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Dietary incorporation of Sweet Potato *Ipomoea batatas* shoots improved growth performance and haematological profile of Tilapia *Oreochromis niloticus* in Hapa Nets

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ABSTRACT

This study was conducted to evaluate the potential of sweet potato *Ipomoea batatas* powder (IbSPP), hot-water extracts (IbSPHWE) and crude ethanolic extracts (IbSPCEE) as growth promoter and immunoenhancer for tilapia cultured in hapa nets. Fish were divided randomly into four Treatments: T1 (control group) was fed a practical diet (PD) while T2, T3 and T4 were fed PD + IbSP powder (P), PD + IbSP hot-water extract (HWE), PD + IbSP Crude Ethanol Extracts (CCE), respectively. The growth indices and haematological profile of cultured fish were recorded after four months of feeding experiment.

The final weight, weight gain, specific growth rate, condition factor, FCR, PER, FER were significantly higher in fishes that received PD +IbSPHWE than those that received either PD, PD + IbSPCEE or PD+ IbSPP only. The same trend was observed with the RBC, Hb, HCT, WBC and the PLT and its indices. The study has demonstrated that incorporation of the hot-water extracts of *I. batatas* could improve the growth performance and increase immunocompetence of *O. niloticus* as evidenced by improved haematological profile.

Keywords: *Oreochromis niloticus, Ipomoea batatas,* Immunostimulants, Hot-Water extracts, Haematological profile

Introduction

The production of commercially-important aquaculture species significantly increased during the past decades. To meet the market demands, fish culture practices in enclosed spaces like ponds, net cages and tanks had intensified. Along with intensive fish culture are increase in stocking density, water quality and environment manipulation, enhanced fish nutrition and feeding management and fish health management. However, with increased stocking density, fish are exposed to a number of stressors such as overcrowding, transport, handling, which adversely affect the health status of cultured fish. Improvement of the health status and growth performance of cultured fish is of great importance to aquaculture. Strengthening the defense mechanism of fish through prophylactic administration of immunostimulants seem to be one of the most promising method of controlling diseases in aquaculture (Li et al, 2004; Robertsen, 1999; Raa et al, 1992). These substances increase the immunocompetence and resistance to pathogens and diseases by enhancing both the specific and non-specific defense mechanisms of fish and other organisms (Zhou et al, 2003).

Recently, increasing attention is being paid to the use of plant products for disease control in aquaculture as an alternative to chemical treatments (Reverter et al, 2014) Numerous studies documented the efficacy of plant extracts as anti-stress, growth promoter, appetite stimulator, immunobooster, and elicit disease resistance and anti-pathogen properties in fish and shrimp aquaculture due to the presence of active principles like alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils (Chakraborty and Hancz, 2011; Citarasu, 2010).

The sweet potato *Ipomoea batatas* (L) is a dicotyledonous plant that belongs to the bindweed or morning-glory family Convolvulaceae. In the Philippines, sweet potatoes (locally known as *camote* or *kamote*) are an important food crop in rural areas. They are often a staple among impoverished families in provinces, as they are easier to cultivate and cost less than rice, it can even be found growing along the backyards of some houses in the rural areas of the country. The young shoots (red or purple in color) and leaves of *I. batatas* are sometimes eaten as a salad or a main ingredient in some stew. The leaves are even used as a cheap source of protein for ruminant feeds.

Various leaf meal and other plant extracts have been studied as a potential feed ingredient for fish and shrimp (Garg et al, 2019; Labh et al, 2017; Wu et al, 2016; Ganzon-Naret, 2014; Baleta et al, 2013; Kaleeswaran et al, 2011; Abd El-Hakim et al, 2010; Olmendo Sanchez et al, 2009; Afuang et al, 2003;), however, utilization of sweet potato leaf meal and extracts as a feed ingredient for animal and fish diets is scarce (Adewolu, 2008; Abonyi et al, 2012). The study was conducted to determine the effects of sweet potato shoots as growth promoter and immunobooster for tilapia particularly on the survival, growth indices and haematological profile.

