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Effect of Monensin Supplementation in the Bovine Diet on the Composition and Anaerobic Digestion of Manure with and without Screening

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Abstract: The incorporation of monensin into cattle diets can significantly alter the physicochemical properties of excreted manure, potentially affecting waste management and treatment systems given the persistence of substantial concentrations of ionophores in the effluent. This study assessed the impact of monensin on the compositional characteristics of cattle manure and its implications for anaerobic digestion efficiency, with and without the separation of manure fractions across two hydraulic retention times (HRTs). Manure samples were collected from cattle fed with doses of monensin at 0, 1.8, 3.6, 5.4, and 7.2 mg per kg of dry matter intake. The HRTs investigated were 20 days (HRT20) and 30 days (HRT30). Increasing monensin inclusion in the diets resulted in a notable decrease in the quantities of total solids (TSs), volatile solids (VSs), and neutral detergent fiber (NDF) per animal per day, accompanied by an increase in lignin content and mass. Fraction separation during anaerobic digestion enhances the reduction of TSs, VSs, and NDF, thereby optimizing biogas and methane production potentials and elevating methane concentrations. The presence of monensin correlated with the reduced degradation of organic components during the anaerobic digestion process. To maximize the efficiency of the anaerobic digestion of manure from cattle diets supplemented with monensin, a 30-day HRT combined with fraction separation is recommended. This approach can enhance biogas yield and methane content, thereby improving the sustainability and efficacy of waste treatment processes.

Keywords: ionophore; screening; solid–liquid separation; waste management

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1. Introduction

Maximizing production is a critical objective in any production system, including dairy farming, in which additives such as growth promoters are commonly used to enhance animal productivity. Among these, monensin is the most widely used [1]. Monensin, a polyether ionophore produced by the bacterium *Streptomyces cinnamonensis*, functions as an antimethanogenic agent and an effective coccidiostat. It modulates the ruminal microbiota, inhibits methanogenesis precursors, and reduces methane production, allocating more energy to productive processes [2]

In Brazil, the use of medicines in animal diets was estimated at 5683 tons in 2010 and is projected to increase to 8447 tons by 2030 [3]. The environmental implications of medicine contamination, particularly the excretion of these compounds, are a growing concern. Animal production is a significant source of such contamination [4], as up to 90% of administered medicines are excreted through feces [5]; in cattle, one of the earliest studies on the use of monensin found a 50% excretion rate [6].

Brazil has the second largest cattle population globally, with 202 million head [7], resulting in substantial daily waste generation. Thus, the treatment of the generated waste is extremely important, especially for reducing the pollutant loads released into the environment. Pharmaceuticals are more frequently detected in areas near animal production facilities [4]. Due to the high density of livestock operations in Brazil, the Central-West, Southeast, and South regions have significant portions of their territory vulnerable to groundwater contamination, including ionophores such as monensin [8]. Given the widespread use of medicines and concerns about their potential for environmental contamination, along with the emergence of resistant genes [9] and soils susceptible to contamination [8], the necessity of treating animal production waste containing monensin before reuse becomes evident to mitigate the environmental load of this compound.

Anaerobic digestion is a widely adopted method for treating cattle waste and offers a potential solution for reducing monensin levels in substrates [10]. However, monensin residues in waste can disrupt the digestion process, leading to decreased methane production, and it may also not lead to a reduction in its content [11], because medicines can affect substrate degradation at all stages in the anaerobic digestion process [12]. Substrate composition is another crucial factor, as waste with high levels of recalcitrant constituents such as lignin, cellulose, and hemicellulose may reduce the efficiency of anaerobic digestion. These cell wall components are significant in ruminant waste and can vary in concentration owing to dietary monensin supplementation [13]. Monensin may influence key fiber-degrading microorganisms in the rumen [14], necessitating research on techniques that facilitate the degradation of monensin-containing substrates with high fibrous content. Such techniques are essential for optimizing digestion, biogas, and methane yields, and fertilizer quality.

The fraction separation technique in substrates for anaerobic degradation can lower antibiotic concentrations in both the solid and liquid fractions, as reported by [15], who found that higher monensin concentrations persisted in the solid fraction. However, the efficiency of this separation can vary depending on the properties of the antibiotic. For instance, [16] found that 64% of veterinary drugs remained more concentrated in the liquid fraction, but nonetheless, there was a reduction in solids post-separation. The separation of fractions combined with anaerobic digestion has been encouraged in a few studies as a post-process utilizing the digestate [17,18]. However, when this technique is used as a pre-process, no studies report the benefits of its application to waste containing antibiotics, ionophores, or any pharmaceuticals with the aim of improving digestion process efficiency.

In cattle production units, waste separation primarily aims to enhance the recycling process efficiency, enabling faster and more effective recycling compared to using intact waste. The solid fraction can be directed towards composting, which not only increases the nutrient concentration in the resulting fertilizer but also facilitates its storage and transport. Additionally, it reduces greenhouse gas emissions and contributes to sustainable agricultural practices [19]. For anaerobic digestion, focusing on the liquid fraction, the reduction in fibrous constituents and coarse particles decreases the hydraulic retention time requirements, thereby improving the biogas and methane yields per unit of treated effluent [20].

