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Anaerobic digestion reduces veterinary ionophore lasalocid in dairy manure

Osman A. Arikan[a,b,c,*], Walter Mulbry[a], Clifford Rice[a], Stephanie Lansing[b]

[a]*Sustainable Agricultural Systems Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD, 20705, USA, Tel. +1 (301) 504 64 17; email: walter.mulbry@ars.usda.gov (W. Mulbry), Tel. +1 (301) 504 63 98; email: clifford.rice@ars.usda.gov (C. Rice)*

[b]*Department of Environmental Science and Technology, University of Maryland, College Park, MD, 20742, USA, Tel. +1 (301) 405 11 97; email: slansing@umd.edu (S. Lansing)*

[c]*Department of Environmental Engineering, Istanbul Technical University, Istanbul, 34469, Turkey, Tel. +90 (212) 285 37 94; email: arikan@itu.edu.tr (O.A. Arikan)*

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ABSTRACT

Lasalocid is an antibiotic from the group of carboxylic ionophores, and it is commonly used in cattle and poultry as a coccidiostat and for growth promotion. The widespread use and persistence of lasalocid have led to its detection in the environment. The objective of this study was to determine the fate and effect of veterinary ionophore lasalocid during the anaerobic digestion of dairy manure. Duplicate plug flow field-scale digesters were operated using either non-amended dairy manure or dairy manure amended with 1 or 5 mg/L lasalocid. Results showed that lasalocid was reduced approximately 75% during anaerobic digestion. Methane production from digesters treating manure amended with 1 and 5 mg/L lasalocid were comparable with CH_4 production from the duplicate digesters operated without added lasalocid. These results suggest that anaerobic digestion can be used to reduce lasalocid levels in dairy manure, and lasalocid did not affect digester stability at concentrations expected in dairy manure.

Keywords: Antibiotic; Biogas; Digester; Lasalocid; Methane

1. Introduction

Lasalocid is an antibiotic from the group of carboxylic ionophores, and it is mainly active against gram-positive microorganisms [1]. It is commonly used in cattle and poultry as a coccidiostat and for growth promotion [1,2–6].

An estimated 1,400 tons of lasalocid (400 tons for cattle and 1,000 tons for poultry) were used for non-therapeutic purposes in the United States in 2001 [2]. Other countries also reporting use of lasalocid include Republic of Korea, Denmark and Norway [7]. Approximately 80% of lasalocid administered to broiler chickens is excreted in manure as a parent compound [1].

The widespread use and persistence of lasalocid have led to its detection in dairy manure [8], chicken litter [3] and surface water [9]. Hansen et al. [10] estimated an EC_{50} value of 3.7 mg/L for soil bacteria for lasalocid. An EC_{50} value of 4.9 mg/kg soil for isopod avoidance was reported [11]. Zizek et al. [6] concluded that lasalocid was potentially harmful to soil organisms in a worst-case scenario using the maximum permissible amount of manure and immediately after application. Lasalocid may also be dangerous for equine species [12,13].

The effect of composting on the persistence of four ionophores in dairy manure and poultry litter was recently studied and results showed that composting is not very effective for removing lasalocid in dairy manure [8]. Anaerobic digestion is an established technology for the treatment of animal manure and is one possible means of reducing the antibiotics that are ultimately released into the environment [14,15]. However, residues of feed additives in manure can effect anaerobic digesters [16–18]. Bak et al. [19] suggested

* Corresponding author.

that further research is needed about the environmental fate and effects of ionophores including lasalocid.

The objective of this study was to determine the fate and effect of lasalocid during anaerobic digestion using replicate, field-scale dairy manure digesters. Duplicate plug-flow field-scale digesters were operated using non-amended dairy manure and dairy manure amended with lasalocid to 1 and 5 mg/L. Lasalocid concentrations in digester effluents were monitored to calculate lasalocid removal rates. Methane (CH_4) production and digester effluent characteristics (pH, alkalinity, soluble COD removal, volatile fatty acids [VFA]) were monitored to evaluate the effect of lasalocid on digester efficiency and stability.

