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Efficacy of Dietary *Saccharomyces Cerevisiae* Supplementation with Inclusion of Q Z Toss™ on Nile Tilapia

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A B S T R A C T

The current study was designed to investigate the probiotic potential of the brewer's yeast (*Saccharomyces cerevisiae*) with and without the water quality improvement product Q Z Toss™ on growth and health performance of Nile tilapia (*Oreochromis niloticus*). Four fish groups were maintained on control diet supplemented with the yeast for one month. Both yeast dietary levels exhibited significant increase in growth parameters as well as in white blood cell count with no negative impacts on both hepatic and renal functions. The histopathological examination revealed better intestinal epithelial status in yeast treated groups than other groups, while gills showed significant improvement due to Q Z Toss™ treatment more than other untreated groups. Therefore, we can recommend the dietary inclusion of yeast in aqua-feed along with Q Z Toss™ application in rearing water as an efficient method to achieve feasible and sustainable fish production.

Keywords: *Saccharomyces cervisiae*,; Q Z Toss; Biochemical; Histopathological

1. Introduction

The aquaculture ability to reduce the resultant exhaustion of wild fisheries and enhance economic development has brought the industry to be the most dynamic food sector. The fish farming intensification puts fish under risk of infectious diseases. One of the solutions to improve the animal health is the use of functional dietary supplements (Ganguly et al. 2013 ; Hoseinifar et al. 2018). The reduction of pH level in the stomach and upper intestine increase the number of the intestinal beneficial flora such as lactic acid producing bacteria (Abu Elala & Ragaa 2015) and inhibit the growth of Gram-negative bacteria through the dissociation of the acids (SCFAs) and production of anions in bacterial cells (Hoseinifar et al. 2017; Nawaz et al. 2018).

Due to the negative effects of chemicals and antibiotics on the environment, followed by the development of mutagenic microbial strains and adversely affected fish health, their application to control disease outbreaks is no longer recommended (Cabello 2006). Therefore, the application of eco-friendly feed additives, such as microbial supplements, to improve the physiology, growth performance, and immune responses of aquaculture-related species have gained much more attention during recent years (Dawood & Koshio 2016). Naturally-occurring microorganisms play a key role in aquatic environments, as they can fulfil a wide range of roles, including recycling nutrients, degrading organic matter, and protecting fish against infections (Bentzon-Tilia et al. 2016). All these roles conduced to use these microorganisms in aquaculture and the development of probiotics. The use of probiotics is one of the alternative approaches to immunoprophylactic control in aquaculture (Esteban et al. 2014). Q Z Toss™ is a probiotic that help improve the water quality on fish and shrimp farms by reducing ammonia and nitrites and by digesting organic matter in the sludge.

The increase of innate immune activities through neutrophil activation, increasing lysozyme secretion, phagocytosis and production of anti-inflammatory cytokines in fish which safeguard the animal against diseases (Ringø et al. 2018).

Brewer's yeast (*Saccharomyces cerevisiae*) contains various immunosaccharides such as β-Glucans, nucleic acids, chitin and mannan-oligosaccharides Martínez Cruz *et al.* (2012). Several literatures approved the ameliorating effects of dietary yeast immunosaccharides on aquatic animal health (Faggio *et al.* 2015; Meena *et al.* 2013). The current study was conducted to assess the efficacy of saccharomyces on health of cultured Nile tilapia along with addition of Q Z Toss™ to enhance the water quality.

2. Material and methods

2.1. Fish

A total number of 120 apparently healthy Nile tilapia *Oreochromus niloticus* (*O. niloticus*) fish with average body weight 50±5 g were used in the experimental work. Fish were obtained from a private fish farm in El Beheira Governorate and transported alive to the experimental facility in aerated plastic tanks.

2.2. Experimental tanks

Fish were kept in 4 prepared concrete tanks (3X4X1 m. each). These tanks were used for holding the experimental fish throughout the acclimatization and experimental period of this study. All fish were acclimatized for 2 weeks prior to the experiment. The tanks were supplied with deep well water (Bexfield & Jurgens 2014). The continuous aeration was maintained in each Q Z using a 3hp electric air pump. Water temperature was kept naturally at 24±1 ºC.