Material and Methods

Experimental Fish

Healthy tilapia (*O. niloticus*) with a mean weight of 17-18 g were obtained from the Partido State University (PSU) Multispecies Hatchery Laboratory, PSU Sagñay Campus. Fish were acclimated for two weeks at the experimental hapa nets (3m x 5m x 1m) installed in 1000 m^2 pond**.** Fish were fed with commercial feed *ad libitum* twice per day until initiation of the experiment. During the feeding experiment, ten (10) fish were stocked at each hapa net (1m x 1m x1m) Water parameters were measured during the conditioning phase and the duration of the feeding experiment. The water temperature, pH and dissolved oxygen ranged between 29 ±2.2 °C, 7.2-7.5 pH and 6.4 ±1.3 mg L^{-1}, respectively during the culture period.

Collection and Preparation of I. batatas Powdered Leaves

The shoots of *I. batatas* were purchased at the local market of the town. Preparation of the feeds were done at the CAS Science Laboratory, Partido State University, Goa, Camarines Sur, Philippines. The leaves were washed thoroughly with tap water and the unwanted parts (stems and the necrotic parts of the leaves) were removed. The cleaned and sorted leaves were air-dried for two weeks at room temperature. The air-dried leaves were oven-dried at 60^0C until a brittle and crispy consistency of the leaves is achieved. The dried leaves were crushed and grinded with a blender and hammer mill until powdered particles are produced. The powdered leaves were stored in an air-tight plastic container at room temperature until use. The ratio of wet to dried powdered *I. batatas* leaves were recorded.

Preparation of the Hot-Water Extracts of I. batatas

The hot-water extract of *Ipomea batatas* shoots were prepared based on the method described by Fujiki et al. (1992), Hou and Chen (2005) and Baleta et al (2013) with modifications. Briefly, 100 g of dried *I. batatas* powder were added to 1000 mL of distilled water and were boiled for 3 h in a water bath set-up. The boiled suspension was passed through a nylon mesh

and the filtrate were frozen until use. The frozen *I. batatas* HWE were thawed and were boiled before use for the experiment.

Preparation of the I. batatas Crude Ethanolic Extracts

Five hundred grams of *I. batatas* powder were soaked in 5 L of 95% ethanol and incubated in the dark condition at room temperature for 72 hrs. Thereafter, the supernatant was filtered using Whatman No. 42 filter paper. The supernatant was then evaporated to dry under reduced condition (40°C) via a rotary evaporator (IKA-100). The obtained extract was stored at a refrigerator.

Preparation of Experimental Diets and Feeding Experiment

Fish were divided randomly into four Treatments (T1, T2, T3 and T4). Each group was divided into two subgroups (A and B; each sub-group had triplicate of 30 fish). Subgroup A was used for growth and survival studies while subgroup B was used for examination of haematological profile. T1 (control group) was fed a practical diet. Experimental diets were formulated from locally available ingredients to satisfy the nutrient requirements of *O. niloticus* (Table 1). The proximate composition of the practical and experimental diets is presented in Table 2 The ingredients were ground, mixed, pelletized to a 1.5 mm diameter and dried at room temperature for a day. Pellets were stored at 4°C until use. T2 was fed practical diet supplemented with 5% *I. batatas* powder (50 g kg^{-1}). T3 and T4 were given with practical diet supplemented with 5% *I. batatas* HWE (50 mL kg^{-1}) and *5% I. batatas* CEE (50 g kg^{-1}), respectively . Food was provided twice daily (8:00 am and 4:00 pm) at the rate of 5% of fish live body weight. The amount of food was readjusted every two weeks according to fish weight.

Table 1. Ingredients (per kg) for the experimental diets of tilapia

	Experimental Groups			
Feed Ingredient	Practical Diet (PD)	PD + IbSP P	PD + IbSP HWE	PD + IbSP CEE
Fish Meal (local)	0.25	0.28	0.28	0.28
Corn Meal	0.12	0.10	0.10	0.10
Soy bean meal	0.28	0.27	0.27	0.27
Rice bran	0.21	0.16	0.16	0.16
Corn Oil	0.05	0.05	0.05	0.05
Vit. And Minerals	0.04	0.04	0.04	0.04
Corn Starch	0.05	0.05	0.05	0.05
IbSP Powder		0.05		
IbSP HWE			0.05	
IbSP CCE				0.05