This study hypothesizes that (1) the inclusion of monensin in diets decreases feed intake and alters waste characteristics, and (2) fraction separation of substrates enhances the reduction of solid constituents, improves composition, and increases biogas production. This study aimed to assess the effect of monensin inclusion in cattle diets on waste characteristics and the subsequent effects on substrate composition, with and without fraction separation, during anaerobic digestion.

2. Materials and Methods

2.1. Location of the Experiment and Animals

The feeding phase and collection of waste generated by the cattle were conducted at the Experimental Farm, and the laboratory analyses were performed at the Agricultural Waste Management Laboratory, both part of the Faculty of Agricultural Sciences at the Federal University of Grande Dourados, Dourados, MS, Brazil (latitude 22°11′38′′ S, longitude 54°55′49′′ W, and 462 m above sea level). The animal experiments were approved and conducted according to the guidelines of the Animal Use Ethics Committee (protocol 16/2021, approved on 30 November 2021).

The animals employed in the experiment were Jersey breeds and housed in individual pens covered with concrete floors and equipped with automatic waterers and individual feeders. Fifteen steers, with an average weight of 350 kg, were used. Initially, the animals were adapted to a diet containing 50% oat hay and 50% concentrate (composed of ground corn, soybean meal, urea, and a mineral and vitamin premix; composition presented in Table 1) for 14 days [21]. After this adaptation period, the experimental phase began with a diet based on 2.5% body weight (BW) in dry matter, ensuring a minimum of 10% feed refusal relative to the amount offered. The diets were supplied twice daily at 7:00 a.m. and 3:00 p.m., with the leftovers removed from the troughs before the first meal of the day.

Table 1. Characterization of the oat hay and concentrate used in the cattle feed.

Components of the Diet	DM (%)	CP (%)	NDF (%)	ADF (%)	EE (%)	MM (%)	NFC%
Oat hay	91.77	6.38	75.23	45.56	1.25	9.89	7.25
Concentrate	91.60	20.83	9.85	4.16	6.81	9.12	53.39

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; MM, mineral matter; NFC, nonfibrous carbohydrates.

2.2. Collection of Waste

The monensin inclusions characterized the experimental treatments and consisted of five doses: 0, 1.8, 3.6, 5.4, and 7.2 mg kg^{-1} of dry matter intake (DMI). The doses were administered to all animals simultaneously over 19 days, with the first 14 days corresponding to the adaptation period and the following five consecutive days dedicated to waste collection. A completely randomized design consisting of five treatments with three replicates (animals) was used.

Monensin was administered daily before the first meal. A small portion of the concentrated feed (±100 g of concentrated) was mixed with the respective monensin dose and placed in the animals' trough, where it was quickly consumed, ensuring total ingestion of each dose.

Waste was collected by scraping the floor without adding water and considering the amount accumulated within 24 h. After collection, the waste was quantified, and the fresh mass was obtained and sent to the laboratory for analysis of total solids (TSs) and pH. After drying, the samples were ground and used to determine volatile solids (VSs), neutral detergent fiber (NDF), and lignin. The remaining waste was frozen in a horizontal freezer (Consul, Dourados, Brazil) for the anaerobic digestion tests.

2.3. Anaerobic Digestion Test

A completely randomized design was adopted to conduct the anaerobic digestion test with a 5 × 2 factorial scheme (monensin dose × screened or unscreened), consisting of three replications.

Semicontinuous biodigesters with a total capacity of 8 L were used. Fraction separation by screening was also employed, resulting in screened (SC) and unscreened (US) substrates, as well as two hydraulic retention times (HRT) of 20 and 30 days (HRT20 and HRT30).

The TS concentration in the influents was set at 2.5% for US substrates containing intact waste, whereas for SC substrates, the TS concentration decreased as the coarser fraction was retained on the screen. The biodigesters were filled daily with the load volume depending on the capacity of each digester and the retention time adopted (Table S1). An industrial blender was used to homogenize the influents, and for the SC condition, fraction separation was performed manually using a 1 mm mesh screen, achieving an average solid fraction retention efficiency of 40%. After separation, only the liquid fraction was used in the biodigester. The characteristics of the waste and substrates used for anaerobic digestion are presented in Table 2.

Table 2. Characterization of the fresh manure and initial substrates employed for anaerobic digestion.

Materials	TS (%)	VS (%)	NDF (%)	pH
Fresh manure control	23.04	84.64	40.70	8.05
Fresh manure—dose 1.8 mg monensin.kg^{-1} DMI	21.74	83.68	41.80	8.38
Fresh manure—dose 3.6 mg monensin.kg^{-1} DMI	20.37	83.15	45.16	8.16
Fresh manure—dose 5.4 mg monensin.kg^{-1} DMI	21.78	83.14	47.09	8.25
Fresh manure—dose 7.2 mg monensin.kg^{-1} DMI	22.85	82.85	47.26	8.26
US substrate control	2.47	85.83	45.50	7.79
US substrate—dose 1.8 mg monensin.kg^{-1} DMI	2.47	85.21	45.13	7.79
US substrate—dose 3.6 mg monensin.kg^{-1} DMI	2.40	85.41	47.73	7.42
US substrate—dose 5.4 mg monensin.kg^{-1} DMI	2.30	85.44	48.31	7.86
US substrate—dose 7.2 mg monensin.kg^{-1} DMI	2.37	84.58	50.83	7.85
SC substrate control	1.44	78.36	28.10	7.68
SC substrate—dose 1.8 mg monensin.kg^{-1} DMI	1.39	78.50	28.12	7.88
SC substrate—dose 3.6 mg monensin.kg^{-1} DMI	1.38	79.12	29.98	7.60
SC substrate—dose 5.4 mg monensin.kg^{-1} DMI	1.41	78.59	32.15	7.92
SC substrate—dose 7.2 mg monensin.kg^{-1} DMI	1.32	77.33	32.70	7.88

