Isolation of *Salmonella* spp. from liquid and solid excreta prior to and following ensilage in ten swine farms located in central Mexico

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Abstract

A study was carried out to define selected bacteriological characteristics of residues from 10 swine farms, 5 with or without prior clinical enteric disease (PCED) and to determine the effect of ensilage on the bacteria present in the solid fraction. At each farm, samples were taken from the sedimentation basin (SB), the solid fraction (SF), and the liquid fraction (LF). For each sample, CFU/g for enteric bacteria were quantified; *Salmonella* spp. were isolated and typified. Solid phase samples from each farm were used to prepare the ensilage, with a mixture of solids (80%), sorghum (12%) and molasses (8%). The quantity of enteric bacteria was significantly greater in farms without PCED ($P < 0.05$). *Salmonella enterica* were isolated from 8/10 of the farms with and without PCED; in 8 from SB; in 6 from LF; and in 5 from SF. Enteric bacteria were not isolated from silage, therefore, ensilage may be an alternative treatment for excreta that allows the elimination of pathogens such as *Salmonella* spp.

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Keywords: *Salmonella* spp.; Swine farm residues; Ensilage

1. Introduction

Of worldwide meat production, pork is the most abundant type with a total annual production of 93.5 million tons. This has brought about a strong incentive to increase the size of the farms in a relatively small area due to increased production efficiency but also resulting in the production of great quantities of residues. Although land spreading of untreated wastes remains a potential disposal method, it can not be applied to animal units with high density population (e.g. pig farms) due to the decrease of available land in the proximity of the farms (Andreadakis, 1992). Most often farms are found in areas that are close to human settlements and have scarce water resources (Pérez, 1997), this has induced the development of processes that can use these liquid and solid wastes for farmland fertilizers. However, fecal residues constitute a serious contamination threat for rivers, lakes and land surrounding the farms, with a significantly high contamination potential for groundwater. This has created a need for the development of a more complete waste treatment in order to limit environmental pollution and transmission of diseases (Taiganides, 1994).

In Mexico, only 76% of pig farms have a residual waste water treatment system that consists of separating solids and liquids and sending the latter to oxidation ditches lagoons. In the case of solid excreta, 23% of farms use it as feed, without prior treatment (Pérez, 1997). This method yields a decrease in feed production costs; however, the use of untreated solid residues without prior evaluation of their microbiological characteristics can result in environmental pollution and the
transmission of enteric diseases for both humans and other species. The transmission of *Salmonella* spp. is particularly risky, because salmonellosis is an important zoonotic infection (Strauch and Ballarini, 1994; Henry et al., 1995), with animal products and byproducts being the most important source of infection for man (Gyles and Thoen, 1993). The pig is the main source of worldwide food-borne salmonellosis (Mead et al., 1999; Duffy et al., 2000). For example, in Denmark in 1993, pork was the most important source of food-borne salmonellosis (Baggesen et al., 1996).

In Mexico, various studies have documented the presence of *Salmonella* spp. in meat products, generally due to contamination of the meat from several materials such as feces, knives, meat cutting tables, and direct contamination from the hands of workers (Barreiro, 1998; Salgado et al., 1999). The main source of *Salmonella* spp. is the animal carrier and it is thought that this carrier state is brought about by direct pig-to-pig contact or through exposure to a contaminated environment (Berends et al., 1996). The carrier state may be characterized by the absence of any evidence of clinical disease in animals that are capable of transmitting the infection to susceptible individuals and eliminating it into the environment. These animals can eliminate up to $10^5$ CFU/g *Salmonella* spp. in feces during a period that can last from months to years, and there are certain factors that increase their susceptibility for excreting the bacteria (Henry et al., 1983), some of these factors include commingling of pigs, transportation, concurrent diseases, and food deprivation.

The potential for *Salmonella* spp. survival in the environment should also be considered. This potential was amply documented in a study by Plym and Ekesbo (1993), who observed that *S. dublin*, *S. senftenberg* and *S. typhimurium* could be found in feces for up to 183 days, in fresh feces from sows, placed on the ground. This emphasizes the sanitary risk faced by using both fresh and solid excreta in animal feed and makes it necessary to evaluate the bacteriologic characteristics of farm residues under different conditions. Recommendations can then be made concerning the treatment of these residues prior to recycling. At the same time, it is necessary to find new treatment methods for solid excreta, such that the latter will serve as a viable and profitable alternative in animal feed, especially in developing nations, where they are a valuable nutritional resource.