Note: IbSP – *Ipomoea batatas Sweet Potato Powder*;
IbSP HWE - *Ipomoea batatas Sweet Potato Hot-water Extract;*
IbSP CCE - *Ipomoea batatas Sweet Potato Crude Ethanolic Extract*

Table 2. Proximate composition of the practical and experimental diets of tilapia

	Experimental Groups			
Feed Ingredient	Practical Diet (PD)	PD + IbSP P	PD + IbSP HWE	PD + IbSP CEE
Crude Protein (N x 6.25)	25.12	25.48	25.25	24.26
Crude Fat	12.45	13.91	12.24	12.23
Ash	10.42	11.05	11.08	10.97
Moisture	12.89	12.93	12.13	13.08
Total Carbohydrates (%)	34.15	34.63	34.20	34.94

Note: IbSP – *Ipomoea batatas Sweet Potato Powder*;
IbSP HWE - *Ipomoea batatas Sweet Potato Hot-water Extract;*
IbSP CCE - *Ipomoea batatas Sweet Potato Crude Ethanolic Extract*

Growth Performance

The experimental fish in sub-group A were counted and weighed at the end of the feeding experiment to assess growth performance. The final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency ratio (FER) and condition factor (CF) were determined according to De Silva and Anderson (1995), Hevroy et al. (2005) and Jafarian et al. (2007).

Haematological Profile, Blood Collection and Plasma Separation

Six fish were randomly collected from each treatment in sub-group B at the end of the feeding experiment. Whole blood were collected from the caudal vein of each fish using 1cc syringe (25 g x 5/8). The collected blood was transferred in 0.5 mL heparin vacutainer tube with EDTA and maintained at low temperature until analyses. The blood parameters red blood cell count (RBC; 10^6/mL), hematocrit (HCT; %), hemoglobin concentration (HB; g/l), mean corpuscular volume (MCV; fl), mean corpuscular hemoglobin (MCH; pg) and mean corpuscular hemoglobin concentration (MCHC; g/dl), white blood cell count (WBC; 10^3/mL), lymphocytes (LYMP#; 10^6/mL), monocytes (Mon#; 10^6/mL), eosinophil (Eos#; 10^6/mL), platelet count (PLT; 10^6/mL), mean platelet volume (MPV; fl), platelet distribution width (PDW; fl), platelet large cell count (P-LCC; 10^6/mL) and platelet large cell ratio (P-LCR; %) were analyzed using Rayto Auto Hematological Analyzer (RT-7600).

Statistical Analyses

All data were analyzed by one-way ANOVA ($P<0.05$) and Tukey's tests. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS v.26 (SPSS, IL, USA).

Results and Discussion

The final weight, weight gain, specific growth rate and condition factor were significantly higher in fishes that received practical diets supplemented with the HWE of *I. batatas* than those that received either PD, PD + IbSPCEE or PD+ IbSPP only for four months (Table 3). Similarly, the feed conversion ratio, protein efficiency ratio, and feed efficiency ratio is significantly higher in T3 (PD +IbSPHWE) as compared to T4 (PD + IbSPCEE), T1 (PD), and T2 (PD + IbSPP) after the feeding experiment (Table 4).

Significant increase in RBC, Hb, HCT and other RBC indices were also observed in fishes that received PD+IbSPHWE as compared to fishes that received either PD + IbSPCEE, PD+ IbSPP, or PD only (Table 5) ($P<0.05$). The same trend was observed with the WBC and other leukocyte indices (Table 6), and even with the PLT and its indices (Table 7).