TSs, total solids; VSs, volatile solids; NDF, neutral detergent fiber; pH, hydrogen ion concentration; US, unscreened; SC, screened; DMI, dry matter intake.

The biodigesters were equipped with gasometers consisting of two cylindrical PVC pipes, one used as a water seal and the other as a gasometer, where the produced biogas was stored. Biogas production was measured by the vertical displacement of the gasometers, and the volume produced was calculated based on the gasometer area and displacement height corrected to standard temperature and pressure conditions [22]. The concentrations of CH_4, CO_2, and O_2 were analyzed using an infrared biogas analyzer, Gasboard-3200 L (Cubic Sensor and Instrument Co., Ltd., Wuhan, China). The biogas was analyzed twice a week, with the same frequency applied to the total solid (TS) measurements in the influents and effluents. The anaerobic digestion test spanned a total of 18 weeks, including a four-week period dedicated to biodigester stabilization.

2.4. Laboratory Analyses

TS, VS, and pH analyses were performed according to the previously described methodology [23]. NDF, ADF, and lignin analyses were performed as previously described [24].

Reductions in TSs and VSs were calculated based on the contents of these constituents at the start (influent) and end (effluent) of the process, along with the load and discharge volumes. To estimate the biogas and methane production potentials, the relationship between volumetric production and the amount of TSs added to the biodigesters was considered.

2.5. Statistical Analysis

In the analysis of variance, interactions between factors were assessed, and if no interaction was observed, independent analyses of factors were conducted using polynomial regression ($p < 0.05$) for monensin doses and Tukey's test ($p < 0.05$) for screening conditions.

If a significant interaction between factors occurred, the analysis was further broken down for screening conditions within each monensin dose, and comparisons of means were performed using Tukey's test ($p < 0.05$). Polynomial regression analysis ($p < 0.05$) was used to evaluate monensin doses within the screening conditions.

The results were evaluated independent of the tested HRT. All analyses were conducted using R software version 4.3.2 (2023).

3. Results and Discussion

3.1. Characterization of Waste

The inclusion of monensin in the diet significantly reduced ($p < 0.05$) the total solids (TSs) mass of waste produced by cattle (Figure 1). Although monensin did not significantly affect ($p > 0.05$) the amount of feed consumed by the animals (an average of 10.7 kg DMI day^{-1}), the existing literature indicates that monensin can reduce the passage rate of forage [25], which, as noted by [26], might enhance the digestibility of the ingested feed and thereby decrease the mass of waste produced.

The decrease in the mass of waste produced is significant for the management and treatment of residues because it results in smaller quantities of material being collected, handled, and treated, thereby reducing the costs associated with the treatment and recycling processes. The production of volatile solids (VSs) and neutral detergent fiber (NDF) (Figure 1) followed the trend observed for total solids (TSs), which decreased with the inclusion of monensin in the diets. However, the NDF content in the waste increased ($p < 0.05$) with monensin supplementation (40.7%, 41.8%, 45.2%, 47.1%, and 47.3% for doses of 0, 1.8, 3.6, 5.4, and 7.2 mg monensin kg^{-1} DMI, respectively). A previous study [27] reported that monensin reduced the digestibility of NDF in dairy cow diets, with the authors noting changes in the microbial population and subsequent inhibition of cellulolytic bacteria. Monensin primarily affects Gram-positive bacteria, such as *Ruminococcus albus*, which are responsible for fiber degradation; however, it can also affect the growth of *Fibrobacter succinogenes*, which explains the higher NDF content and mass in the waste when this ionophore is adopted [14]. This factor should be considered for waste management and treatment, as longer or more intensive digestion conditions may be required to degrade the fibrous fraction.

In this context, the lignin content should also be monitored as this fraction acts as a limiting factor or at least slows the degradation of substrates [28]. In the present study, for the range of monensin doses tested, the curve for lignin mass (Figure 1) showed an increasing trend ($p < 0.05$) with the inclusion of monensin. This was due to the concentration of lignin, as animals fed the control diet had lignin concentrations of 4.68% in the TSs, whereas animals receiving diets with maximum monensin inclusion had 11.36% lignin. Similarly, [13] reported higher lignin levels in the waste of cattle fed monensin than in those that did not receive the ionophore.

