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Chemical and microbiological characteristics of rice husk bedding having distinct depths and used for growing–finishing swine

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ABSTRACT

This study compared the effects of different bedding depths on the chemical and microbiological characteristics of the bedding material used to raise pigs during growing and finishing. The experiment was conducted in two pens housing 5 pigs from 60 to 145 days of age, with rice husk beddings 0.50 or 0.25 m deep. Four lots of pigs (replicates) were raised over time in each bedding depth: each bedding was used by two consecutive lots. Bedding samples were collected quarterly to determine the most probable number (MPN) of thermophilic and mesophilic bacteria, fungi and actinomycetes. Contents of N, P, K, C, organic, mineral and dry matter, C:N ratio and pH were also determined. The MPN of thermophilic bacteria was higher for the 0.50 m than for the 0.25 m bedding ($p < 0.05$). The compost of 0.25 m deep bedding had a higher N, P and K content than that from the 0.50 m bedding ($p < 0.05$). Thus, the use of the 0.25 m deep bedding would be recommended due to its greater agronomical value in comparison with the deeper bedding.

1. Introduction

In Brazil, as is common in many other countries, the swine inventory is highly concentrated in a relatively small area (Corrêa et al., 2000, 2009). This situation results in manure accumulation on farms that usually have little or no area for manure application (Zhang and He, 2006). Since manure has a high pollution potential and many swine producers have limited resources for manure management investment (Gentry et al., 2002), swine production systems are considered as one of the livestock activities having the greatest potential negative impact on the environment (Corrêa et al., 2000).

Deep bedding systems were developed as low-cost swine production systems versus conventional solid floors (made of concrete, iron or plastic) using cellulose-rich beddings (Honeyman, 1996). Deep beddings absorb feces and urine, thereby reducing humidity in contact with the pigs and favoring the expression of their typical exploratory behavior (Oliveira et al., 1999; Corrêa et al., 2000; Honeyman and Harmon, 2003).

In deep bedding systems, manure accumulates in the bedding and is exposed to the action of bacteria, fungi and actinomycetes (Morten and Baath, 1998). This microbiota is responsible for biological changes in the beddings, breaking complex molecules and modifying temperature, humidity, carbon:nitrogen (C:N) ratio and pH (Tang et al., 2004; Wang et al., 2004). The organic material is degraded and the resulting compost is rich in humus (Tiquia et al., 2002; Ishii and Takii, 2003; Charest et al., 2004). The physical and chemical changes occurring in the bedding determine the level of stabilization of its compost (Vuorinen and Saharinen, 1999). However, during the thermophilic phase of the composting process, the temperature limit recommended by Veit and Troutt (1999) for finishing pigs (12–21 °C) may be surpassed, influencing negatively their environmental comfort, especially under warm weather (Oliveira et al., 1999; Corrêa et al., 2000; Rinaldo et al., 2000). Therefore, understanding microbial processes during the bedding stabilization process is necessary to minimize potential negative effects on the environmental comfort of pigs raised in deep bedding systems (Kapuinen, 2001; Tang et al., 2004). Although some studies have been conducted for this purpose (Tiquia et al., 2002; Ishii and Takii, 2003; Zhang and He, 2006), there are limited descriptions about the association between distinct bedding depths and the microbial, physical and chemical characteristics of such beddings. The objectives of this study were to evaluate the effect of distinct bedding depths on the chemical and

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physical characteristics of beddings used on the growing and finishing phases of swine production, and to associate those characteristics with the dynamics of the microbiota.

2. Methods

The experiment was conducted from July 2003 to July 2004, at the UFPel Experimental Station (31°45′S latitude and 52°21′W longitude). The experimental units consisted of two 7 m^2 pens having a solid concrete floor, one conventional feeder and a nipple drinker. Each pen received five F1 Landrace × Large White piglets (two castrated males and three females), that were raised from 60 to 145 days of age. The piglets were fed an *ad libitum* diet consisting of 19% crude protein and 3350 kcal ME/kg during growing and 17% crude protein and 3200 kcal ME/kg during finishing (National Research Council – NRC, 1988).

In each pen, rice husk bedding was placed over the floor at two depths (treatments): 0.25 and 0.50 m. The bedding volume per animal was equal to 0.35 m^3 for 0.25 m deep beddings and to 0.70 m^3 for 0.50 m deep beddings. Four lots of pigs (replicates) were raised over time for each treatment: the first from July to September 2003; the second from October to December, 2003; the third from February to April, 2004; and the fourth from May to July 2004. Each bedding was used in two consecutive lots, without addition of rice husk, although the beddings were revolved between lots. At the end of the second lot, the bedding used on the first two lots was removed and replaced with fresh rice husk.