2.3. Fish diets

Fish were fed floating fish pellets containing 30% crude protein (Aller Aqua Egypt). According to the used fish size, the diet was daily provided at 5% of body weight as described by **(Eurell *et al.* 1978).** The daily amount of food was offered on two occasions over day (at 9 AM and 1 PM).

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Table 1: Body weight, blood and serum biochemical parameters

	Control -Ve	Q Z Toss	Saccharomyces + Q Z toss	Saccharomyces
Body weight (g)	71.27 ± 0.37	74.33 ± 0.33	78.67 ± 0.33*****	74.67 ± 0.67
AST (U / ml)	11.23 ± 0.15	11.19 ± 0.46	10.81 ± 0.16	10.20 ± 0.46
ALT (U / ml)	36.00 ± 1.53	36.33 ± 0.88	31.30 ± 0.35 *	31.67 ± 0.88 *
Urea (mg/ dl)	3.58 ± 0.07	3.97 ± 0.26	3.430 ± 0.18	3.71 ± 0.05
Creatinine (mg/dl)	0.51 ± 0.02	0.56 ± 0.01	0.49 ± 0.01	0.58 ± 0.04
TLCs x 10^3/mm^3	4.80 ± 0.17	4.94 ± 0.12	4.70 ± 0.06	5.30 ± 0.21

Data represented as means± SE. Within rows values with different superscripts indicating that their corresponding means are significantly different at ($p \leq 0.05$) according to one way ANOVA followed by *Tukey* -b *test*.