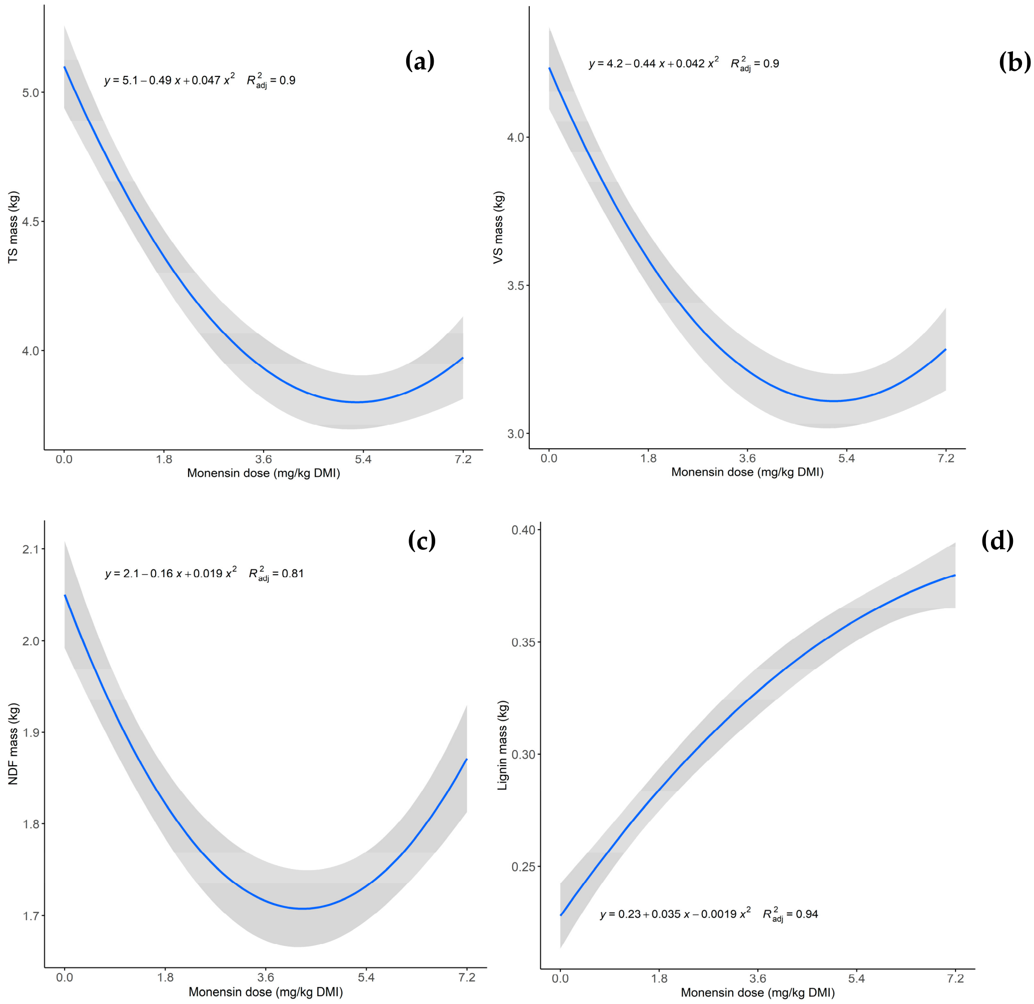

Figure 1. Masses (kg) of (**a**) total solids (TSs), (**b**) volatile solids (VSs), (**c**) neutral detergent fiber (NDF), and (**d**) lignin produced by cattle fed a diet supplemented with monensin. The shadow area corresponds to the confidence interval (95%).

The results of the characterization of waste from cattle fed increasing doses of monensin highlight the need for tailored waste management strategies. Although smaller waste masses are generated, which benefit transportation and handling, the waste has a more resistant composition with higher concentrations of fibrous constituents and lignin. According to [19], solid–liquid separation for waste treatment is a viable alternative, as it can provide more uniform digestion for fibrous waste substrates, such as pure ruminant waste, plant-based residues, digestate, and various effluents. The authors suggest that this practice is an important component of sustainable agriculture with reduced greenhouse gas emissions, leading to improved waste treatment systems, such as anaerobic digestion.

3.2. Anaerobic Digestion

For the 20-day hydraulic retention time, reductions in TSs were influenced ($p < 0.05$) by monensin inclusion in the animal diet under both screening conditions (Figure 2a). The highest monensin dose (7.2 mg kg^{-1} DMI) resulted in the smallest reduction (35.80% and 32.06% for the SC and US conditions, respectively). However, under the SC condition, the maximum TSs reduction was 47.44% (at 3.31 mg monensin kg^{-1} DMI), whereas, for US substrates, the maximum reduction occurred with a dose of 2.29 mg monensin kg^{-1} DMI (40.95%). This behavior may be associated with the beneficial effects of screening, which may have effectively reduced monensin concentrations in the liquid fraction intended for digestion [15] compared to the US substrates. Additionally, screening retained the coarser fibrous fraction, resulting in lower NDF levels (Table 2) and making the substrate more digestible to microorganisms, as indicated by the reductions in VSs (Figure 2b), with maximum reductions of 53.11% (SC) and 47.26% (US). These results exceed those found by [17], who observed a maximum of 31% VSs reduction when digesting dairy cow manure, reaching 59% only with co-digestion. However, it is noted that lower VSs reductions may be a consequence of using a shorter HRT than designed for the digesters, which results in lower process efficiency, as these results were evaluated on several farms and not under experimental conditions.