To the best of our knowledge, there are no published data on the fate and effect of lasalocid during anaerobic digestion. In addition, results on the fate and effect of antibiotics in the literature are presented from laboratory-scale rather than field-scale systems. Based on results from our recent field-scale study for monensin (another ionophore) [20], we predicted that extractable levels of lasalocid would decrease during anaerobic digestion and methane production would be partially inhibited in digesters containing 1 and 5 mg/L lasalocid, respectively.

2. Materials and methods

2.1. Chemicals

Lasalocid A sodium salt (100 mg/L in acetonitrile) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The structure of lasalocid is shown in Fig. 1. HPLC grade acetonitrile (C_2H_3N) and methanol (CH_3OH) were purchased from Fisher Scientific (Pittsburg, PA, USA). All reagents used in this study were analytical grade. Water used for analysis was purified using a Picotech UV-plus purification system (Hydro Service and Supplies, Durham, NC, USA). The system includes mixed bed deionizers, an activated carbon absorption column, and a 265 nm UV light to remove total organic carbon content below 5 ppb.

2.2. Substrate

Dairy manure was obtained from the USDA's Dairy Research Unit at the Beltsville Agricultural Research Center (BARC) (Beltsville, MD, USA). The dairy's free stall barn houses approximately 120 dairy cows and uses sawdust as bedding on top of rubber pillows. Manure is mechanically scraped into holding pits and pumped daily from the pits to a screw-press solid separator system. The solids are collected and composted, and the solids-separated liquid manure (containing roughly 1%–2% total solids [TS]) is pumped over the course of a day into the full-scale completely mixed BARC digester (400 m^3 working volume) for treatment. The solids-separated liquid manure used in this study (referred to hereafter as dairy manure) was obtained daily from the post-separated manure pit and was the same influent as that used in the BARC digester. Table 1 shows characteristics of the dairy manure used as feedstock in this study. The dairy manure did not contain background lasalocid (<0.01 mg/L).

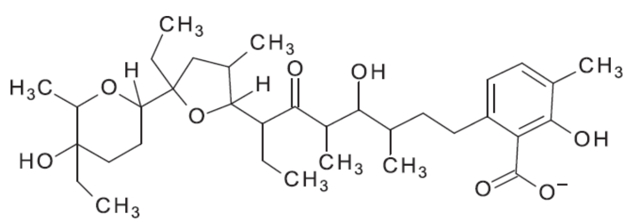

Fig. 1. Chemical structure of lasalocid. Adapted from Clarke et al. [21] (M_w = 591 g/mol, pK_a = 4.4, log K_{ow} = 1.4–2.8).

2.3. Field-scale anaerobic digestion

Anaerobic digestion experiments were carried out using six modified Taiwanese-model plug-flow field scale (FS) digesters (Fig. 2) at the BARC dairy [22]. Each FS digester has a total capacity of 3 m^3 and a working volume of 2 m^3.

Prior to the study, each FS digester was filled with 1 m^3 of inoculum from the BARC digester and 1 m^3 of dairy manure. Each digester was subsequently loaded with dairy manure and maintained at 30°C ± 2°C.

After steady-state conditions were achieved, duplicate FS digesters were operated for additional 42 d using dairy manure (non-amended) and dairy manure amended with lasalocid at either 1 or 5 mg/L. These two amendment levels were chosen as they spanned the range of values expected using

Table 1
Characteristics of dairy manure used as feedstock in the study (mean ± standard deviation)

Parameter	Concentration
Total solids (TS), g/L	14.3 ± 1.4
Volatile solids (VS), g/L	9.8 ± 1.1
pH	7.3 ± 0.1
Alkalinity, g/L as $CaCO_3$	4.3 ± 0.3
Total COD, g/L	17.2 ± 1.9
Soluble COD, g/L	5.3 ± 1.1
Total Kjeldahl nitrogen (TKN), g/L	0.9 ± 0.2
Total phosphorus (TP), g/L	0.18 ± 0.03