Bedding samples were collected quarterly, from five different points of the bedding, at half of its depth, mixed to make a homogenous sample and subjected to chemical and microbiological analysis. The most probable number (MPN) method (Charest et al., 2004), was used to estimate the concentration of bacteria and fungi, by plating 10 µL of decimal dilutions (10^{-1} to 10^{-8}) in phosphate saline buffer at pH of 7.2 on plates with media for the microorganisms under study, in triplicate. Samples for isolation of actinomycetes were heated at 65 °C during 4 h before being diluted (McCarthy and Williams, 1990). Trypticasein soy agar (DIFCO Labs) supplemented with 20 µg ml^{-1} of benomyl and 50 µg ml^{-1} of cycloheximide was used to estimate the MPN of aerobic bacteria (Charest et al., 2004). The Martin medium was used for isolation of fungi (Menzies, 1965) and the caseinate-dextrose agar was used for isolation of actinomycetes (Clark, 1965). Half of the plates were incubated at 27 °C (for mesophilic microorganisms), and the other half at 50 °C (for thermophilic microorganisms). The MPN for bacteria and fungi were estimated after 7 days of incubation and actinomycetes after 21 days.

The chemical content of the beddings was analyzed as described by Page et al. (1982). Dry matter was determined after 24 h in incubator at 105 °C and mineral matter after heating in electric oven at 250 °C. Organic matter was estimated by the difference between the dry and the mineral matter. Nitrogen contents were determined by the Kjeldhal method, while carbon contents were determined by the method of Walkley–Black and the C:N ratio was determined by calculation. Potassium content was determined by flame photometry and phosphorus content was determined by atomic absorption spectrophotometry. The pH was measured by a digital pH meter (PH-208, Lutron Electronic Enterprise Ltd., Taipei, Taiwan).

The effects of the treatments on the chemical and microbiological characteristics of bedding samples were evaluated by analysis of variance, with comparisons of means by Tukey test, considering beddings samples as the experimental units. Effects of replicates, month of sample collection and potential interactions were tested. Orthogonal contrasts were used for comparisons between new (first and third lots) and used beddings (second and fourth lots), and also for comparisons between the first (first and second lots) and the second beddings (third and fourth lots). Means of contrasts were compared through the Schefeé test. Multiple linear regression models were generated to evaluate the variation in the presence of mesophilic and thermophilic microorganisms as a function of the chemical characteristics of the beddings, for each depth. As none of the estimated microbial counts followed a normal distribution, those variables were transformed to a logarithmic scale. All statistical analyses were conducted with the Statistix® software (2003).

3. Results

Carbon (C), nitrogen (N), phosphorus (P) and potassium (K) contents were higher and the content of dry matter and C:N ratio were lower ($p < 0.05$) for the 0.25 m compared to the 0.50 m deep beddings (Table 1). The contents of both mineral and organic matter and the pH did not differ between beddings ($p > 0.05$). No significant difference was observed ($p > 0.05$) for any chemical parameters between the first and second bedding (Table 2). Used beddings contained higher C, N, P, K and other mineral matter contents ($p < 0.05$) than fresh beddings (Table 3), whereas the organic and dry matter contents and the C:N ratio were higher for new than for used beddings ($p < 0.05$).

The MPN of thermophilic bacteria was higher ($p < 0.05$) for the 0.50 m depth than for 0.25 m deep beddings (Table 4), but the MPN for the other microbial populations did not differ across bedding depths ($p > 0.05$). The only difference in microbial counts between the first and second beddings was observed for mesophilic fungi (Table 5), which were higher for the second than for the first beddings ($p < 0.05$). New beddings presented higher MPN of mesophilic bacteria and actinomycetes ($p < 0.05$) than used beddings (Table 6).

No chemical parameter was associated ($p > 0.05$) with the MPN of mesophilic actinomycetes in 0.50 m deep beddings. The P content and the C:N ratio were negatively related ($p < 0.05$) to the MPN of thermophilic actinomycetes and bacteria and of mesophilic bacteria (Table 7). Organic matter content was associated with increased MPN of thermophilic bacteria and actinomycetes and with reduced MPN of mesophilic bacteria ($p < 0.05$). Mineral matter content was associated with an increased MPN of thermophilic bacteria and actinomycetes ($p < 0.05$), whereas the MNP of mesophilic and thermophilic fungi were negatively associated with the dry matter content ($p < 0.05$). An increased K content was associated with reduced MPN of mesophilic bacteria, whereas increased pH was related to the decreased MPN of mesophilic fungi ($p < 0.05$).