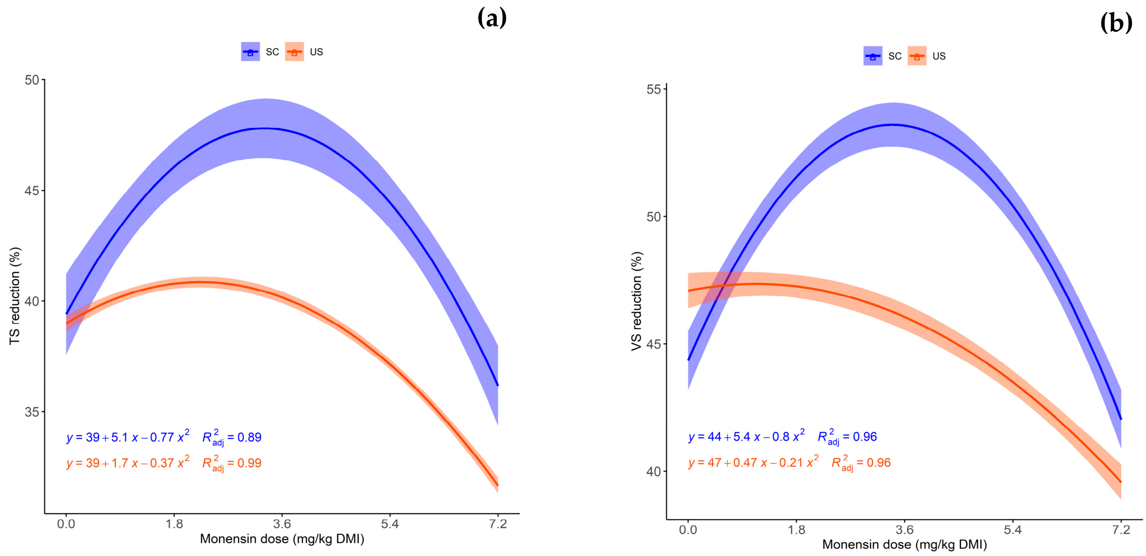

Figure 2. (**a**) Reduction of total solids (TSs, %) and (**b**) volatile solids (VSs, %) in the anaerobic digestion of waste produced by cattle fed a diet supplemented with monensin and a 20-day hydraulic retention time. SC: screened, US: unscreened. The shadow area corresponds to the confidence interval (95%).

In the 30-day hydraulic retention time, similar to the 20-day HRT, a beneficial effect of screening was observed on the reduction of solids (Figure 3), with maximum reductions of 52.82% and 59.64% for total solids (TSs) and volatile solids (VSs), respectively. These higher reductions are likely associated with the longer residence time of the substrates during digestion, as under mesophilic conditions, an increased HRT enhances the exposure time of the antibiotic to microorganisms and facilitates its biodegradation and adsorption, particularly owing to reduced microbial activity [29]. The inclusion of monensin in cattle diets also influenced the reduction in TSs and VSs in US substrates. However, under US conditions, the decrease in TSs degradation showed a linear effect, representing a more direct negative influence compared to the SC substrates. Maximum reductions in TSs and VSs were observed in the control treatment for US substrates (48.62% and 56.00%, respectively), while screening did not influence the reduction in TSs and VSs at a monensin dose of 7.2 mg.

Antibiotics can affect the degradation of organic components in substrates from the initial stage, characterized by hydrolysis, because hydrolytic bacteria are hindered by these products [12]. Despite the expectation of reduced solid reduction in the presence of antibiotics, the reductions achieved in our study exceeded those reported by [30], which were 31.41% and 33.17% for TSs and VSs, respectively, in substrates prepared with dairy cattle waste and a 30-day HRT without antibiotic use. In our study, even the highest doses of monensin resulted in greater reductions in TSs and VSs, possibly due to a lower percentage of solids used (2.5%), whereas the cited authors used 8% total solids in their substrates.

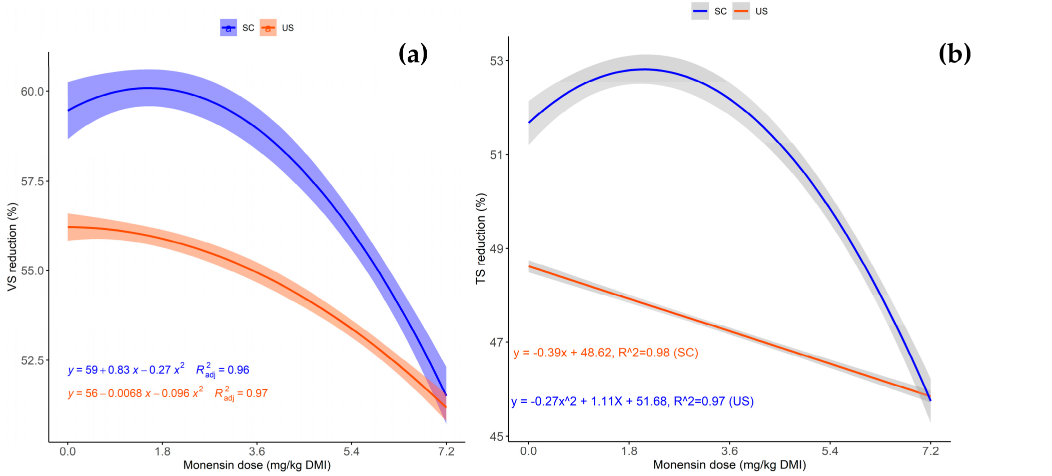

Figure 3. (**a**) Reduction of volatile solids (VSs, %) and (**b**) total solids (TSs, %) during the anaerobic digestion of waste produced by cattle supplemented with doses of monensin and a 30-day hydraulic retention time. SC: screened, US: unscreened. The shadow area corresponds to the confidence interval (95%).