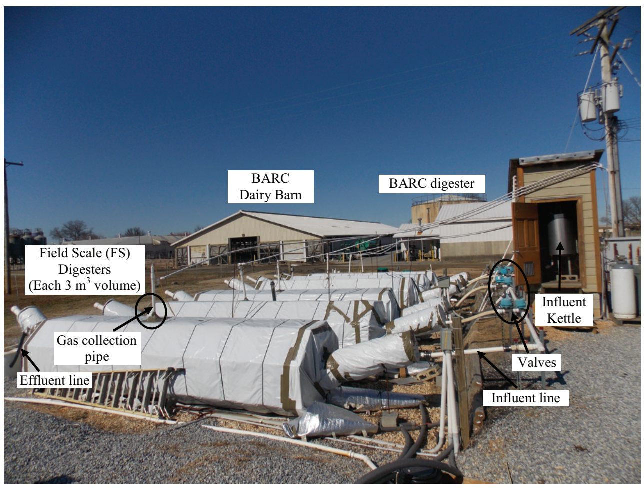

Fig. 2. Field-scale (FS) anaerobic digesters.

the minimum (60 mg/cow/d) and maximum (300 mg/cow/d) dosage rates [23], with an estimated 85% excretion rate and a daily liquid manure rate of 50 L per cow [24]. Dry mix feed grade Bovatec 91 (containing 20% lasalocid) (Zoetis Inc., Kalamazoo, MI, USA) was used for lasalocid addition. Bovatec 91 was added into the influent kettle while the kettle was being filled with dairy manure. The kettle was stirred continuously to thoroughly mix lasalocid with the dairy manure.

The digesters were loaded 5 d a week with approximately 240 L of dairy manure, corresponding to a hydraulic retention time (HRT) of 12 d. The mean organic loading rate (OLR) was approximately 1.0 kg volatile solids (VS)/m^3-d during the study. After feeding was completed each day, the influent kettle and manure pipes lines were rinsed with water in order to minimize carryover of lasalocid. Steady-state conditions were reached in the digesters after they were operated for a minimum of two HRT.

Digesters were emptied at the end of the study. Multiple samples were collected from each digester as it was emptied in order to determine the amount of lasalocid that had settled with manure solids in the bottom of the digesters. A mass balance of lasalocid was calculated for each digester using the total mass of lasalocid fed to individual digesters over the 42-d period, the total mass of lasalocid in the effluent, and the quantity that stayed in the digester within the settled solids.

2.4. Analytical methods

Digester temperatures were continuously monitored using thermocouples connected to a Labview™ software program (National Instruments Corp., Austin, TX, USA). The produced biogas was monitored using low-pressure cumulative gas meters (model PGM.75, EKM Metering, Santa Cruz, CA, USA). Although biogas readings were recorded daily, biogas production values were calculated using weekly averages. Biogas samples were analyzed for CH_4 weekly using a mobile gas analyzer (Biogas 5000, Landtech North America, Colton, CA, USA).

Influent and digester effluent samples were collected weekly for determination of pH, alkalinity, soluble chemical oxygen demand (COD), according to Standard Methods [25]. Concentrations of VFA (acetic, propionic, butyric and valeric acids) were measured using 7890A gas chromatograph (Agilent, CA, USA) equipped with a flame ionization detector, an injection temperature of 250°C, a detector temperature of 300°C, an initial oven temperature of 100°C, and a He carrier gas flow rate (FR) of 1.8 mL/min. The temperature was maintained at 100°C for 2 min followed by an increase of 10°C/min for a total run time of 10 min. Total VFA content was calculated by summing the concentrations of acetic, propionic, butyric and valeric acids, and values are expressed as mg/L acetic acid. Total Kjeldahl nitrogen and total phosphorus content values of influent samples were determined by block digestion and flow injection analysis (Lachat Instruments, Milwaukee, WI, USA). TS, VS and total COD values of influent samples were determined according to Standard Methods [25].

