feed utilization, and hematological, immunological, and biochemical variables of *O. niloticus*, who used 40% saponins. Similarly, the absolute growth rate of O. niloticus fry was confirmed to be in the positive direction of the effect of *Tribulus terrestris* seed extract when its level increased in fry feed up to 2.5 g/kg in fry feed (Figure 3). This finding is consistent with that reported by Gültepe. *et al*. (2014) on effects of dietary *Tribulus terrestris* extract on growth performance of and feed utilization for fingerling of *O. niloticu*s weighed 2.61±0.35g and there is no significance difference in treatmen1 (0.062±0.008c) and disagree in general factors of treatment, dosage uses and fish size. The obtained survival rates were significantly different ($p < 0.05$) among the four groups of *O. niloticus* fry fed with different levels of *Tribulus terrestris* extract, which can be attributed to the interruption of electricity power during the experimental period, especially at the second and tenth weeks of the experiment. However, the highest mortality was recorded in the fry group fed with 3.00 g/kg inclusion level of the *Tribulus terrestris* extract (Figure 4). In general, high mortality was recorded among the fry groups throughout the experimental period. The superior growth performance observed at the 3.0 g/kg inclusion level can be attributed to the bioactive constituents of Tribulus terrestris, particularly, steroidal saponins. Saponins act as natural growth promoters by stimulating the endocrine system. Previous studies have suggested that these compounds may enhance the endogenous secretion of androgens, which play a critical role in protein metabolism (Hassona, et al., 2020). This hormonal modulation likely improved the retention of nitrogen and the efficiency of protein synthesis in the fry, resulting in the observed increase in body weight and specific growth rate. However, the decline in performance at higher doses might be due to the anti-nutritional effects often associated with excessive saponin intake, which can interfere with nutrient absorption.

3.2 Feed conversion ratio of fish fries

significant differences were found among the *O. niloticus* groups fed with different levels of *Tribulus terrestris* extract (Figure 5). The improvement in the feed conversion ratio of *O. niloticus* fry groups was parallel with the increase in the extract levels in fry feeds. Similarly, Sakineh et al. (2017) reported the same finding in their study on the effects of extract on the growth and reproductive performance of male convict cichlid, *Cichlasoma nigrofasciatum*.

Conclusion

Dietary inclusion of *Tribulus terrestris* seed extract effectively enhances the growth performance and feed efficiency of *O. niloticus* fry. While the 3.00 g/kg inclusion level provided maximum growth, the discrepancy in survival rates suggests that a concentration between 2.50 and 3.00 g/kg may represent the optimal balance for production efficiency. Further research is recommended to elucidate the physiological mechanisms driving these outcomes.

Acknowledgment

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Data availability

All data analyzed for this study is available in this article

Conflict of interest

The authors declare no known source of conflict of interest.

Fund declaration

This research received no specific funding.

Ethical approval and consent to publish

This research is original was conducted by the authors.

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