# ACTIVATORS OF BIOLOGICAL TRANSFORMATION AND THEIR EFFECT ON PIG MANURE QUALITY AND BARN CONDITION AGGREGATE

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**Abstract.** In order to reduce the consumption of industrial fertilizers used in agriculture, production of higher quality pig manure acting as their partial substitution should be enhanced. The production involves utilization of the agents on the basis of organic matter biological transformation activators. The objective of this paper is to verify the efficiency of such an agent, i.e. Z`fix, from the viewpoint of the barn condition parameters, of the pig manure quality and manure nutrient content, and mainly from the viewpoint of ammonia emissions. From January 2018 to January 2019, 80 samples of pig manure were collected in a farm with the total of 84 livestock units (LU), where 1 LU = 500 kg live weight (L.W.). On the farm, the pigs were stabled in 2 barns with straw bedding, and with 2 pens each. In the first barn, the agent Z`fix was applied, the second one represented a control. In each barn, fattening pigs were divided in 2 pens (40-70 ± 5 kg and 70-110 ± 5 kg L.W.). The frequency of pig manure removal varied from 150 to 180 days. An accredited laboratory examined the manure chemical parameters. Ammonia emissions were evaluated every two weeks (Data Logger $NH_3$ apparatus measuring 25 cm above the bedding surface during 48 hours). With regard to the pig manure production, the results confirmed ($P < 0.05$) that the treatment of the straw bedding by the Z`fix agent had a positive influence on the pig manure quality, the course of the fermentation process, and on the microclimate in the barn. Compared to the control (without Z'fix application), higher contents of the following compounds were found: N-$NH_4^+$ (by 54 %); N (by 37 %); $K_2O$ (by 10 %); $P_2O_5$ (by 46 %). Furthermore, maximum humidity decreased by 10 % and the pH value increased from 8.7 to 9.1. Finally, maximum levels of the mean ammonia emissions in the barn decreased by 38 %. In agreement with the requirements posed by the Gothenburg Protocol, the agent's utilization is desirable considering its contribution to ammonia emission reduction and pig manure production quality enhancement.

**Keywords:** pig, manure, agent, ammonia, barn.

## Introduction

Since early 1990s, arable land management in the Czech Republic has undergone substantial changes in connection with the applied agricultural policy. Czech farmers have asked themselves how to prevent increasing degradation of arable land and thus the loss of quality organic matter, which significantly contributes to the quality of humus and to the soil fertility. One of the ways to remedy the described situation is increased production of high-quality pig manure, especially by using agents on the basis of the organic matter biological transformation activators.

In order to attain stabilized balance of organic substances in the soil in the conditions of the Czech Republic, 4.0-4.5 tons of organic substances should be applied annually per 1 hectare of the farm land [1]. This amount is partly covered by the post-harvest residues (2.0-2.5 $t \cdot ha^{-1}$), and the rest (1.5-2.0 $t \cdot ha^{-1}$) is desirable to be covered by manure. Currently, as mentioned by Richter *et al*. [2], only 0.6-0.7 $t \cdot ha^{-1}$ originates from manure. It is therefore necessary to look for alternative sources or to increase the production of quality manure. One of the possibilities to reduce the consumption of industrial fertilizers applied onto the soil is to increase the production of higher quality (composted) pig manure with the use of the agents on the basis of the organic matter biological transformation activators. The agent Z`fix application in the in straw bedding has positive influence on the barn's microclimate, pig manure quality and fermentation process rectification (temperature, humidity and ammonia production). Different agents' applications in pig barns were monitored and verified [3;4].

Agriculture is also a major producer of ammonia. It is estimated that 80-95 % of total emissions in Europe come from agricultural practices, with emissions from animal excrement being over 80 % and emissions from the use of fertilizers less than 20 %. High levels of ammonia have been found mainly in areas with intensive livestock farming in Europe. The Gothenburg Protocol sets the ceiling and levels to reduce ammonia emissions in housing systems by at least 20 %.

In combination with naturally ventilated housing systems, straw allows the animals to self-regulate their temperature with less ventilation and heating, thus reducing the energy consumption.

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Optimal straw amount plays an important role in pig manure production by affecting its absorption of urine and liquid manure in the barn, and its microbial activity (temperature and ammonia production) during fermentation and manure production. Gilhespy et al. [5] tested additional straw and its optimal amount in straw bedding with regard to the ammonia emission production in fattening pigs. The only significant difference in emissions between the treatments was found between 4.0 kg·LU$^{-1}$ and 2.0 kg·LU$^{-1}$, whereby emission increased by 39 % in response to halving the daily straw use. In the Czech Republic, a daily amount of straw ranges from 8.0 to 15.0 kg·LU$^{-1}$ in the straw bedding.