2.5. Extraction of lasalocid

Digester effluent samples were collected weekly and extracted for analysis of lasalocid using a slight modification of the method developed for the ionophore monensin [26]. Briefly, 10 mL dairy manure samples were extracted with 20 mL of acetonitrile followed by an extraction with 20 mL of an acetonitrile: ethyl acetate solution (50:50 v:v). After each solvent addition, samples were mixed for 1 min using a vortex mixer, followed by centrifugation (5 min, 1,200 × g). Extracts from the second solvent addition (acetonitrile: ethyl acetate) were sonicated for 10 min in a sonication bath (Elma, E100H, Elmasonic, Germany) prior to centrifugation. Following centrifugation, the upper extraction layers were decanted into 250 mL glass beakers and allowed to evaporate in a fume hood at room temperature (approximately 2 d). Dried samples were dissolved in 40 mL of methanol and aliquots of this material were transferred to amber autosampler vials prior to analysis of lasalocid by liquid chromatography mass spectrometry (LC/MS/MS).

2.6. LC/MS/MS analysis for lasalocid

Lasalocid concentrations were determined using a Waters 2695 LC and Micromass Quattro Ultima MS (Waters Corp., Milford, MA, USA) with an electrospray source using an XBridge C_{18} column (150 mm × 2.1 mm i.d., 5 μm) (Waters Corp., Milford, MA, USA) in conjunction with an XBridge C_{18} guard column at 60°C. Positive ionization modes were used for detection. The injection volume was 5 μL. A gradient separation was utilized involving a mixture of solvent A (0.1% formic acid: acetonitrile, 50:50, v/v), solvent B (100% water), solvent C (100% methanol) and solvent D (100% acetonitrile). The solvent gradient program was as follows: 0–2 min. 100% B, FR 0.2 mL/min; 8–20 min a linear gradient to 100% A, FR 0.4 mL/min; 20–23.5 min. 20% A, 80% D, FR 0.4 mL/min; 23.5–27 min 100% C, FR 0.3 mL/min; 27–32 min 100% D, FR 0.3 mL/min and 32–33 min returning to the starting condition. The column was stabilized for 7 min with 100% B prior to injection of the next sample. The total run time was 40 min. Lasalocid was detected using multiple reaction monitoring methods available on the triple quadrupole mass spectrometer. The parent and daughter ions used for lasalocid identification and quantitation were 613 and 377 Da, respectively. Lasalocid concentrations were calculated by the external standard method using five point standard calibration curves (fits with > 0.9 r^2 values). Peak integration and quantitation were performed automatically using the MassLynx 4.0 software (Waters Corp., Milford, MA, USA).

2.7. Determination of extraction efficiencies for lasalocid

To determine extraction efficiencies, duplicate samples of effluent manure were spiked with lasalocid and extracted as described above. Recovery results were calculated as means of duplicate samples at each concentration. Average recovery values of lasalocid from digester effluents at 0.1, 0.5, 1 and 5 mg/L spiked concentrations were 92% ± 4%, 92% ± 1%, 88% ± 3% and 80% ± 2%, respectively. Recovery values decreased with increasing initial concentration.

2.8. Statistical analyses

Statistical significance between digesters amended with lasalocid and non-amended digesters was evaluated.

Differences in CH_4 production and content, effluent pH, alkalinity, soluble COD removal and total VFA at the steady state were statistically analyzed separately. One-way ANOVA and Tukey's test were used for comparing the effects of different treatments. Differences were considered significant for p-values less than 0.05. All statistical analyses were performed using R statistical software [27].

3. Results and discussion

3.1. Fate of lasalocid during anaerobic digestion

Effluent lasalocid levels from digesters containing lasalocid amended manure increased gradually after addition of lasalocid (Fig. 3). Steady-state mean effluent lasalocid concentrations were 0.27 ± 0.06 and 1.29 ± 0.1 mg/L from the digesters containing manure amended with 1 and 5 mg/L lasalocid, respectively. These values correspond to approximately 75% reduction of lasalocid (73% ± 6% and 74% ± 2% for the 1 and 5 mg/L lasalocid amended digesters, respectively) at a 12-d HRT. However, mass balance results of lasalocid show that roughly 35% of input lasalocid removal was due to settling of insoluble lasalocid residues in the bottom of these unmixed digesters. Since solids settling is common in these unmixed plug flow digesters [28], accumulation of lasalocid in the digesters was not surprising.

