

## Sustainable Agriculture through ICT innovation

**Microbiological analysis of effluents and influents of manure from broilers fed diets with probiotic and exogenous enzymes after treatment in batch biodigesters**

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Poster presentation

Environmental information systems/Environmental management systems

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**ABSTRACT**

The objective was to quantify total and thermotolerant coliforms in effluents and influents from the manure of broilers that were fed a diet with probiotic and exogenous enzymes, treated in batch biodigesters. Samples of manure were collected from broilers (Cobb®), ages between 43 and 48 days, raised in cages, and fed different diets: negative control (NC, a control diet without feed additives); probiotic (PRO, the NC diet + 500 ppm of a product containing *Bacillus subtilis*); exogenous enzymes blend (ENZ, the NC diet + 20 ppm phytase+200 ppm protease+200 ppm xylanase); and a treatment combining both feed additives in the NC diet (P+E). The manure samples were identified according to the diet and prepared (4% total solids). The volume per batch was the same (1.8 kg) for all biodigesters, with manure (0.301 kg) + water (1.499 kg). Samples were collected from the influent at the beginning of the trial and from the effluent after 142 days of treatment. Coliforms were determined using the Most Probable Number (MPN) of Total (35°C) and Thermotolerant (44°C) Coliforms. Results were expressed as MPN/100 ml influent and effluent and efficiency percentage of treatment in the batch biodigesters. Total (NC-  $14 \times 10^3$ ; PRO-  $7,8 \times 10^3$ ; ENZ-  $4,5 \times 10^4$ ; P+E-  $14 \times 10^3$ ) and thermotolerant coliforms (NC-  $11 \times 10^3$ ; PRO-  $7,8 \times 10^3$ ; ENZ-  $4,5 \times 10^4$ ; P+E-  $14 \times 10^3$ ) were present in the influent samples from all treatments. The ENZ treatment had the lowest amount of coliforms in the influent and coliforms were absent in the effluents from all treatments. It was found that the microbiological treatment in batch biodigesters had 100% efficiency when the influent and effluent samples are compared. It can be concluded that adding enzymes to poultry diet reduces the

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## Sustainable Agriculture through ICT innovation

organisms present in broiler manure affluent. The same observation was made in batch biodigesters, with 100% efficiency in all treatments.

**Keywords:** Digester, total and thermotolerant coliforms, *Bacillus subtilis*.

## 1. INTRODUCTION

Expansion of poultry production in Brazil and the increment in technology being used in production systems have resulted in increased amounts of waste being generated, and they are often disposed of in the environment.

The high contamination rates of natural resources and decrease in quality of life in large production areas are an indication that a large portion of poultry production effluents are being released directly or indirectly on the soil and water bodies without an adequate treatment. When carefully chosen and well carried out, the adopted management will allow the wastes to be fully used according to the conditions determined in each farm (Angonese & Campos, 2006).

Anaerobic biodigestion can be used to treat solid or liquid wastes, reducing their polluting ability and producing biogas and also biofertilizer as a by-product, with several practical applications in the farm (Toledo, 1996). The process has shown results in decreasing the environmental impact of animal wastes, not only by reducing the presence of solids but also by reducing undesirable organisms in the effluents.

The aim of this study was to evaluate the efficiency of batch anaerobic biodigester in reducing the number of organisms indicators of fecal contamination (total and fecal coliforms) manure of broilers fed different diets with additives, the objective being to use the effluent as biofertilizer (ferti irrigation).

## 2. MATERIAL AND METHODS

The objective was to quantify total and thermotolerant coliforms in effluents and effluents from the manure of broilers that were fed a diet with probiotic and exogenous enzymes, treated in batch biodigesters. Samples of manure were collected from broilers (Cobb®), ages between 43 and 48 days, raised in cages.

The treatments consisted of different diets for broiler chickens containing exogenous enzymes and *Bacillus subtilis*. The diets were based on corn and soybean meal, and were supplemented with minerals, vitamins and amino acids to meet the nutritional requirements according to Rostagno et al. (2005) recommendations. A nutritional matrix of each enzyme was used to ensure the proper diet formulation. The added *Bacillus subtilis* is present in a commercial product currently tested in birds. Treatments were as follow: a negative control (NC, a control diet without feed

P0311

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## Sustainable Agriculture through ICT innovation

additives); probiotic (PRO, the NC diet + 500 ppm of a product containing *Bacillus subtilis*); exogenous enzymes blend (ENZ, the NC diet + 20 ppm phytase + 200 ppm protease + 200 ppm xylanase); and a treatment combining both feed additives in the NC diet (P+E).

The manure samples were identified according to the diet and prepared (4% total solids). The volume per batch was the same (1.8 kg) for all biodigesters, with manure (0.301 kg) + water (1.499 kg). Samples were collected from the affluent at the beginning of the trial and from the effluent after 142 days of treatment. Coliforms were determined using the Most Probable Number (MPN) of Total (35°C) and Thermotolerant (44°C) Coliforms (Silva et al. 1997). Results were expressed as MPN/100 ml affluent and effluent, and percentage of treatment efficiency in the batch biodigesters.