Table 3. Growth Performance of *O. niloticus* under different diets with sweet potato *I. batatas* powder and extracts after 16-wk culture period

Parameters	Feeds			
	Control	**Experimental Treatments**		
	Feed 1 (0 g kg^{-1} IbSP)	**Feed 2 (PD + IbSP Powder)**	**Feed 3 (PD + IbSHWE)**	**Feed 4 (PD + IbSCEE)**
Length (Initial) cm	9.50 ±0.35^{a}	10.13 ±0.12^{a}	9.23 ±0.44^{a}	9.47 ±0.43^{a}
Length (Final) cm	16.23 ±0.40^{b}	16.11 ±0.35^{b}	18.49 ±0.44^{a}	16.86 ±0.33^{b}
Weight (Initial) g	14.03 ±0.44^{a}	14.65 ±0.23^{a}	14.23 ±0.20^{a}	14.93 ±1.09^{a}
Weight (Final) g	78.80 ±5.69^{b}	78.67 ±4.83^{b}	120.24 ±5.69^{a}	85.40 ±5.42^{b}
Weight gain (g)	60.84 ±5.20^{b}	60.81 ±14.23^{b}	101.25 ±4.71^{a}	67.26 ±6.25^{b}
Survival rate (%)	100 ±0.00^{a}	100 ±0.00^{a}	100 ±0.00^{a}	100 ±0.00^{a}
Average Daily weight gain (g)	0.57 ±0.05^{b}	0.56 ±0.04^{b}	0.93 ±0.05^{a}	0.62 ±0.05^{b}
Average Daily length gain (g)	0.06 ±0.01^{ab}	0.05 ±0.00^{b}	0.08 ±0.01^{a}	0.06 ±0.01^{ab}
Specific Growth rate	1.51 ±0.09^{b}	1.47 ±0.05^{b}	1.87 ±0.03^{a}	1.53 ±0.05^{b}
Condition Factor	1.51 ±0.03^{a}	1.52 ±0.02^{a}	1.67 ±0.04^{a}	1.54 ±0.05^{a}

Means with the same letters as superscripts are not significantly different ($P>0.05$)

Values are expressed as mean ± standard error, calculated from the mean-square for error of the ANOVA

Table 4. Parameters of feed and nutrient utilization of *O. niloticus* under different diets with sweet potato I. *batatas* powder and extracts after 16-wk culture period

Parameters	Feeds			
	Control	**Experimental Treatments**		
	Feed 1 (0 g kg^{-1} IbSP)	**Feed 2 (PD + IbSP Powder)**	**Feed 3 (PD + IbSHWE)**	**Feed 4 (PD + IbSCEE)**
Feed intake (g feed g^{-1} fish)	95.94 ±0.08^{b}	96.52 ±0.64^{b}	98.59 ±0.56^{a}	93.85 ±0.76^{c}
Feed conversion ratio	1.51 ±0.15^{a}	1.52 ±0.11^{a}	0.94 ±0.04^{b}	1.35 ±0.11^{a}
Protein Efficiency Ratio	2.15 ±0.20^{b}	2.13 ±0.16^{b}	3.53 ±0.18^{a}	2.34 ±0.18^{b}
Feed efficiency ratio	67.52 ±6.41^{b}	66.30 ±4.65^{b}	107.47 ±5.05^{a}	75.12 ±5.91^{b}

Means with the same letters as superscripts are not significantly different (P>0.05)
Values are expressed as mean ± standard error, calculated from the mean-square for error of the ANOVA

Table 5. Erythrocyte indices of *O. niloticus* under different diets with sweet potato *I. batatas* powder and extracts after 16-wk culture period.

Treatment	Erythrocyte Indices						
	Red Blood Cell RBC (10^6/mL)	**Hemoglobin Hb (g/dL)**	**Hematocrit HCT (%)**	**Mean Corpuscular Volume MCV (fl)**	**Mean Corpuscular Hemoglobin MCH (pg)**	**Mean Corpuscular Hemoglobin Concerartion MCHC (g/dL)**	**Red Blood Cell Distribution Width RDW-CV**
PD	1.69 ±0.03^{c}	78.00 ±2.85^{c}	28.13 ±1.09^{c}	169.01 ±3.81^{c}	49.64 ±0.42^{b}	254.33 ±8.29^{b}	10.05 ±0.49^{b}
PD+IbS Powder	1.72 ±0.03^{ac}	84.87 ±1.02^{b}	29.52 ±0.58^{c}	172.84 ±3.66^{bc}	48.05 ±0.83^{b}	269.67 ±4.42^{b}	15.75 ±1.79^{a}
PD+IbS HWE	2.20 ±0.04^{a}	106.33 ±3.09^{a}	39.68 ±1.12^{a}	185.53 ±2.49^{a}	53.27 ±0.94^{a}	329.53 ±10.66^{a}	20.57 ±2.40^{a}
PD+IbS CEE	1.82 ±0.03^{b}	88.33 ±1.99^{b}	32.26 ±0.69^{b}	181.25 ±2.96^{ab}	49.28 ±0.55^{b}	274.33 ±3.75^{b}	17.06 ±1.54^{b}