To the best of our knowledge, there are no published data on the fate of lasalocid during anaerobic digestion. However, the results are comparable with our recent field-scale study in which approximately 70% reduction of monensin (another ionophore) was observed during the anaerobic digestion of dairy manure spiked at 1 and 10 mg/L at 30°C with a 17-d HRT and 1.4 ± 0.1 kg VS/m^3-d OLR (pH in between 7 and 7.6) using similar plug flow digesters [20]. In addition, our previous study showed that composting was not very effective (25%–50% reduction within 42 d) for removing lasalocid in dairy manure [8]. Zizek et al. [6] reported similar results for the removal of lasalocid by composting in poultry manure. They found that lasalocid levels in a composted wood chip/chicken manure mixture declined 50% within 21 d (half-life of 18 d). In chicken manure stored at ambient temperature, lasalocid levels decreased 50% within the first 21 d, but did not decline further during the 49-d experiment [6]. However, levels of lasalocid in soils appear to decrease rapidly at ambient temperatures. Sassman and Lee [29] reported a very short half-life (less than 4 d) for lasalocid in moist soils at 23°C. Using ^{60}Co-sterilized soils, they found that abiotic degradation of lasalocid is small relative to the microbial processes.

3.2. Effect of lasalocid on anaerobic digestion

Before the addition of lasalocid, weekly CH_4 production and CH_4 content values were not significantly different ($p > 0.05$) among the six digesters. The average weekly CH_4 production and CH_4 content values from the digesters were 4.1 ± 0.4 m^3/week and 66.7% ± 0.8%, respectively (Fig. 4). The average specific CH_4 production value of the digesters was 0.33 ± 0.02 m^3/kg-VS. This value is within the range of the CH_4 productivity values (0.14–0.34 m^3/kg-VS) reported for separated dairy manure at mesophilic temperatures [30]. After addition of lasalocid, CH_4 production and content values from lasalocid amended and non-amended (control) digesters were not significantly different, although CH_4 production values fluctuated during the study because of OLR variations due to changes in water use at the dairy (Fig. 4; Table 2). The average steady-state weekly CH_4 production values of the digesters fed with non-amended manure and manure

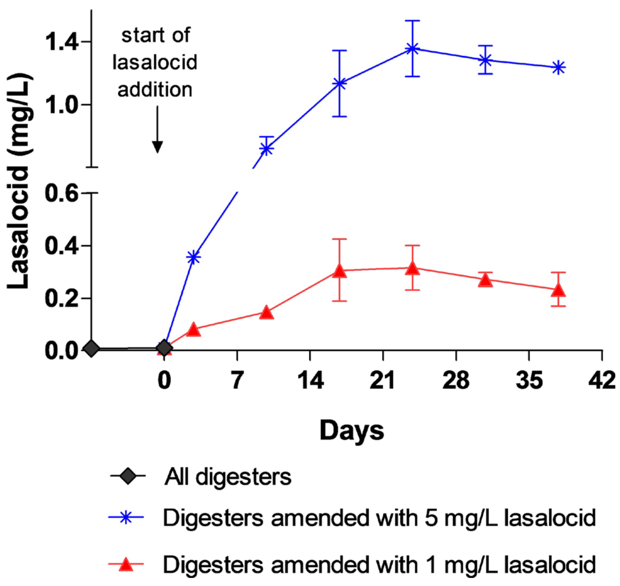

Fig. 3. Effluent lasalocid concentrations from the field-scale (FS) digesters operated using dairy manure (non-amended) and dairy manure amended with lasalocid to 1 and 5 mg/L. Values are the means from duplicate digesters. Standard deviations are shown as error bars. (Start-up of digesters is not shown.)