There are problems with application of untreated pig manure to the soil today. Too many pigs carry pathogens or parasitic worms resulting in a host of organisms in their manure. In order to reduce these risks as well as the loss of nutrients, manure could be made using composting (field dunghill), or using combination of manure production (barn) treated with agent Z`fix application. Armstrong et al. [6] tested the application of composted pig bedding litter. Temperature during fermentation is an important selective factor for the development of microbial (e.g. actinobacterial) populations in compost [7], and high temperature can result in the absence of the mesophilic microbial community in manure [8].

The aim of this paper is to verify the Z`fix agent's efficiency from the viewpoint of the barn condition parameters, of the pig manure quality and of its nutrient content, and mainly from the viewpoint of decrease in ammonia emissions.

## Materials and methods

In the Czech Republic at a representative farm with fattening pig production the monitoring was conducted on the total number of 84 LU (1 LU = 500 kg L.W.) of fattening pigs (40-110 kg L.W.) from January 2018 to January 2019 . Total agricultural area of the farm was 1,153 ha (arable land), the main field crops being cereals, maize, alfalfa and sugar beet. Fattening pigs came from the farm's own breeding (cross breeder Large White × Danish Landrace). Total monitored annual pig manure production reached 700 tons.

On the farm, fattening pigs were fed with using automatic feeders in each pen. Complete compound feed mixture (all at dry matter basis) consisted of 80 % of cereal components (wheat, barley and corn), 16 % of protein components (soybean meal, rapeseed meal), and of 4 % of minerals, premix of vitamins, amino acids and trace elements in accordance with the Feeding Standards for pigs [9]. According to the Kjeldahl method (Kjeltec type 2200), a total of 15 feed samples were analysed during the monitoring period for the crude protein determination in a laboratory.

The fattening pigs were stabled in 2 barns (straw bedding) with 2 pens in both (40-70 ± 5 kg and 70-110 ± 5 kg L.W.). Cut straw was landed twice a week. The frequency of pig manure removal varied from 150 to 180 days. The barns on the farm were made from a lightweight structure with natural ventilation and open spaces (windows) equipped by roller shutters during bad weather conditions. The function of the Z`fix agent (PRP Technologies Comp., France) is based on the "Mineral Inducer Process" consisting of trace elements, magnesium and calcium carbonate, and the essential oils. In the first barn, the agent's application at the rate of 0.5 kg·LU$^{-1}$ was conducted weekly to the bedding (both pens) during the whole time of the monitoring [10]. The second barn was used as the control.

In a laboratory accredited for chemical analyses, the 80 collected samples of pig manure were analysed after drying and milling, or lyophilized according to the methods of the Central Institute for Supervising [11; 12]. Samples for qPCR analysis were lyophilized. DNA was extracted from 0.2 g of lyophilized composted manure using DNeasy PowerSoil Kit (Qiagen, USA) following the manufacturer's instructions. Before extraction each sample was spiked with the DNA of plasmid vector derived from pUC18, which served as an internal quantification standard for valuation of the yield efficiency and contamination with PCR inhibitors. Isolated DNA was quantified photometrically (Picodrop; Picodrop Limited, UK). SYBR-green assays were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories). Partial bacterial and fungal rDNAs were quantified by qPCR using 1108F (5' ATGGYTGTCGTCAGCTCGTG 3') and 1132R (5'GGGTTGCGCTCGTTGC 3') primers for bacteria, and FF390 (5'AICCATTCAATCGGTAIT 3') and FR1 (5'AICCATTCAATCGGTAIT 3') primers for fungi. DNA of pUC18-derivate (internal standard) was quantified by qPCR using SQP (5'GTTTTCCCAGTCACGAC 3') and SQPR2 (5'CTCGTATGTTGTGTGGAA 3') primers.

During 48 hours (per 1 point; data recording every 30 second) measurements of humidity, ammonia emissions and temperature were conducted at the height of 25 cm above the bedding surface at 5 points at the same time in each barn in 14-day intervals together with the manure sampling at these points. Measurements of the $NH_3$ emissions were carried out using the apparatus Data Logger $NH_3$ (Comp. Bauer Technics Ltd. CZ; $NH_3$ value 0-100 ppm ± 0.1 ppm; humidity 0-100 % ± 0.5 %; temperature value 0-200 ºC ± 0.1 ºC).

The following parameters were estimated: concentrations of $N\text{-}NH_4^+$ ($kg \cdot t^{-1}$), $N_{tot}$ ($kg \cdot t^{-1}$), $K_2O$ ($kg \cdot t^{-1}$), and of $P_2O_5$ ($kg \cdot t^{-1}$), pH, humidity (daily mean %), ammonia emissions (mean of maximum ppm value), maximal temperature (mean of maximum ºC) of bedding reached by fermentation in the barn, amount of available $N_{tot}$ ($kg \cdot t^{-1}$ and $EUR.t^{-1}$), total and daily consumption of straw ($kg \cdot LU^{-1}$) for pig manure production were determined according to the certified methodology [10].