In 0.25 m deep beddings, the K content was negatively associated ($p < 0.05$) with an increased MPN of thermophilic bacteria and actinomycetes and mesophilic fungi (Table 8). An increased C:N ratio was related with reduced MPN of mesophilic bacteria

Table 1
Chemical characteristics of the rice husk and of rice husk beddings having distinct depths.

Variable	Bedding depth (m)			SEM
	Rice husk	0.50	0.25	
Organic matter (%)	84.8	74.8[a]	75.3[a]	0.28
Dry matter (%)	88.8	74.2[a]	70.2[b]	0.74
Mineral matter (%)	15.2	25.3[a]	24.7[a]	0.29
N (%)	0.3	1.0[b]	1.2[a]	0.03
P (%)	0.1	1.6[b]	1.8[a]	0.04
K (%)	0.3	1.1[b]	1.2[a]	1.12
C (%)	28.7	29.2[b]	30.6[a]	0.40
C:N	92.8	28.2[a]	24.9[b]	0.02
pH	7.3	8.1[a]	8.1[a]	0.09

Least square means ± standard error of the mean (SEM) having distinct superscripts in the same line differ by at least $p < 0.05$.

Table 2
Chemical characteristics for first and second rice husk beddings.

Variable	Bedding		SEM
	First	Second	
Organic matter (%)	75.6	75.3	0.80
Dry matter (%)	74.2	72.1	2.10
Mineral matter (%)	24.5	24.7	0.82
N (%)	1.2	1.0	0.09
P (%)	1.6	1.5	0.12
K (%)	1.1	1.1	0.07
C (%)	29.0	30.8	1.13
C:N ratio	29.7	30.1	0.06
pH	8.2	7.9	0.26

First: lot 1, 07/2003 to 09/2003 and lot 2, 10/2003 to 12/2003; second: lot 3, 02/2004 to 04/2004 and lot 4, 05/2004 to 07/2004.
Least square means ± standard error of the mean (SEM) did not differ ($p < 0.05$).

Table 3
Chemical characteristics for new and used rice husk beddings.

Variable	New	Used	SEM
Organic matter (%)	79.5[a]	71.3[b]	0.80
Dry matter (%)	76.1[a]	70.2[b]	2.10
Mineral matter (%)	20.5[b]	28.7[a]	0.82
N (%)	0.8[b]	1.4[a]	0.09
P (%)	0.7[b]	2.5[a]	0.12
K (%)	0.7[b]	1.5[a]	0.07
C (%)	29.0[b]	30.9[a]	1.13
C:N ratio	37.5[a]	22.7[b]	0.06
pH	7.9[a]	8.1[a]	0.26

New: lot 1, 07/2003 to 09/2003 and lot 3, 02/2004 to 04/2004; used: lot 2, 10/2003 to 12/2003 and lot 4, 05/2004 to 07/2004.
Least square means ± standard error of the mean (SEM) having distinct superscripts in the same line differ by at least $p < 0.05$.

Table 4
Mesophilic (27 °C) and thermophilic (50 °C) microbial counts (most probable number) for rice husk beddings having distinct depths.

Microorganisms	Temperature (°C)	Bedding depth (m)		SEM
		0.50	0.25	
Bacteria	27	5.81[a]	5.69[a]	0.12
	50	6.11[a]	5.79[b]	0.11
Fungi	27	4.35[a]	4.44[a]	0.10
	50	4.57[a]	4.34[a]	0.14
Actinomycetes	27	4.70[a]	4.57[a]	0.13
	50	4.80[a]	4.79[a]	0.09

Least square means ± standard error of the mean (SEM) (expressed in logarithmic scale) having distinct superscripts in the same line differ by at least $p < 0.05$.

Table 5
Mesophilic (27 °C) and thermophilic (50 °C) microbial counts (most probable number) for first and second rice husk beddings.