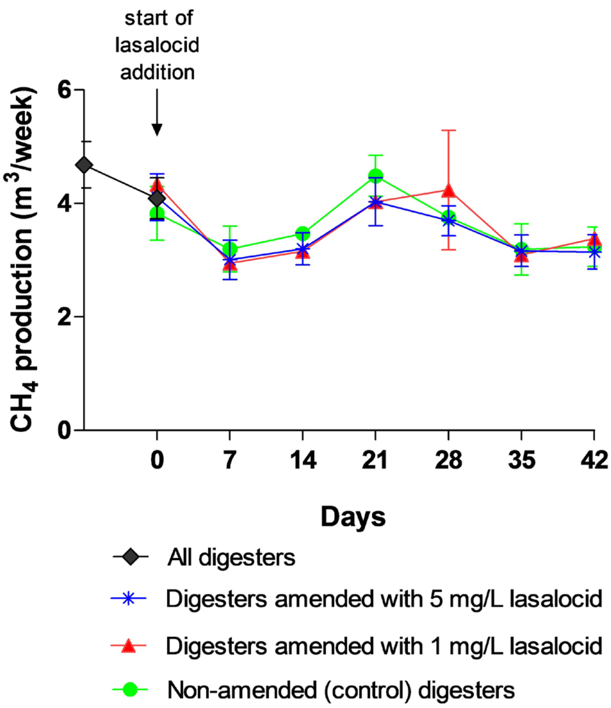

Fig. 4. Weekly CH_4 production from the FS digesters. Values are the means from duplicate digesters. Standard deviations are shown as error bars. (Start-up of digesters is not shown.)

Table 2
Steady-state results of field-scale (FS) digesters operated using dairy manure (non-amended) and dairy manure amended with lasalocid to 1 and 5 mg/L (mean ± standard deviation)

Parameter	Non-amended (control) digesters	Digesters amended with 1 mg/L lasalocid	Digesters amended with 5 mg/L lasalocid
Weekly CH_4 production, m^3/week	3.4 ± 0.4	3.6 ± 0.7 (0.60) [1]	3.3 ± 0.4 (0.79)
CH_4 content, %	66.4 ± 0.9	66.7 ± 1.1 (0.64)	65.6 ± 0.7 (0.12)
Effluent pH	7.55 ± 0.05	7.57 ± 0.12 (0.65)	7.58 ± 0.08 (0.40)
Effluent alkalinity, g/L as $CaCO_3$	5.3 ± 0.3	4.9 ± 0.3 (0.09)	5.1 ± 0.2 (0.39)
Effluent soluble COD removal, %	61 ± 5	53 ± 3 (<0.01)	53 ± 4 (<0.01)
Effluent total VFA, mg/L as acetic acid	381 ± 39	786 ± 62 (<0.01)	711 ± 79 (<0.01)

[a]Numbers in parenthesis show *p*-values between lasalocid amended digesters and non-amended digesters.

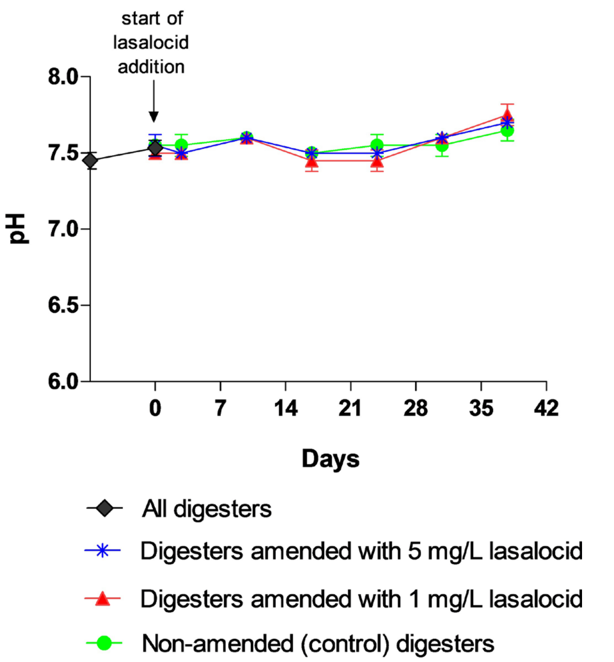

Fig. 5. Effluent pH from the FS digesters. Values are the means from duplicate digesters. Standard deviations are shown as error bars. (Start-up of digesters is not shown.)

