Supplementary Materials

Effects of Dietary Protein and Energy Sources on Digestibility and Methane Production Potential of Nile Tilapia Feces

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Supplementary Methods

Method S1. Anaerobic digestion assay with different inoculum-to-fish feces ratios

The anaerobic digestion assay was conducted at the Experimental Warehouse and Agricultural Waste Management Laboratory of the Faculty of Agricultural Sciences, Federal University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brazil (22°13′18″ S, 54°48′23″ W). The experiment evaluated different inoculum-to-fish feces ratios (1:1, 2:1, 3:1, 4:1, and 5:1) under batch anaerobic digestion conditions using a completely randomized design with four replicates per treatment, in which each biodigester represented one experimental unit.

The inoculum used in the experiment originated from previously stabilized anaerobic digesters operating with active biogas production and methane concentrations above 75%, indicating adequate methanogenic activity and microbial stability. Prior to biodigester loading, the inoculum and fish feces were homogenized according to the proposed inoculum-to-substrate proportions. Water was added when necessary to standardize the total solids concentration of the substrates.

The substrates were placed into bench-scale batch biodigesters with a working capacity of 500 mL. The biodigesters consisted of two interconnected PVC cylinders, one functioning as a hydraulic seal and the other as a gasometer for biogas storage, equipped with a discharge valve for gas release, following the configuration described by Oliveira et al. (2022).

The biodigesters were maintained under sheltered laboratory conditions at ambient temperature throughout the experimental period, protected from direct sunlight and rainfall. Biogas production was monitored daily by measuring the vertical displacement of the gasometers, and gas volume was corrected to standard temperature and pressure conditions according to the methodology described by Kunz et al. (2019). Methane concentration was determined whenever biogas production occurred using a Gasboard-3200L infrared gas analyzer (Cubic Sensor and Instrument Co. Ltd., Wuhan, China) equipped with sensors for O_2, CO_2, and CH_4 detection.

Biogas and methane production potentials were calculated relative to the amounts of total solids (TS) and volatile solids (VS) added to the biodigesters. The reductions in TS and VS were determined from the difference between influent and effluent concentrations measured at the beginning and end of the anaerobic digestion process, respectively. Total solids, volatile solids, and pH analyses were performed according to the standard methods described by APHA (2012).

Supplementary Tables

Table S1. Effects of inoculum inclusion level on biogas potential, methane potential, and final pH during anaerobic digestion of feces waste from fish farming.

Parameter		Inclusion level					*p-value*
		1:1	2:1	3:1	4:1	5:1	
Biogas	(L)	0.56 ± 0.07	0.65 ± 0.29	0.64 ± 0.21	0.41 ± 0.21	0.27 ± 0.10	ns
	(L kg^{-1} TS)	117.2 ± 30.0^{a}	106.4 ± 43.1^{ab}	63.7 ± 16.8^{ab}	80.3 ± 42.0^{ab}	52.4 ± 20.5^{b}	*
	(L kg^{-1} VS)	151.9 ± 36.8^{a}	137.8 ± 55.9^{ab}	82.6 ± 20.4^{ab}	105.4 ± 55.7^{ab}	68.9 ± 26.4^{b}	*
Methane	(L)	0.37 ± 0.10	0.24 ± 0.10	0.14 ± 0.10	0.14 ± 0.10	0.06 ± 0.06	ns
	(L kg^{-1} TS)	51.7 ± 15.7	55.3 ± 18.1	38.1 ± 4.2	54.4 ± 11.6	29.4 ± 3.6	ns
	(L kg^{-1} VS)	73.6 ± 26.0	74.2 ± 26.1	49.0 ± 4.1	77.0 ± 22.4	39.1 ± 4.6	ns
pH		7.40 ± 0.04	7.42 ± 0.07	7.33 ± 0.10	7.49 ± 0.05	7.51 ± 0.07	ns

Values are presented as mean ± standard deviation (n = 4). Different lowercase letters within the same line indicate significant differences among inclusion levels according to Duncan's test ($p < 0.05$). TS = total solids; VS = volatile solids.