Microbial counts	Temperature (°C)	Bedding		SEM
		First	Second	
Bacteria	27	5.77[a]	5.64[a]	0.32
	50	5.92[a]	5.93[a]	0.28
Fungi	27	4.21[b]	4.55[a]	0.28
	50	4.30[a]	4.51[a]	0.36
Actinomycetes	27	4.49[a]	4.80[a]	0.40
	50	4.78[a]	4.72[a]	0.19

First: lot 1, 07/2003 to 09/2003 and lot 2, 10/2003 to 12/2003; second: lot 3, 02/2004 to 04/2004 and lot 4, 05/2004 to 07/2004.
Least square means ± standard error of the mean (SEM) (expressed in logarithmic scale) having distinct superscripts in the same line differ by at least $p < 0.05$.

Table 6
Mesophilic (27 °C) and thermophilic (50 °C) microbial counts (most probable number) for new and used rice husk beddings.

Microbial counts	Temperature (°C)	New	Used	SEM
Bacteria	27	5.71[a]	5.70[a]	0.32
	50	6.11[a]	5.74[b]	0.28
Fungi	27	4.40[a]	4.35[a]	0.28
	50	4.44[a]	4.37[a]	0.36
Actinomycetes	27	4.86[a]	4.63[a]	0.40
	50	4.85[a]	4.46[b]	0.19

New: lot 1, 07/2003 to 09/2003 and lot 3, 02/2004 to 04/2004; used: lot 2, 10/2003 to 12/2003 and lot 4, 05/2004 to 07/2004.
Least square means ± standard error of the mean (SEM) (expressed in logarithmic scale) having distinct superscripts in the same line differ by at least $p < 0.05$.

and fungi and thermophilic bacteria and actinomycetes ($p < 0.05$). Dry matter content was positively associated with the MPN of mesophilic bacteria and thermophilic fungi, whereas mineral matter content was negatively associated with the MPN of thermophilic fungi ($p < 0.05$). The organic matter content presented a negative association with the MPN of mesophilic actinomycetes, but a positive association with the MPN of thermophilic actinomycetes ($p < 0.05$). An increased N content was related to an increased MPN of mesophilic actinomycetes ($p < 0.05$).

4. Discussion

As observed in the present study, during the bedding stabilization process, changes on the microbiota were related to simultaneous changes in temperature, humidity, pH and C:N ratio, with progressive transformations of complex substances in simpler molecules (Klamer and Balath, 1998; Tiquia, 2005). The bedding stabilization process starts at an ambient temperature by the activity of mesophilic microorganisms, but, as microbial activity intensifies, the temperature inside the bedding increased due to decomposition of the light fraction of the organic matter (Larney et al., 2000; Tiquia et al., 2002; Ishii and Takii, 2003). The temperature gradually increases up to 60 °C, when thermophilic microorganisms replace mesophiles (Kapuinen, 2001; Tang et al., 2004). Thus, the changes occurring during the bedding stabilization process would affect the environmental comfort of pigs raised in deep bedding systems, depending on the characteristics of the material used and of the depth of the bedding. In fact, the temperature inside 0.25 m deep beddings was lower than inside 0.50 m deep beddings (Corrêa et al., 2008, 2009), which, as our results indicate, could be due to their lower counts of thermophilic bacteria. For both bedding depths tested in this study, decreased counts of thermophilic bacteria were associated with increased K contents and C:N ratio. Therefore, the K content is apparently the chemical parameter having the most characteristic association with the concentrations of thermophilic bacteria recovered.

Overall microbial counts were higher for 0.50 m than for 0.25 m deep beddings, but the latter showed lower C:N ratio and higher contents of both C and N. As a consequence, 0.25 m deep beddings had higher content of organic matter and thus their compost would have better agronomic value than 0.50 m deep beddings, which could be attributed to the lower bedding volume available per animal. Nevertheless, the compost produced in both depths can be considered non-stable, because their C:N ratios were above 15/1 limit for stable composts (Tiquia et al., 1997; Suszek et al., 2005; Venglovsky et al., 2005). The results of this study indicate that 0.25 m deep beddings were more efficient in transforming rice husk into fertilizer than 0.50 m deep bedding. Therefore, the use of 0.25 m deep bedding would be recommended for swine operations, especially in southern Brazil, where rice husk is an abundant

Table 7
Multiple linear regression for mesophilic and thermophilic microbial counts (most probable number expressed in logarithmic scale) for rice husk 0.50 m deep beddings.