Fraction separation and monensin dose also influenced the degradation of neutral detergent fiber (NDF) in the substrates, as well as biogas and methane production. For the 20-day hydraulic retention time, the highest reduction in NDF was observed in the SC substrates, with a maximum reduction of 69.79%, compared to the US conditions (54.24%, Figure 4). As previously mentioned, waste produced from animals that received higher monensin doses showed a higher lignin content, which may have resulted in decreased degradation of the fibrous fraction as well as reduced biogas production and methane concentrations. The reductions in NDF reported by [31] using whole manure were 45%. However, the authors used 4% TSs in the influent in batch biodigesters, which may have contributed to a lower reduction due to the higher initial TSs content. A similar pattern was observed in the substrates retained for 30 days, under US or SC conditions (Figure 4). However, the highest reductions in NDF were observed in the control treatment substrates, with reductions of 71.95% and 60.70% under SC and US conditions, respectively.

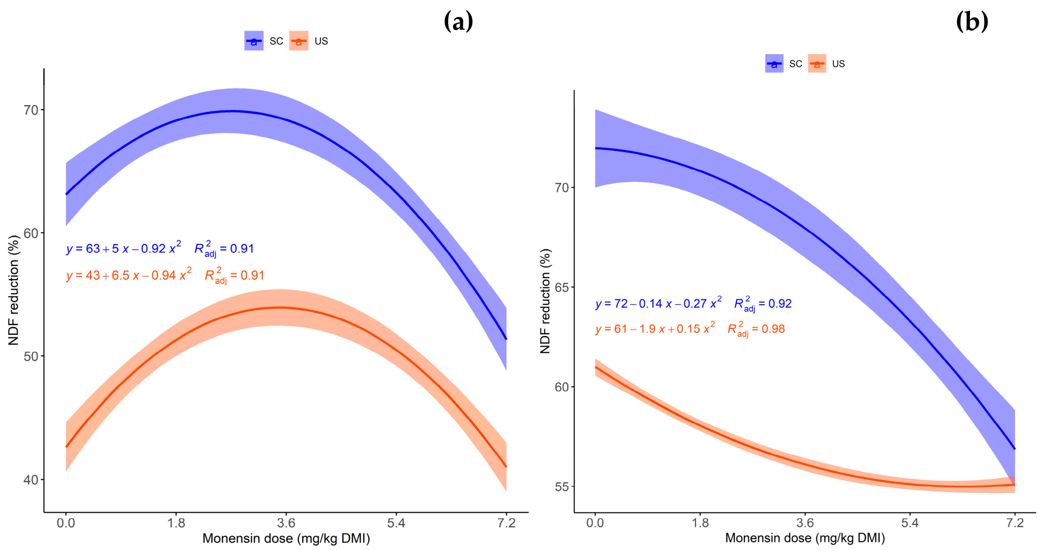

Figure 4. Reduction of neutral detergent fiber (NDF, %) in the (**a**) 20-day hydraulic retention time and (**b**) 30-day hydraulic retention time during the anaerobic digestion of waste produced by cattle supplemented with doses of monensin. SC: screened, US: unscreened. The shadow area corresponds to the confidence interval (95%).

Monensin is a potent inhibitor of enteric methane production in livestock, leading to a negative effect on biogas production and energy potential, particularly at short retention times [32]. This effect was evident in this study, with decreases in biogas production, methane concentration, and methane production when monensin was added to the diet of the animals.

The results for biogas production potential showed a clear influence ($p < 0.05$) of fraction separation for the 20-day HRT (Figure 5), with the highest potential achieved in SC substrates (157.28 L/kg of TSs added) at the dose of 1.53 mg monensin and the lowest potential with the dose of 7.2 mg kg^{-1} DMI. In the US substrates, the maximum potential reached was 103.42 L/kg of TSs added, with minimum production occurring at the maximum monensin dose. Using monensin at two dosages, [10] achieved a biogas production of 7.1 m^3 per week with 1 mg of monensin L^{-1}, while 10 mg resulted in only 2.0 m^3 with a 17-day HRT. For the 30-day HRT, the SC substrates also resulted in higher biogas production potentials, with a maximum yield of 199.83 L/kg of TSs added and a minimum yield at the dose of 7.2 mg kg^{-1} DMI (142.91 L/kg of TSs added). In the US substrates, the maximum production was 151.29 L/kg of TSs added, and the minimum was observed at the dose of 7.2 mg monensin.