One of the most simple and economical methods for treating solid excreta is ensilage. This method for the anaerobic preservation of pig and other animal excreta, is based upon fermentation and the production of acids that can significantly change the concentration of soluble carbohydrates in the mixture. Ensilage improves odor, preserves nutritional content (Castrejón, 1993) and destroys enteric bacteria present in the excreta, including *Salmonella* spp. (Martínez-Gamba et al., 2001). However, it is not known whether this method can be applied to the destruction of pathogens in farm residues under various conditions such as: prior sanitary conditions, feed nutritional quality, humidity content, type of solids separator, residue retention time in the basins, and environmental conditions at the farm, nevertheless there is a constant characteristics in pig farms waste, this mean the presence of enteric bacteria; therefore the objective of these study was to determined the capacity of silage process to eliminated those enteric bacteria of solid excreta, arising from pigs farms under different production systems.

2. Methods

2.1. Geographical area and farms evaluated

Ten farms were evaluated, containing a minimum of 150 and a maximum of 1500 breeder sows; the following states in central Mexico were included in the study: Queretaro (2 farms), Mexico (2 farms), Michocan (3 farms), Morelos (1 farm) and Puebla (2 farms). All the farms had a solids separating system for pig excreta, five of them reported having enteric bacterial problems in the two years prior to the study (Table 1).

2.2. Farm classification

Farm classification was done with information obtained from interviews made by the principal author of this study to the general manager of each pig operation, and this information was confirmed by some of the other authors of this study. The farm number was assigned in the order of visited and sampled farm.

In order to facilitate farm classification, the following data were obtained:

(I) General characteristics, such as: location, animal breed inventory and total number of animals, feed distribution procedure, water source, water treat-

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<th>Farm</th>
<th>Prior disease</th>
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<td>1</td>
<td>Salmonellosis</td>
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<td>2</td>
<td>Salmonellosis</td>
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ment, and cleaning and disinfection of water deposits were recorded to have a general view of the conditions of each farm.

(II) Prior sanitary conditions, such as diseases that have been present in the last two years, immunization programs, medications added to water and feed, and animal movements within the farm (all in/all out or continuous flowing management).

(III) Type of equipment used to separate liquids and solids.

All this information was used to classify the farms into two groups: by type of solids separator (cylindrical or cascade-type) and the presence or absence of prior enteric disease.

2.3. Sampling

Samples were obtained from three specific areas in each farm: (1) the collection basin or general sedimentation basin prior to excreta processing, (2) solids obtained after the separation process, and (3) liquids discarded after the separation treatment process. One liter of liquid from the sedimentation basin (SB) and from five different places at 50 cm depth was collected from each farm. A closed plastic tube that opened upon insertion into the SB was used for collecting the samples (Seconsa, México Cat. 201114). Samples were deposited in sterile glass containers (Quiromed, México Cat. 56) and pH was measured using reactive strips (ElCrisol, MéxicoCat.Caj-015). Containers were labeled and placed in a polyurethane box with cooling packs (Polimex, México Cat. Inmeg-ban1172) and then pouring the content into one liter capacity containers. Ten litres were obtained in the same manner (Martinez-Gamba et al., 2001).

Liquid fraction (LF) samples were obtained from the drainage pipe that joined the solids separator with the fermentation lagoon or with the outside of the farm. Each sample consisted of one liter of residual water, obtained by filling five 200 ml sterile containers (El Crisol, México Cat. Inmeg-ban 1172) and then pouring the contents of these into one liter capacity containers. Ten minutes after the initial sampling, a second liter of liquid was obtained in the same manner (Martinez-Gamba et al., 2001).

The solid fraction (SF) that exited from the separator were sampled in five different places, obtaining 500 g from each sampling spot. The five samples were mixed, homogenized and 500 g were collected into a 1 kg capacity sterile plastic bag, the last considered the sample for solids to be used for ensilage and bacteriological identification. Two kg of the mixture were kept at laboratory conditions for 144 h so as to evaluate the behavior of a non-treated sample (control). Sampling was repeated at each farm, as described by Monteith and Shannon (1986), obtaining a total of 60 samples.