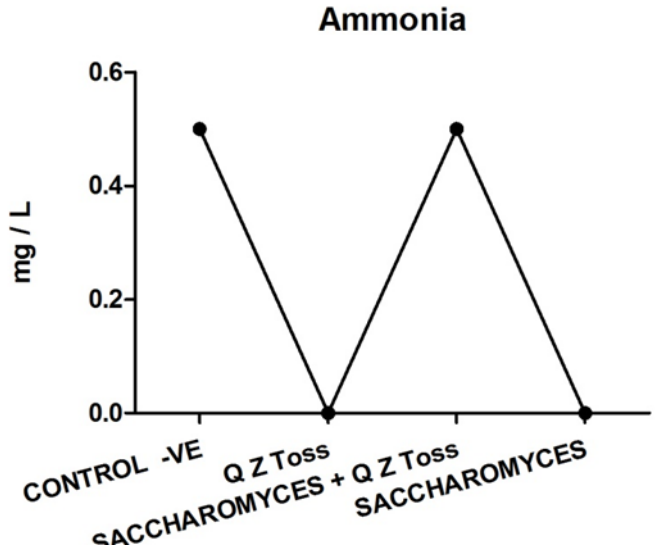

Figure 1: water levels of ammonia in fish groups

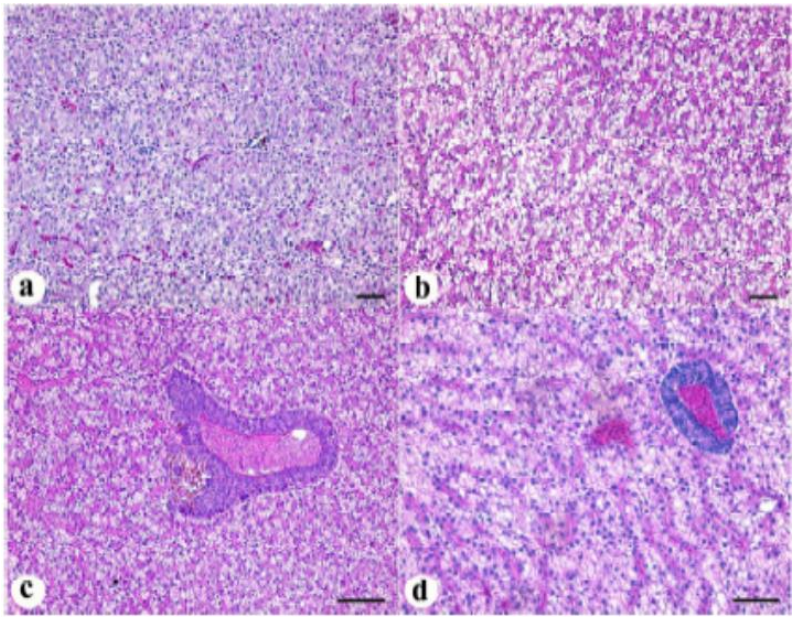

Figure 2: Hepatopancreas of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing mild congestion in sinusoidal spaces with some vacuolar degeneration in hepatocytes. (b) Q Z Toss™ only group showing diffuse vacuolar degeneration in hepatocytes. (c) *Saccharomyces cervicae* 500 gm/ton + Q Z Toss™ group showing congestion in main blood vessels and sinusoidal spaces with activation of melano-macrophage centers. (d) *Saccharomyces cervicae* 500 gm/ton only group showing congestion in main blood vessels and sinusoidal spaces. Hematoxylin & Eosin stain (Bar = 50 µm).

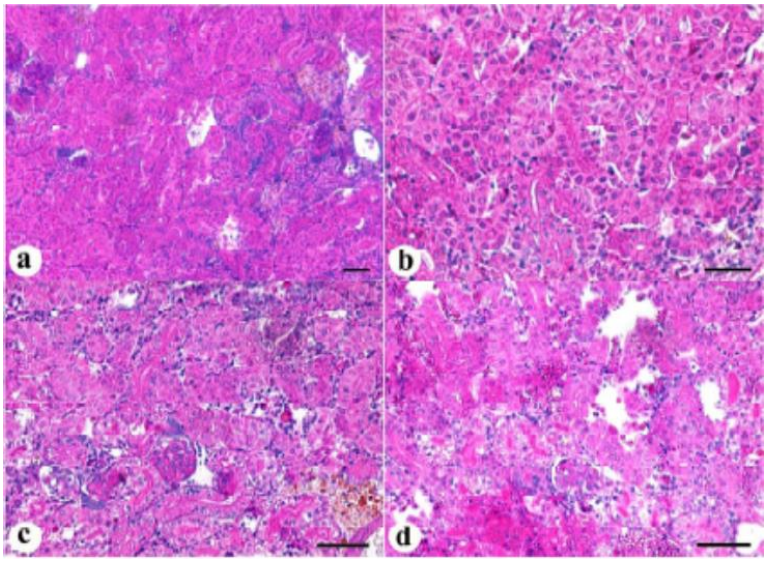

Figure 3: Posterior kidney of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing focal areas of renal tubular necrosis. (b) Q Z Toss™ only group showing normal tubular structure. (c) *Saccharomyces cervicae* 500 gm/ton + Q Z Toss™ group showing multifocal tubular degeneration and necrosis with activation of melano-macrophage centers. (d) *Saccharomyces cervicae* 500 gm/ton only group showing multifocal tubular degeneration and necrosis. Hematoxylin & Eosin stain (Bar = 50 µm).

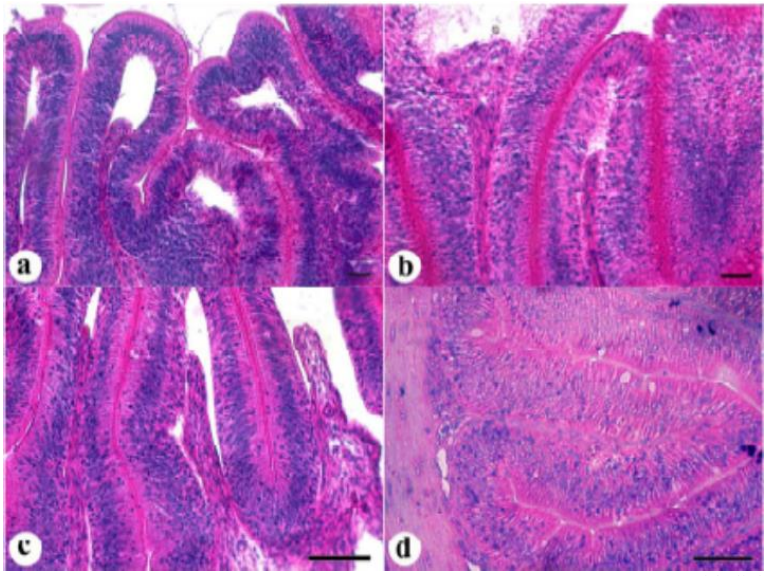

Figure 4: Intestine of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing moderate sub-epithelial edema. (b) Q Z Toss™ only group showing mild sub-epithelial edema. (c) *Saccharomyces cervicae* 500 gm/ton + Q Z Toss™ group showing normal healthy villar epithelial structure. (d) *Saccharomyces cervicae* 500 gm/ton only group showing normal healthy villar epithelial structure. Hematoxylin & Eosin stain (Bar = 50 µm).