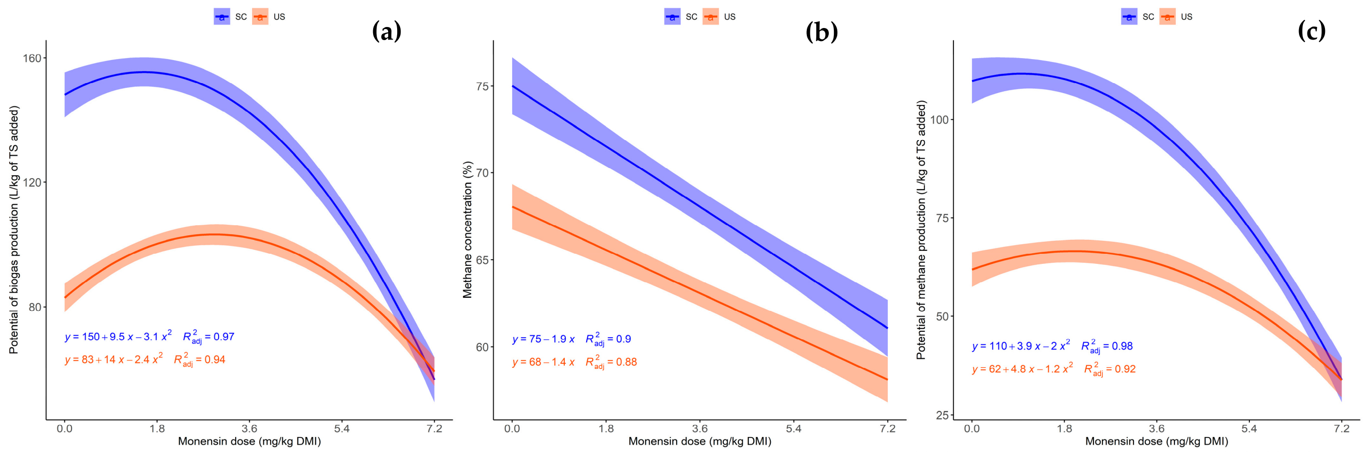

Figure 5. (**a**) Potential of biogas production (L/kg of TSs added); (**b**) methane concentration (%); (**c**) potential of methane production (L/kg of TSs added) during anaerobic digestion of substrates produced from cattle manure with monensin in the diet and a 20-day hydraulic retention time. SC: screened, US: unscreened. The shadow area corresponds to the confidence interval (95%).

In the 20-day HRT, as with other analyzed parameters, the SC condition also improved methane concentrations (Figure 5), reaching up to 75% CH_4, while in the US condition, the highest concentration was 68%, both in the control treatment. These concentrations are similar to those found by [10], who observed no differences between the control substrates and 1 mg of monensin (66% of CH_4), whereas 10 mg resulted in only 47%. When using lasalocid, [33] found no differences between the treatments, with 66% of CH_4, though the maximum dose used was 5 mg L^{-1}. The concentrations directly influence the capacity of ionophores to hinder digestion; however, the exact threshold for this effect remains unclear. Another factor is that ionophores have different physicochemical properties, which, depending on their degree of hydrophobicity, may also result in lower degradation rates during the process [20]. However, for the 30-day HRT, methane concentrations (Figure 6) did not differ between the SC and US conditions, only between the different doses of monensin, with the highest percentage in the control treatment (an average of 73.2% CH_4) followed by a decrease up to the maximum dose of monensin (7.2 mg monensin, an average of 68.18% CH_4). Thus, it is considered that a longer retention time was beneficial for the possible adaptation of the microorganisms in the digestion medium, as previously reported, which is advantageous for reducing the impact of monensin within the digesters [29]

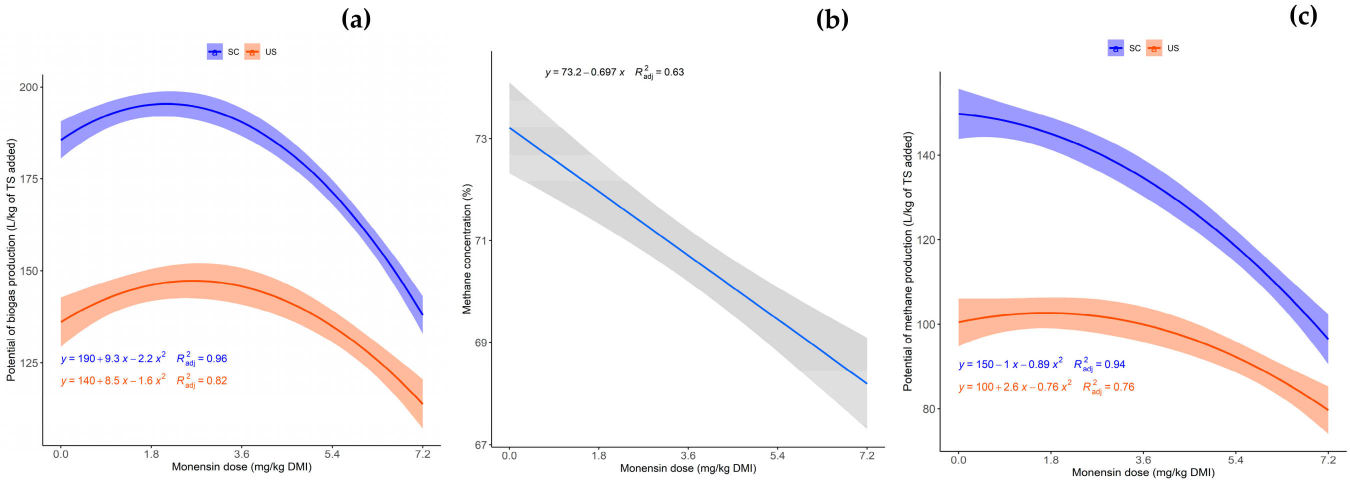

Figure 6. (**a**) Potential of biogas production (L/kg of TSs added); (**b**) methane concentration (%); (**c**) potential of methane production (L/kg of TSs added) during anaerobic digestion of substrates produced from cattle manure with monensin in the diet and a 30-day hydraulic retention time. SC: screened, US: unscreened. The shadow area corresponds to the confidence interval (95%).