2.4. Bacteriologic isolation

Once all samples had been collected, they were sent to the diagnostic laboratory of the Swine Production Department in the Faculty of Veterinary Medicine at the National Autonomous University of Mexico. To avoid bacterial load modification during transport, samples were kept under refrigeration at 4 °C. The maximum transport time was 4 h.

2.4.1. Isolation and identification of Salmonella spp.

Separately, five ml of LF and 5 g of SF were inoculated into 50 ml of Sodium Selenite Broth (Bioxon, Becton Dickinson, Cat. 220300), as well as into Tetrathionate Broth (Bioxon, Becton Dickinson, Cat. 211683), and incubated at 37 °C for 18–24 h. After that, another sample from the original inoculation was placed via the swab procedure, onto plates containing Salmonella-Shigella Agar (SS) (Bioxon, Becton Dickinson, Cat. 211683) and Brilliant Green Agar (BG) (Bioxon, Becton Dickinson, Cat. 211708); a second passage was carried out on SS and BG (Carter, 1979) 24 h after; and a third one 24 h later using the previous media. Plates were examined daily and the colonies with Salmonella spp. characteristics were selected: transparent colonies with a central black point (in SS agar plates). A smear was prepared from each colony and those that corresponded to Gram-negative bacilli were transferred to plates containing SS agar (Henry et al., 1995).

Plates with SS agar were examined 24 h later and once the colonies had been detected they were identified using biochemical tests, which consisted of inoculating them onto Triple Sugar Iron (TSI) Agar (BBL, Cat. 252515), SIM-medium (Merck, Cat. 5470), Simmons Citrate Agar (BBL, Cat. 252514), urea (BBL, Cat. 252575), malonate, and sugars such as rhamnose, arabinose and threulose; and incubating for 24 h at 37 °C. The information gathered was used to determine the serotype (Carter, 1979).

The serogroup was corroborated in the following manner: 50 μl of polyvalent antiserum of Salmonella somatic group O, Antiserum Poly A-I & Vi (Difco, Mexico, Cat. 222641) were placed in a glass plate, and a bacteriologic loop was used to remove colonies from the TSI Agar that had previously been inoculated with Salmonella spp. Homogenization using the same bacteriologic loop was carried out in the next three minutes, while agglutination was assured as being representative of a positive reaction.

Once Salmonella spp. had been identified, 50 μl of the following antisera were placed in a glass plate: one from group C of the Salmonella somatic group O, Group C1, factors 6, 7 (Difco, Mexico, Cat. 222641); and one from Group D1, factors 1, 9, 12 (Difco, Mexico, Cat. 2951470). Subsequently colonies were extracted with a bacteriologic loop from TSI Agar that had been
inoculated previously with Salmonella spp. This test was done in order to differentiate S. cholerae suis from S. enterica. The percentage of positive isolation of Salmonella spp. by farm with or without prior enteric disease and type of solids separator equipment was determined from these results.

2.5. Enteric bacteria count

In order to enumerate the enteric bacteria, a tenfold serial dilution was carried out. One gram of the sample was placed in 9.0 ml of sterile saline solution (pH 7.0) and homogenized in a Vortex (Vortex-genie-2, Scientific Industries Inc., USA). One ml was subsequently transferred to another tube for the next dilution, and so on until the tenth tube (10⁻¹⁰). Fifty µl from each dilution were inoculated onto MacConkey agar (Bioxon, Becton Dickinson, Mexico, Cat. 210900) and incubated at 37 °C for 24 h, after which the number of colony forming units per gram (CFU/g) were observed and recorded (Martínez-Gamba et al., 2001). For the enteric bacteria count, the Minimum Detection Limit (MDL) was 1 × 10⁻¹.

2.6. Solid fraction exposed to the environment for 144 h

One portion (2 kg) of the solid samples was deposited in a 10 kg capacity plastic container and placed on a shelf, leaving it exposed to an average temperature of 18 °C for 144 h. This was done to determine Salmonella spp. persistence in solid excreta without silage process. Following this period these were sampled for enteric bacteria counts, isolation and typification of Salmonella spp.