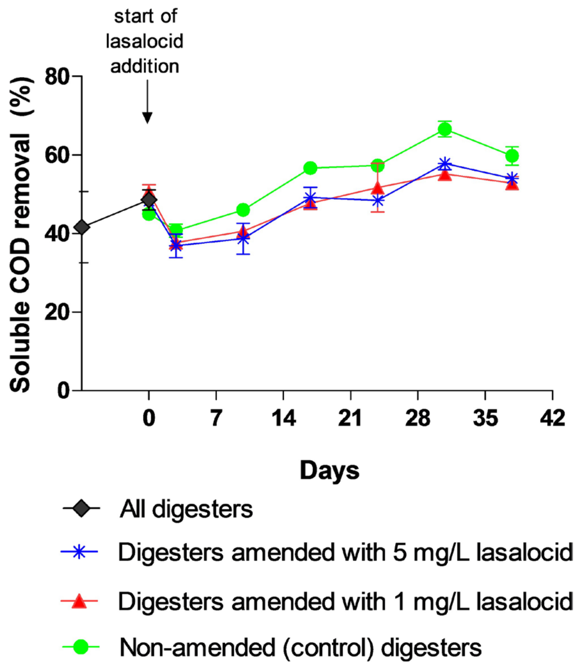

Fig. 6. Effluent soluble COD removal rates from the FS digesters. Values are the means from duplicate digesters. Standard deviations are shown as error bars. (Start-up of digesters is not shown.)

amended with 1 and 5 mg/L lasalocid were 3.4 ± 0.4, 3.6 ± 0.7 and 3.3 ± 0.4 m^3/week, respectively (Fig. 4; Table 2). The average CH_4 content values in the biogas from non-amended, 1 and 5 mg/L lasalocid amended digesters were 66.4% ± 0.9%, 66.7% ± 1.1% and 65.6% ± 0.7%, respectively (Table 2).

Neither 1 nor 5 mg/L lasalocid amendment significantly affected digester stability. Average pH values for digesters were 7.5 ± 0.1 before the lasalocid addition (Fig. 5). pH values remained constant during the study, and the average steady-state pH values of all the digesters were similar (7.6 ± 0.1) (Fig. 5; Table 2). Average steady-state alkalinity values of the digesters fed with non-amended manure and manure amended with 1 and 5 mg/L lasalocid were 5.3 ± 0.3, 4.9 ± 0.3 and 5.1 ± 0.2 g/L as $CaCO_3$, respectively, and were not significantly different (Table 2). At steady state, although 1 and 5 mg/L lasalocid amendments resulted in significantly lower (approximately 15% compared with non-amended) soluble COD removal (Fig. 6; Table 2) and significantly higher total VFA concentrations (786 ± 62 and 711 ± 79 mg/L, respectively) compared with non-amended (381 ± 39 mg/L) the digester stability was not affected (Table 2). The ratio of VFA and alkalinity value is used as an indicator of digester process stability. Callaghan et al. [31] suggested that a VFA:alkalinity ratio less than 0.4 is characteristic of stable digesters. In our study, all digesters achieved VFA:alkalinity values less than 0.2 (Table 2).

To our knowledge, there are no published data examining the effect of lasalocid during anaerobic digestion. However, our recent study with ionophore monensin showed similar results at 1 mg/L in which monensin did not have significant effect on CH_4 production and process stability [20]. In contrast, a concentration of 10 mg/L monensin significantly reduced CH_4 production (75% less compared with control) and resulted in digester instability and 40-fold higher total VFA levels compared with control [20].

4. Conclusions

Our study showed that approximately 75% removal of lasalocid was achieved by anaerobic digestion of dairy manure. About 35% of this reduction was due to lasalocid associated with solids that settled in the digesters. Further studies on mechanisms of the lasalocid reduction (degradation, mineralization or binding of lasalocid to the organic matrix) are still needed. The results also demonstrated that lasalocid did not have significant effect on CH_4 production and process stability. These results suggest that anaerobic digestion can be used to significantly reduce lasalocid levels in dairy manure prior to land application of the manure to reduce environmental presence of lasalocid in the environment.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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