Methane production was influenced by fraction separation in the 20-day HRT (Figure 5) with maximum production reaching 111.90 and 66.80 L/kg of TSs added in the SC and US conditions, respectively. For methane production in the 30-day HRT (Figure 6), both fraction separation and monensin doses had a significant effect ($p < 0.05$), with positive results for the separation process, with maximum production in the control treatment (149.16 L/kg of TSs added) and a decrease in production with increasing monensin doses (96.66 L/kg of TSs added, 7.2 mg monensin/kg DMI). A similar pattern was observed in the US condition, but the maximum potential occurred at the 1.71 mg monensin/kg DMI dose (102.22 L/kg of TSs added).

Studies that better elucidate the behavior of ionophores during anaerobic digestion, or how the process evolves in the presence of these compounds, are still scarce in the literature. However, research on other groups of antibiotics suggests that they may stimulate the process, increasing methane production [34]. Nevertheless, it cannot be conclusively stated that ionophores have a stimulatory effect on biogas or methane production, as biodigesters fed with loads containing monensin may take up to six months to stabilize [35].

The use of monensin in cattle diets, which targets Gram-positive bacteria, results in reduced enteric methane emissions. However, these emissions were dose-dependent, as observed in previous meta-analyses [2,36]. A similar effect may occur in the anaerobic digestion of cattle manure, depending on the antibiotic concentration within the digesters. Disturbances and reductions in methane production may or may not occur.

As reported previously [10], the addition of monensin to substrates led to varying effects depending on the dosage. At a concentration of 10 mg monensin per liter of substrate, there was a 75% reduction in specific methane production, whereas a dose of 1 mg monensin per liter resulted in only a 12% reduction. The authors noted that higher monensin concentrations caused instability in the digesters, although the threshold concentration at which the instability began was not determined.

Conversely, [11] reported decreases in methane production with just 0.2 mg monensin per liter of substrate during the digestion of dairy cattle manure, and greater instability in the microbiome at 5 mg monensin per liter of substrate. However, the authors observed an adaptation to monensin when the amount was gradually increased.

The results of this study highlight the beneficial effects of fraction separation on improving the efficiency of anaerobic digestion. Fraction separation has been studied for its potential to reduce greenhouse gas emissions when biofertilizers are applied to soil or during manure storage [17]. However, there is limited literature on the positive effects of solid fraction separation before anaerobic digestion and its influence on substrate degradation efficiency. In Brazil, because of the large number of cattle, the use of ruminal modulator antibiotics in cattle production is a concern, necessitating urgent recommendations for the treatment of monensin-containing waste. By separating the fractions, it is possible to reduce antibiotic concentrations in the feces destined for the digester and direct the solid fraction to the compost, where microbial degradation may be more intense and potentially more efficient in reducing monensin, even at higher concentrations [15,20].

Another benefit of anaerobic digestion with fraction separation is the reduction of fibers and coarse particles [20], which can decrease the HRT and volume of digesters in the field, thus enhancing the economic viability of waste treatment systems. Despite the reduction in the amount of waste generated with the use of monensin, this waste required a longer degradation time, as higher lignin levels were found in the manure with increasing monensin levels in this study, making the substrate more resistant to degradation.

Hydraulic retention times of 20 to 30 days are commonly utilized for anaerobic digestion, with even shorter retention times being employed in some cases [10,33]. Extended retention times, such as 40 to 50 days, may not be required to achieve process efficiency. Additionally, it is crucial to consider the duration needed to produce high-quality digestate and to optimize biogas and methane production.

4. Conclusions

The characterization of the manure revealed higher percentages of fibrous content with increasing doses of monensin, which may have influenced the substrates for anaerobic digestion, resulting in substrates with a lower potential for biogas and methane production. Therefore, fraction separation is beneficial for the anaerobic digestion process, and the use of screening for substrates with 20- and 30-day HRTs is recommended, as it reduces the solid content in the substrates by 40%, facilitating the degradation of solids by the microorganisms involved in the process or even reducing the monensin content in both fractions.

However, studies aimed at evaluating the impact of monensin on anaerobic digestion are still scarce in the literature. Future research is needed to clarify the concentration limits that may cause disturbances, the time required for microorganisms to adapt to these products, which are widely used as animal performance enhancers, or the most effective pre- or post-treatment methods to reduce monensin concentrations before using the biofertilizer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10090474/s1, Table S1: Volume, loads, discharges, and average daily production of semi-continuous experimental-scale digesters operated with manure from cattle fed with monensin doses (mg kg-1 of dry matter intake).

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