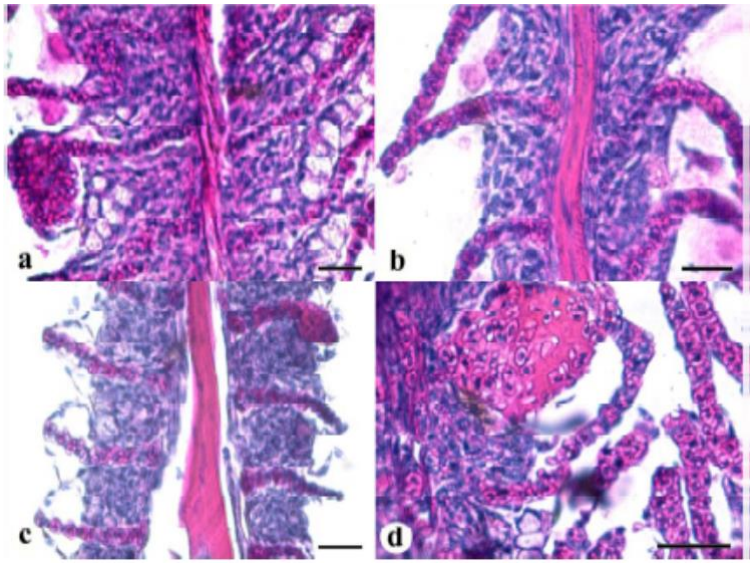

Figure 5: Gills of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing severe hyperplasia in malpegian cell layers with pronounced telangiectasis. (b) Q Z Toss™ only group showing moderate hyperplasia in malpegian cell layers. (c) *Saccharomyces cervicae* 500 gm/ton + Q Z Toss™ group showing moderate hyperplasia in malpegian cell layers with separation of the epithelial lining of the secondary gill lamellae. (d) *Saccharomyces cervicae* 500 gm/ton only group showing severe hyperplasia in malpegian cell layers and severe telangiectasis. Hematoxylin & Eosin stain (Bar = 50 µm).

2.4. Probiotics

1. Brewer's yeast (*Saccharomyces cerevisiae*)

2. Q Z Toss™

As confirmed by the manufacturer (Keeton Industries USA), it is a blend of bacillus species contains 2×10^{12} cfu/kg namely, *Bacillus subtilis* $9X10^{11}$ cfu, *Bacillus amyloliquefaciens*, $8X10^{11}$ cfu, and *Bacillus lichinformis*, 3×10^{11} cfu/kg.

2.5. Preparation of experimental feed

The diets were prepared by mixing the yeast in a ratio of 250 g/ton feed with sunflower oil, applied and mixed with the feed and then left for drying.

2.6. Experiment

One hundred eighty *O. niloticus* fish were distributed randomly in 4 concrete tanks, 30 fish / tank which filled with aerated deep well water. Fish in the 1st tank were fed regular feed till the end of experiment (30 days) and act as negative control. Fish in 2nd tank were fed regular feed, as well as Q Z Toss™ in a dose of 2g/ m^3 was added to the water, after that Q Z Toss™ was added again as 1 g/m^3 each week till the end of experiment. Fish in the 3rd tank were fed on ration containing 500 gm/ ton yeast with addition of Q Z Toss™ exactly like the 2nd group. Fish in the 4th tank were fed on ration containing 500 g/ ton yeast without application of Q Z Toss™ till the end of experiment.

The water in tanks receiving Q Z Toss™ remained without change till the end of experiment, while the water in the tanks without Q Z Toss™ was changed daily. The amount of ration was re-adjusted every week according to the fish body weight. Fish were kept under observation for any up normal signs. The level of ammonia in each tank was determined by ammonia kits at the end of experiment. At the end of the experiment the fish were weighted to estimate the growth rate and FCR.