Means with the same letters as superscripts are not significantly different (P> 0.05)
Values are expressed as mean ± standard error, calculated from the mean-square for error of the ANOVA

Table 6. Leukocyte indices of *O. niloticus* under different diets with sweet potato *I. batatas* powder and extracts after 16-wk culture period.

Treatment	Leukocyte Indices				
	White Blood Cell WBC (10^6/mL)	**Lymphocytes LYMP# (10^6/mL)**	**Monocytes MON (10^6/mL)**	**Nuetrophils NEUT (10^6/mL)**	**Eosinophils EOS (10^6/mL)**
PD	67.45 ±0.84^{c}	67.02 ±1.06^{c}	67.02±1.06^{c}	15.84 ±5.77^{a}	2.34 ±1.21^{a}
PD+IbS Powder	78.05 ±2.38^{b}	72.16 ±1.92^{b}	72.16 ±1.92^{b}	16.59 ±10.37^{a}	2.26 ±0.62^{a}
PD+IbS HWE	88.75 ±1.86^{a}	82.23 ±1.30^{a}	82.23 ±1.30^{a}	22.48 ±5.47^{a}	1.38 ±0.74^{a}
PD+IbS CEE	78.44 ±2.09^{b}	79.28 ±1.32^{a}	79.28 ±1.32^{a}	22.92 ±11.42^{a}	1.51 ±1.08^{a}

Means with the same letters as superscripts are not significantly different (P>0.05)
Values are expressed as mean ± standard error, calculated from the mean-square for error of the ANOVA

Table 7. Platelet indices of *O. niloticus* under different diets with sweet potato *I. batatas* powder and extracts after 16-wk culture period.

Treatment	PLT Indices					
	Platelet PLT (10^6/mL)	**Mean Platelet Volume MPV (fl)**	**Platelet Distribution Width PDW (fl)**	**Plateletcrit PCT (%)**	**Platelet-Large Cell Count P-LCC (10^6/mL)**	**Platelet-Large Cell Ratio P-LCR (%)**
PD	321.93 ±22.47^c	9.85 ±2.23^a	4.75 ±0.34^a	0.21 ±0.03^b	6.53 ±0.61^a	28.80 ±3.72^a
PD+IbS Powder	446.27 ±26.06^b	5.13 ±0.07^b	4.19 ±0.08^a	0.24 ±0.01^b	7.11 ±0.47^a	34.27 ±2.83^a
PD+IbS HWE	694.33 ±43.45^a	4.91 ±0.07^b	4.17 ±0.09^a	0.35 ±0.03^a	6.17 ±0.42^a	37.86 ±4.56^a
PD+IbS CEE	413.00 ±17.68^b	5.17 ±0.11^b	4.12 ±0.09^a	0.28 ±0.02^b	7.03 ±0.77^a	36.27 ±3.96^a

Means with the same letters as superscripts are not significantly different (P>0.05)
Values are expressed as mean ± standard error, calculated from the mean-square for error of the ANOVA

Incorporation of feed additives; such as vitamins, minerals and prebiotics are considered as promising options in aquaculture particularly in the enhancement of growth, disease prevention and improvement of aquatic animal health.

Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured fish (Harikrishnan et al., 2012; Pavaraj et al., 2011; Takaoka et al., 2011). In the study of Shalaby et al. (2006), food intake, specific growth rate and final weight of Nile tilapia (*Oreochromis niloticus*) increased when garlic was incorporated in the diet. Punitha et al (2008) found out that grouper *Ephinephelus tauvina* fed diets with a mixture of methanolic herb extracts (Bermuda grass (*Cynodon dactylon*), Long pepper (*Piper longum*), stonebreaker (*Phyllanthus niruri*), coat buttons (*Tridax procumbens*) and ginger (*Zingiber officinalis*)) exhibited 41% increase in weight of grouper than the control group. Aside from gain in weight, several studies demonstrated that plant extracts somehow improve digestibility and availability of nutrients resulting in an increase in feed conversion and leading to a higher protein synthesis (Citarasu, 2010; Nya and Austin, 2009; Talpur et al., 2013). For example, Putra et al. (2013) showed that supplemented diet with 1% of ethanolic katuk extract (*Sauropus androgynous*) stimulated appetite, growth and improved food utilization (lower feed conversion ratio) in grouper *Ephinephelus coioides*. The health status of the fish can also be determined by evaluating the Specific growth rate (SGR) and condition factor (CF). Extracts from plants and other products may control, limit and inhibit the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in fish guts. The incorporation of these plant extracts in the diets of fish may provide greater efficiency in the feed utilization, which may result in improved growth and feed efficiency (Jain et al, 2008; Bedford, 2000). Our study demonstrated that the incorporation of *I. batatas* extracts in the practical diets of tilapia significantly improve the gain in weight, specific growth rate, feed conversion ratio, protein efficiency ratio, feed efficiency ratio and condition factor of tilapia.

Haematological profile is a pathophysiological reflector of the entire body and the counts of various parameters in blood give an indication to the health status of fish by determining any abnormality brought about by using these immunostimulants (Tewary and Patra, 2011). The results of the present study indicated that inclusion of *I. batatas* in the fish diet increased the RBC counts and the values appeared to increase with increasing dietary inclusion levels of *I. batatas* in healthy fish. The result of the present study is in agreement with observed increase in RBC in: *C. carpio* fed extract of *Eurphobia hirta* (Pratheepa and Sukumaran, 2014), and *Clarias gariepinus* fed *Morus alba extract* (Sheikhlar et al, 2014). These results also correspond with those by Nya and Austin (2009) who reported the counts of RBC were significantly higher in rainbow trout fed with the garlic-added and ginger-added diets. The apparent increase in RBC after dietary supplementation with *I. batatas* may be related to presence of iron, vitamin A, vitamin B, vitamin C and vitamin B12 which are required for RBC production (Dugency et al, 2003).

The nutritive status of fish can be linked to the health condition of an animal and potential way they deal with stress resulting from their surrounding environment. In our study, a clear link between increased weight and length gains with the increased number of RBCs and other haematological parameters can be observed. The primary function of WBC's is to defend the body

against foreign pathogenic organisms. In the current study, the amount of WBC's, neutrophils, RBC's and hemoglobin concentration was greater in treatment 3 (PD+IbSPHWE) which confirms that this increase was the underplaying factor for the enhanced growth of the fish. Also, result of haematological profile of our study denotes that the health status of tilapia was at the optimal level as well as no or minimal infection or pathogenic activity in the fish body.

Hematological parameters are used to provide information about the health and physiological status of fish, feeding conditions and water quality in which they live (Fazio et al., 2013). WBC, RBC, Hct, and Hb values are particularly recommended on a routine basis to monitor the health of the stock in fish farms. In the previous study on the effects of herbal immunostimulants on hematology and the immune system, it has been reported that plants bioactive substances caused an increase in blood cells counts, and this triggered the immune system and enhanced a natural defense in distinct fish species (Nya and Austin, 2009a; Talpur et al., 2013; Ajeel and Faragi, 2013; Haghighi and Rohani, 2013).

Conclusion

The study demonstrated that incorporation of *I. batatas* hot-water extract in the practical diets of *O. niloticus* is a potential feed ingredient that significantly improved the growth performance as evidenced by enhanced weight gain, reduced feed conversion ratio, increased protein and feed efficiency ratios. Moreover, the immunocompetence of *O. niloticus* is also improved as evidenced by the hematological profile. These results indicate that diets enriched with plant extracts have beneficial effects on fish health and enhance the immune system and hence they could play an important role in preventing disease outbreaks in aquaculture systems, thereby improving growth performance.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Experimental design and fish handling of the current study had been approved by the Research Ethics Committee of Partido State University, Goa, Camarines Sur, Philippines.

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Disclosure: -

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