Microbial counts	Constant	P	K	C:N ratio	Organic matter	Mineral matter	Dry matter	pH	R^2
Bacteria (27 °C)	21.3775	−0.59454	−1.77259	−0.03629	−0.15230	NS	NS	NS	0.5120
Bacteria (50 °C)	−57.1942	−0.48819		−0.03309	0.66319	0.61778	NS	NS	0.6669
Actinomycetes (27 °C)	−37.7516	−0.70549		−0.02532	0.43027	0.48873	NS	NS	0.6987
Actinomycetes (50 °C)	10.9474	NS	NS	NS	NS	NS	−0.04636	−0.38947	0.2432
Fungi (27 °C)	9.3258	NS	NS	NS	NS	NS	−0.06391		0.2390

All models have significant equations ($p < 0.05$).
NS: not significant.

Table 8
Multiple linear regression for mesophilic and thermophilic microbial counts (most probable number expressed in logarithmic scale) for rice husk 0.25 m deep beddings.

Microbial counts	Constant	K	C:N ratio	Organic matter	Mineral matter	Dry matter	N	R^2
Bacteria (27 °C)	3.4126	NS	−0.02462	NS	NS	0.04267	NS	0.2988
Bacteria (50 °C)	7.7826	−1.00339	−0.02573	NS	NS	NS	NS	0.4457
Actinomycetes (27 °C)	19.9010	−4.04824	NS	−0.17496	NS	NS	2.26074	0.3583
Actinomycetes (50 °C)	1.47507	NS	−0.1922	0.05147	NS	NS	NS	0.3435
Fungi (27 °C)	5.77407	−0.68209	−0.0169	NS	NS	NS	NS	0.2293
Fungi (50 °C)	12.2539	NS	NS	NS	−.08171	0.08392	NS	0.6081

All models have significant equations ($p < 0.05$).
NS: not significant.

byproduct of rice production, with low added value (Corrêa et al., 2008, 2009), and that is potentially damaging for the environment because its silica content makes its degradation extremely slow. Additionally, in comparison with 0.50 m deep bedding, the use of 0.25 m deep bedding allows better environmental comfort for pigs in the growing and finishing phases (Corrêa et al., 2008, 2009) and the production of compost with greater agronomic value.

The association between dry matter content and microbial counts found in this study emphasizes the important role of the water content in the bedding stabilization process. Dry matter content was higher for 0.50 m than for 0.25 m deep beddings, probably due to the higher bedding volume present in the deeper beddings. However, the higher volume of rice husk in the 0.50 m deep bedding would incur in higher costs related to waste management, transportation, revolving and disposal. In 0.50 m deep beddings, dry matter content was negatively associated with fungi counts, whereas actinomycetes counts were negatively influenced by increased P content and C:N ratio. On the other hand, in 0.25 m deep beddings, the MPN of both thermophilic actinomycetes and fungi appear to be restricted with higher mineral matter content and C:N ratio, which could maintain the temperature near that recommended for growing–finishing pigs (Corrêa et al., 2009). In systems where 0.50 m deep beddings are currently in use, procedures to increase K, P, organic and mineral matter contents and the C:N ratio could be implemented to change the microbial dynamics inside the bedding, thus reducing the negative effects of high temperatures during the thermophilic phase of the bedding stabilization process. However, procedures to increase K and P content in the bedding would likely be more feasible.

Used beddings had higher N, P, K and overall mineral matter content than fresh beddings, which is due to the higher amount of dejects accumulated in used beddings, after two consecutive lots. Inside the bedding, however, there would be significant losses of N due to denitrification and of C as CO_2 (Tang et al., 2004). On the other hand, fresh beddings presented higher dry matter content. Thus, their higher count of thermophilic bacteria and actinomycetes, in comparison with used beddings, would occur during the thermophilic phase of the bedding stabilization process, resulting in increased temperatures in the bedding surface (Corrêa et al., 2009). Simultaneously with increased temperature, microbial population dynamics could also be altered (Vuorinen and Saharinen, 1999), although no differences in microbial populations were identified between new and used beddings in the present study.

The lack of differences in microbial counts and chemical characteristics between the first and the second beddings was expected, since they are replicates conducted over time. However, the increase in mesophilic fungi observed in the first bedding may have been due to some factors that were not controlled.

5. Conclusions

Different bedding depths were associated with changes in the chemical and microbiological characteristics of the beddings used to raise growing–finishing pigs. Rice husk beddings 0.50 m deep contained a higher total waste volume due to its higher dry matter content, but such volume can be attributed to its higher bedding volume, which makes such bedding more expensive. Besides its lower cost, 0.25 m deep beddings are preferable because they presented compost with greater agronomic value and lower counts of thermophilic bacteria, which provide better environmental comfort for the pigs.

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