2.7. Growth parameters

The nutritional performance in terms of feed intake Faggio et al. (2015) (g), initial weight (g), final weight (FW) (g), weight gain (WG) (g), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (Ruyet et al.) were calculated bi-weekly for two months (Abu Elala & Ragaa 2015).

2.8. *Sampling*

At the end of the experiment, the fish was immobilized on absorbent paper towel and kept motionless. The body surface was then cleaned and blotted dry. The blood samples were collected from the caudal vein on EDTA to determine haemoglobin and white blood cell count. Other blood samples were collected without anticoagulants for serum separation. The serum samples were stored at −20 °C for biochemical analysis. After complete necropsy of the fish, fresh tissue specimens were collected from hepatopancreas, posterior kidney, intestine and gills were rapidly fixed in Davidson's fixative for 24 hours then transferred to 70% ethanol till processing proceeds, for histopathological examination.

2.9. Determination of biochemical parameters

The serum samples were used to measure alanine aminotransferase (ALT) and aspartate aminotransferase (Borges et al.), they were determined colorimetrically according to the methods described by (Reitman & Frankel 1957), respectively. Serum urea and creatinine were determined colorimetrically according to the methods described by (Fawcett & Scott 1960) and (Bartels et al. 1972), respectively.

2.10. Total leucocyte count (TLC)

The total leucocyte count was determined by haemocytometry. For this, the blood specimen is diluted (usually in 1:20 ratio) with the help of WBC diluting fluid (commonly the Turk's Fluid) which preserve, stains and fix the White blood cells and Lysis the Red Blood Cells. The Turk's fluid is isotonic to the White blood cells and does not cause any damage to it. After diluting the specimen, the content is charged on Hemocytometer chamber and the cells are counted in the areas specific for WBC count.

2.11. Histopathological examination

The fixed tissue specimens were processed through the conventional paraffin embedding techniques (Layton & Suvarna 2013). Paraffin blocks were cut as 4 µm-thick tissue sections. Then 2 replicates from the same section were mounted on slides then processed for hematoxylin-eosin (H&E) staining, cover-slipped then visualized by Light Microscope (Olympus BX43).

2.12. Statistical analysis

All data were statistically analysed using one-way Analysis of Variance (ANOVA) using GraphPad Prism 5 (San Diego, USA). All declarations of significance depended on ($p < 0.05$).

3. Results

Growth performance

The growth performance of *O. niloticus* fish fed on yeast is summarized in (Table 1). The results revealed that both yeast supplemented groups showed increase in live body weight gain which was apparently significant in yeast with Q Z Toss™ group ($P \leq 0.05$) compared to control one.

Ammonia levels in water

Inclusion of Q Z Toss™ in fish tanks water decreased ammonia levels in water as showed in figure (Mart *et al.*), where both groups of Q Z Toss™ (Q Z Toss™ only and yeast with Q Z Toss™) showed zero levels of ammonia in water compared to a mean level of (0.5) ammonia in groups without Q Z Toss™.

Haematogram and serum parameters

The blood picture of fish group fed yeast revealed higher TLC count. All experimental fish groups have normal haematological and serum biochemical findings (Table 1). Liver enzymes showed no significant different for AST, while yeast groups showed significant decrease in ALT activity as compared to control group. Moreover, renal function tests represented in urea and creatinine showed normal values as control for treated groups, excluding any drawbacks of yeast supplementation on kidneys function.

Histopathological findings

Hepatopancreas of all treated fish showed congestion in main blood vessels and sinusoidal spaces, with some vacuolar degeneration in hepatocytes (Figure 2), in yeast + Q Z Toss™ group there was mild activation of melano-macrophage centers (Figure 2c). Posterior kidney of all treated fish showed multifocal tubular degeneration and necrosis except Q Z Toss™ only group showing normal tubular structure (Figure 3), while in yeast + Q Z Toss™ group there was activation of melano-macrophage centers (Figure 3c). Intestine of all yeast treated fish groups showed normal healthy villar epithelial structure (Figure 4c,d), while in control –ve group and Q Z Toss™ only group mild sub-epithelial edema was observed (Figure 4a,b). Gills of all treated fish showed milder degrees of pathology in Q Z Toss™ treated groups represented by moderate hyperplasia in malpegian cell layers (Figure 5b,c), while control –ve group and yeast only groups showed severe hyperplasia in malpeghian cell layers with separation of the epithelial lining of the secondary gill lamellae and severe telangiectasis (Figure 5a,d).