2.7. Solid excreta ensilage

Solids obtained from each of the 10 farms were used to produce ensilage (microsilos), in the following manner: 24.6 kg of excreta (82%), 3.0 kg of ground sorghum (10%) and 2.4 kg of molasses (8%) were placed in a 30 kg capacity plastic container. Once the mixture had been manually prepared, 3.0 cm layers were placed in 500 g capacity sterile plastic jars. The material was compacted after each layer had been added, until the jars were filled to capacity (five layer). The jars were then closed. Three replicates were constructed for each farm.

2.8. Microbiological analysis of silage

After 11 days of ensilage, the microsilos were opened under sterile conditions. To avoid the presence of possible contaminated material, at the time of opening the containers, the top 4-5 cm were removed and eliminated using a sterile aluminum spatula. Subsequently 5 g of the material were inoculated on different media so as to corroborate the presence of Salmonella spp. and enumerate the enteric bacteria, following the previously described procedure.

To confirm the presence of Lactobacillus spp., another gram of silage was inoculated on to Petri dishes containing Columbia Agar (Bioxon, Becton Dickinson, Mexico, Cat. 224000). These dishes were placed in anaerobiosis jars, in which CO₂ was generated with anaerobiosis packets (Difco Laboratories, Cat. 1952248) and incubated overnight at 37 °C. Colonies that appeared as dew drops were selected because these suggested the presence of Lactobacillus spp. and these were smeared and Gram stained. Purification of all Gram-positive “coccobacilli” were done by reincubating onto Columbia Agar and incubating for 24 h, after which the colonies were examined through biochemical tests, inoculated on SIM-medium and arabinose, maltose and salicin sugars, in order to differentiate between Erysipelothrix rhusiopathiae, Listeria spp. and Lactobacillus spp.

2.9. Statistical analysis

The results for colony forming units per gram (CFU/g) for samples from the sedimentation basin, effluent and solid were transformed to obtain the base 10 logarithm. Transformed data were used for an analysis of variance, using the SAS statistical package (Statistic Analysis System, 1990).

3. Results

3.1. Farm classification

Following the physical inspection and interview at each farm, to determine the sanitary conditions present at the 10 farms, those that had prior enteric diseases were designated as numbers one, two, five, eight and nine; while those that did not have prior enteric disease were designated as numbers three, four, six, seven and ten. The farms that had a cylindrical solids separator were: one, two, three, seven and eight; while those with a cascade-type solids separator were: four, five, six, nine and ten respectively (Table 1).

3.2. Enteric bacteria counts

The mean enteric bacteria count in all samples was 7.5 × 10⁶ CFU/g ± 2.2 × 10⁷ SD. The CFU/g of enteric bacteria, obtained from the SB material, the LF and the SF is presented in Table 2, where the greatest concentration can be seen to be in the solids. General counts of enteric bacteria from the different types of samples, arising from either the farms with prior enteric disease and those that did not report prior enteric disease, in the two years prior to the study period are shown in
Table 3. Farms with prior enteric disease, had fewer enteric bacteria (CFU/g) \( (P < 0.05) \) when compared to those without prior disease (Table 3). It should be noted that there was no difference between the enteric bacterial count, observed in SB, LF and SF. The relationship between the type of sample and the type of separator is presented in Table 4, also exhibited no interaction between these two factors \( (P > 0.05) \). Likewise, when comparing the quantity of CFU/g in the samples from farms with a cylindrical solids separator and a cascade-type solids separator, no differences were found \( (P > 0.05) \).

### 3.3. Isolation and typification of Salmonella spp.

Isolation of Salmonella spp. was achieved in 8 of 10 farms. A total of 27 of the 60 samples (45\%) were positive; and 4/5 farms with prior enteric disease and 4/5 without prior enteric disease were positive (80\%) (Table 5). From sedimentation basin material, eight farms (80\%) and 14 out of 20 samples (70\%) were positive. From liquids, six farms (60\%) and seven out of 20 samples (35\%) were positive. From solids, five farms (50\%) and six out of 20 samples (30\%) were positive (Table 5). For the cylindrical and cascade-type solids separator, the bacteria were isolated in 4 of 5 farms (Table 5). Following isolation and serotyping of Salmonella spp., 100\% of the analyzed colonies corresponded to Salmonella enterica.