### 3. RESULTS AND DISCUSSION

Total (NC-  $14 \times 10^3$ ; PRO-  $7,8 \times 10^3$ ; ENZ-  $4,5 \times 10^1$ ; P+E-  $14 \times 10^3$ ) and thermotolerant coliforms (NC-  $11 \times 10^3$ ; PRO-  $7,8 \times 10^3$ ; ENZ-  $4,5 \times 10^1$ ; P+E-  $14 \times 10^3$ ) were present in the affluent samples from all treatments. The ENZ treatment had the lowest number of coliforms in the affluent and coliforms were absent in the effluents from all treatments. When the affluent and effluent samples were compared, it was found that the microbiological treatment in batch biodigesters was 100% efficient. It can be concluded that adding enzymes to poultry diet reduces the organisms present in broiler manure affluent (Table 1).

According to the Resolution from CONAMA (Brazilian Council for the Environment) (2005), the water to be used in irrigation is categorized as class 2, with a maximum limit of 1000 thermotolerant (fecal) coliforms in a 100 mL sample. Thus, the effluent produced by the batch biodigester during the experimental period is within the standard range and can be used in ferti irrigation.

Augusto (2007) evaluated the behavior of coliform MPN in the anaerobic biodigestion of fresh and stored manure from layers, and also found total elimination of coliforms as of the 12<sup>th</sup> and 13<sup>th</sup> week of treatment. On the other hand, in a study with anaerobic biodigestion of broiler litter, Orrico Júnior et al. (2010) reported that they found  $3.6 \times 10^5$  MPN of total coliforms when the biodigesters were loaded and  $1.1 \times 10^3$  MPN/100mL<sup>-1</sup> when unloaded. However, the 99.7% reductions did not eliminate the polluting ability of the effluent.

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P0311

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## Sustainable Agriculture through ICT innovation

Table 1. Total and thermotolerant coliforms (MPN/100 ml) and efficiency of treating in batch biodigesters the manure from birds fed a diet containing probiotic and exogenous enzymes.

Treatments	Characteristics					
	Total coliforms		Efficiency	Thermotolerant coliforms		Efficiency
	Affluent	Effluent		Affluent	Effluent	
NC	14x10 <sup>3</sup>	0	100	11x10 <sup>3</sup>	0	100
PRO	7.8x10 <sup>3</sup>	0	100	7.8x10 <sup>3</sup>	0	100
ENZ	4.5x10 <sup>1</sup>	0	100	4.5x10 <sup>1</sup>	0	100
P+E	14x10 <sup>3</sup>	0	100	14x10 <sup>3</sup>	0	100

NC= control diet without feed additives; PRO= NC + 500 ppm of product containing *Bacillus subtilis*; ENZ= NC + 20 ppm phytase, 200 ppm protease and xylanase; P+E= NC+PRO+ENZ

#### 4. CONCLUSION

The same observation was made in batch biodigesters, with 100% efficiency in all treatments.

#### 5. ACKNOWLEDGEMENTS

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#### 6. REFERENCES

- Angonese, A. R.; Campos, A. T.; Zacarkim, C. E.; Matsuo1, M. S. & Cunha F. 2006. *Eficiência energética de sistema de produção de suínos com tratamento dos resíduos em biodigestor*. Revista Brasileira de Engenharia Agrícola e Ambiental, v.10, n.3, p.745–750.
- Augusto, K. V. Z. 2007. *Caracterização quantitativa e qualitativa dos resíduos da produção de ovos: compostagem e biodigestão anaeróbia..* 131f. Dissertação (Mestrado em Zootecnia) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.
- Conselho Nacional do Meio Ambiente. Resolução n° 357, de março de 2005. *Estabelece classificação para as águas doces, salobras e salinas do Território Nacional*. DOU. N° 53. Seção 1. p.58. Brasília-DF, de 18 de março de 2005.

P0311

Praes, M.F.F.M.; Lucas Junior, J.; Amaral, L.A; Sorbara, J.O.B.; Borges, L.; Branco, P.M.P.B. "Microbiological analysis of effluents and effluents of manure from broilers fed diets with probiotic and exogenous enzymes after treatment in batch biodigesters". EFITA-WCCA-CIGR Conference "Sustainable Agriculture through ICT Innovation", Turin, Italy, 24-27 June 2013.

## Sustainable Agriculture through ICT innovation

- Orrico Junior, M.A.P.; Orrico, A.C.A.; Lucas Junior, J. 2010. Biodigestão anaeróbia dos resíduos da produção avícola: cama de frango e carcaças. *Engenharia Agrícola, Jaboticabal*, v.30, n.3, p.546-554.
- Rostagno, H.S., Albino, L.F.T., Donzele, J.L. et al. 2005. *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais*. 2.ed. Viçosa: Universidade Federal de Viçosa. 189p.
- SAS Institute. (2002). *SAS user's guide: statistics*, pp. 466 (Cary).
- Silva, N.; Junqueira, V.C.A.; Silveira, N.F.A. 1997. *Manual de métodos de análise microbiológica de alimentos*. São Paulo: Varala. 295p.
- Toledo, A. A. G. 1996. *Tratamento de águas residuárias de pocilgas, utilizando biodigestores UASB sob quatro tempos de retenção hidráulica*. 1996. 67f. Dissertação (Mestrado em Agronomia) – Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista, Botucatu, 1996.

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P0311

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