4. Discussion

Dietary supplementation of the brewer's yeast (*Saccharomyces cerevisiae*) improved the weight gain and in Nile tilapia. Previous studies on the dietary inclusion of yeast presented a significant increase in both weight gain and feed utilization efficiency in rainbow trout and pacific white shrimp (Staykov 2007; Zhang et al. 2012). Moreover, (Do Huu et al. 2016; Shelby et al. 2009), stated a significant increase in both feed intake and growth parameters of Nile tilapia treated with yeast extract in their diets.

Collectively, it has been reported that dietary yeast has the different mode of actions; it adsorbs the pathogenic flora, passing them outside the intestinal tract, and preventing them from host invasion and colonization (Refstie et al. 2010), which might increase the amino acids utilization of the host (Rawles et al. 1997). Also, the degradation of glucan by glucanase in digestive glands promotes the use of more protein for growth (Liranço et al. 2013), this surely can explain the observed healthy intestinal epithelial status in yeast treated groups in comparison to other groups.

The positive impact of yeast on haematological and serum biochemical parameters was evidenced by increasing the white blood cell count, hepatic and renal function tests, and their histopathological picture in comparison to the control groups. The previous literature stated that the incorporation of yeast in fish diet increase the TLC count, total protein, and albumin concentrations ((Meena et al. 2013). This could be attributed to potential non-specific responses in fish.

Maintaining good water quality is important in aquaculture as the quality of water affects the health and growth of the fish. Good water quality can also help improve the Feed Conversion Ratio which in turn improves fish size and profits. Ammonia concentrations are regulated by passive diffusion down the partial pressure gradient of NH_3 across the gill (Wilson et al. 1994). This gradient is maintained by the simultaneous active excretion of protons from the gills. At the gill surface, protons bind to ammonia molecules, resulting in the formation of ammonium ions.

Excess levels of total ammonia present a major obstacle to intensive fish culture, as high volumes of uneaten feed and fecal matter lead to the accumulation of nitrogenous waste (Borges et al. 2003). Unmanaged total ammonia concentrations in aquaculture are known to compromise fish health, retard growth and cause mortality (Ruyet et al. 1997).

Gut is the preliminary target for immunosaccharides especially in case of immune modulation because it's the place where they establish and interact with host body. The interaction between yeast immunosaccharides and pattern recognition receptors (PRRs), such as β-glucan receptors that expressed in macrophage cell wall, stimulates and initiates a cascade of reactions involving cytokines, interleukins and tumor necrosis factors (Selim & Reda 2015). Better intestinal tissue status in histopathology and activation of melanomacrophage centers in yeast group as a result of macrophage activation due to yeast.

Furthermore, yeast extract is not digested by intestinal enzymes but in fact is a substrate for growth of beneficial bacteria like lactic acid producing bacteria. Pathogens cannot utilize manna oligosaccharide, cannot multiply and are starved to death. Further, yeast is capable to bind and block the glycoprotein receptors on pathogens prevent their attachment and colonization (Hoseinifar et al. 2015).

The present study revealed that concentrations of ammonia were observed to be low in treated Q Zs than in the control Q Z. Probiotics are instrumental in maintaining good water quality, higher beneficial and lower pathogenic bacteria loads in fish Q Zs (Mart et al. 2012).

In conclusion, this study revealed no deleterious effects due to supplementation of Nile tilapia feed with brewer's yeast on the function and integrity of hepatopancreas and posterior kidney with an improvement in water quality via Q Z Toss. Altogether, we can recommend the dietary inclusion of yeast in aqua-feed along with Q Z Toss™ application in rearing water as an efficient method to achieve feasible and sustainable fish production.

Competing Interests

The authors have no conflict of interest.

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