### 3.4. Solid fraction exposed to the environment for 144 h

The average number of enteric bacteria from solid samples exposed to 144 h at environmental temperature was \( 1.5 \times 10^7 \) CFU/g, which was not significantly \( (P > 0.05) \) different from the average CFU/g of solid samples obtained from the solids separator at initial sampling. In the solid samples exposed for 144 h to environmental temperatures, that came from the five positive farms to Salmonella spp., the isolation of the bacteria were confirmed from farms four, five and seven, but not from farms six and eight.

### 3.5. Microbiological analysis of silage

Neither Salmonella spp. or other enteric bacteria (MDL \( 1 \times 10^{-1} \)) were isolated from the 11-day ensilage.
The only bacteria detected at the 11th days were Lactobacillus spp.

4. Discussion

4.1. Farm classification

The characteristics of the 10 farms that were evaluated are very similar concerning their production system. All farms had a manual solid recollection system, except for farms one, two and three, where there were hydraulic systems using flush tanks and pools. These systems dilute excretions and increase flow of liquids to the sedimentation basin and solids separators.

4.2. Bacteriological findings

The mean bacterial counts found in the present study from the SB, LF and SF, were $3.9 \times 10^5 \pm 5.3 \times 10^5$ DS, $1.5 \times 10^5 \pm 2.1 \times 10^5$ DS and $2.2 \times 10^7 \pm 6.8 \times 10^7$ DS, respectively. These concur with those reported by Martínez-Gamba et al. (2001), who observed values of: $8 \times 10^4$, $1.6 \times 10^5$, $2 \times 10^5$ and $4 \times 10^6$, from samples obtained from the top and at one meter depth of a SB, from LF and SF, respectively. In this study the concentrations of enteric bacteria recovered from the SB differed from data reported by Mateu et al. (1992), who sampled SB at 10, 14 and 18 days of retention, finding an initial count of $2.11 \times 10^9$, that decreased to $2.87 \times 10^7$ by day 18; however there are several factors that may affect bacterial load, such as fermentation or degradation, been aerobic or anaerobic conditions; volatile fatty acids concentration with bactericidal activity; decreased pH; and constant or periodic removal of material from the SB (Inigo et al., 1991; Mateu et al., 1992). It is important to point out that in this study the increased enteric bacteria load in the SF, as compared with the material from the SB, may be due to the concentration of solids that occurs following extraction of the liquid phase.

The findings from this study indicate that there are no variations in the count of enteric bacteria between farms despite the varying excreta collection systems, nutritional management, environmental conditions, population size and sanitary conditions. Secondly, the mechanical separation treatment process, either cylindrical or cascade-type, did not affect the bacterial load in solids or in liquids. This is confirmed by not having found differences in the enteric bacterial counts, either in solids or liquids when farms were classified by type of solids separator. The use of a cylindrical or cascade-type solids separator did not appear to influence the enteric bacteria load. Apparently, these systems do not diminish bacterial load as both solids and liquids present similar loads, thus maintaining a risk for the transmission of pathogens.

There were no differences in enteric bacteria quantities found in LF or SF samples when comparing the farms’ sanitary practices. This may be partially explained by the fact that considerable numbers of the enteric bacteria found in this study would not be considered pathogenic for pigs, as well as by the fact that there are enteric pathogens that are not considered such as Enterobacteriaceae such as Brachyspira hyodisenteriae, Clostridium spp. or Lawsonia intracellularis.

Thus it is necessary to take into consideration what conditions the feces are exposed in the stall or in the drainage system, such as: contact with disinfectants, the exposure to drying conditions, and time spent in the SB. All of these may alter the conditions that favor pathogenic bacteria survival, and not having isolated them from feces in this study does not rule out their presence in the animals. Also in many occasions pathogens can only be isolated from feces if they are present in high numbers or if specific cultures techniques are employed (Strauch and Ballarini, 1994). Conversely, the presence of a bacterial pathogen in feces is not consid-
ered a definitive diagnostic of disease, given that for disease to develop other conditions must be considered. These conditions include: level of immunity, the animal’s stress level, and the interaction with other pathogenic microorganisms.

The *Salmonella* spp. isolation percentage found in the present study was lower than that reported by Letellier et al. (1999), who found *Salmonella* spp. in 61% of the fecal material sampled from pens. However the concentration of bacteria isolated from the SB in the present study was slightly higher than those of Letellier et al. (70%). These are greater than those reported by Rajic et al. (2002b), who isolated the same bacteria in 19–42% of samples taken in four samplings of feces found on the floor of 90 pig farms.

The recovery of *Salmonella* spp. in 53.33% of the samples from the SB, LF and SF from farm without prior enteric disease, differs from that reported by Letellier et al. (1999), who, upon sampling farms without enteric disease, found the bacteria in only 17 of 93 samples (18.27%). Previously discussed data allows us to conclude that the lack of clinical signs of infection in swine is not related to the possibility of isolating a bacterial pathogen. In the present study, recovery was achieved in 11 of 30 (36.66%) samples from the SB, LF and SF from farms with prior enteric disease. These results concur with those of Vidal et al. (2002), who isolated *Salmonella* spp. in 26 of 84 farms (31%) with prior enteric problems. Likewise, Van der Wolf et al. (2000) analyzed 326 feces samples from animals with signs of enteric infection, where only *Salmonella* spp. as the pathogenic agents, were isolated from 40% of the samples. These results differ from those obtained by Letellier et al. (1999), who, from 115 samples from animals with clinical signs, found 70 (60.86%) to be positive for *Salmonella* spp. Similarly, Stege et al. (2000), when sampling feces accumulated of the floors of pens, isolated this pathogen in 77% of the farms.

Serotyping of *Salmonella* in the present study yielded that 100% of the isolated colonies analyzed corresponded to *S. enterica*. These data concur from those reported by various authors also in isolates from pigs, such as Baggesen (1996), who reports 30 different serotypes of *S. enterica*; Rajic et al. (2002a), who found 15 different serotypes of *S. enterica*; Jung et al. (2002), who typified 27 different serovarieties; and, Vidal et al. (2002), who identified 11 different serovars. It is important to point out that, in this study, priority was given to the identification of *Salmonella* spp., without placing any emphasis upon the serovarieties, as a potential risk factor for human and animal health, through exposure to untreated liquids or solid excreta, destined for animal feed.

### 4.3. Microbiological analysis of silage

In the present study, enteric bacteria were not recovered in solids following 11 days of silage, as concurs with results reported by Hernández (1997) and Martínez-Gamba et al. (2001), who did not observe bacterial growth in microsilos elaborated with ground sorghum,
the solid excreta fraction and molasses, inoculated with *S. choleraesuis* and *Escherichia coli*.

### 4.4. Solid fraction exposed to the environment for 144 h

In three of the five positive farms and from solid material maintained at 18 °C for 144 h, isolation of *Salmonella* spp. was still possible. Therefore the capacity of this agent for surviving under adverse conditions was shown. The fact that isolation was not possible in the five samples can be explained by results found by Funk et al. (2000), who, when studying the effect of conservation on samples for *Salmonella* spp. recovery, found only 11.4% of positive samples when using one gram of feces. When working with 10 and 25 g of feces, the proportion of isolation increased to 22.4% and 24.1%, respectively. In the present study only 5 g were used, which may be an explanation as to why isolation was not possible in 100% of the samples.

Conclusions draw from the results can be summarized as follows: Having found *Salmonella* spp. in the majority of the evaluated farms could indicate its high prevalence in the studied region, this highlighting the relevance of treating excreta prior to using them in animal feed. The use of a solids/liquid separator, be it cylindrical or cascade-type, has no effect on the quantity of enteric bacteria or *Salmonella* spp. recovered from liquid/solid waste samples. A 144-h time period is not sufficient for the destruction of enteric bacteria or *Salmonella* spp. in solid samples exposed to ambient temperature. It is thus necessary to implement some other treatments. The ensilage process for solid excreta reduces below $1 \times 10^{-1}$ the presence of enteric bacteria from day 11 of ensilage. Therefore, ensilage is an alternative treatment for excreta that allows the elimination of pathogens, thus rendering the excreta ready for animal feed, whether these come from a cylindrical or a cascade-type solids separator.

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### References