For the sake of elimination of pathogens and parasitic worms in pig manure, every batch of pig manure removed from the barn was composted over 4-6 months prior to application in a field (mixed with fresh soil at a ratio of 8-10: 1, deposited in blocks and covered in field heaps). Maximal mummification takes place at temperatures of 40-60 ºC and at an optimal humidity of 60-80 %. Compost remixing was performed every 4-6 weeks. The maximal temperature (mean of maximum ºC) was monitored during the fermentation process of composting pig manure (dunghill).

Data were analysed (level of significance $P < 0.05$) with utilization of software R (R Foundation for Statistical Computing, Austria) and software Microsoft Excel (MS Corp., USA) for the whole period of the monitoring. The general linear models were used for statistical evaluation of the data, where the agent application factors, ammonia emission and concentration of the chosen nutrients were assessed.

## Results and discussion

Table 1 displays the mean values of chemical analyses and differences between the tested samples of pig manure, i.e. between the manure treated with the Z`fix agent and the control. Within the fattening pig system using straw bedding (without biological agent application) Klír et al. [13] reported results for the mean content of $P_2O_5$ at 8.8 $kg \cdot t^{-1}$, the mean content of $N_{tot}$ at 8.5 $kg \cdot t^{-1}$ and the mean content of $K_2O$ at 7.0 $kg \cdot t^{-1}$ in the pig manure.

Table 1

**Chemical analyses of tested samples of pig manure – barn (2018)**

| Parameter | Agent Z`fix application | Control | Index | *P* value |
|---|---|---|---|---|
| Tested samples, n | 40 | 40 | - | - |
| Dry matter, n | 24.1 | 24.1 | - | - |
| N-total, $kg \cdot t^{-1}$ | 9.30 | 6.80 | 1.37 | 0.0244 |
| $N\text{-}NH_4^+$, % | 0.83 | 0.54 | 1.54 | - |
| $P_2O_5$, $kg \cdot t\text{-}^1$ | 9.20 | 6.30 | 1.46 | 0.0389 |
| $K_2O$, $kg \cdot t^{-1}$ | 7.60 | 6.90 | 1.10 | 0.0488 |
| C:N, ratio | 21.1:1 | 23.3:1 | 0.82 | - |
| $NH_3$, maximum mean value ppm | 10.20 | 16.30 | 0.62 | 0.0215 |
| Tmax, maximum mean value ºC | 20.00 - 27.00 | 30.00 - 42.00 | - | - |

Note: $NH_3$ (measured 25 cm above the bedding during 24 hours per 5 points in each barn);
Tmax (measured in the collected samples per 5 points in each barn);
Index (the ratio of the value with Z`fix treatment and the value of the control sample).

By the controlled biological fermentation process of manure, by the optimal amount of straw while reducing fermentation losses (barn), and by better manure management during its composting, storage (dunghill) and field application, the higher manure content of the monitored nutrients was proved with the application of the Z`fix agent compared to the untreated control. This is likely to result from the chemical composition of the Z`fix agent and its controlled application. The production of high quality composted pig manure and its application to the soil corresponds also to the recommendations of other authors [1;2;14].

With Z`fix treatment, a significant decrease of maximum levels of mean ammonia emissions by 38 % (control: 16.6 ppm) was determined compared to the control. The maximum mean of humidity in the barn also decreased from 73-75 % to 63-66 % , as did from 30-42 ºC to 20-27 ºC the maximum mean of the pig manure fermentation temperature (straw bedding), whereas the pH value of the pig manure content rose from 8.7 to 9.1. Practical differences during 24 h monitoring of $NH_3$ emissions (means of 10 measurements at 5 points in 2018) are displayed in Figure 1.

Palkovičová *et al*. [3] found the decrease of ammonia emissions by 20-60 % with the utilization of biological agents; whereas in another study [4], the emissions decreased by 24 %. The Gothenburg Protocol sets the ceiling and levels to reduce ammonia emissions in housing and feeding systems by at least 20 %. In our study, the application of the Z`fix agent brought good results when used in the barns. As a decisive environmental aspect, it is necessary to ensure that the concentrations of nutrients in the animal diet correspond to those recommended by the norms for the individual categories of animals. In laboratory tests [15] monitoring ammonia emission production the pH value, temperature during the manure fermentation process and using wheat straw two peaks were confirmed corresponding to the population dynamics of proteolytic bacteria and amino acid-degrading bacteria respectively.

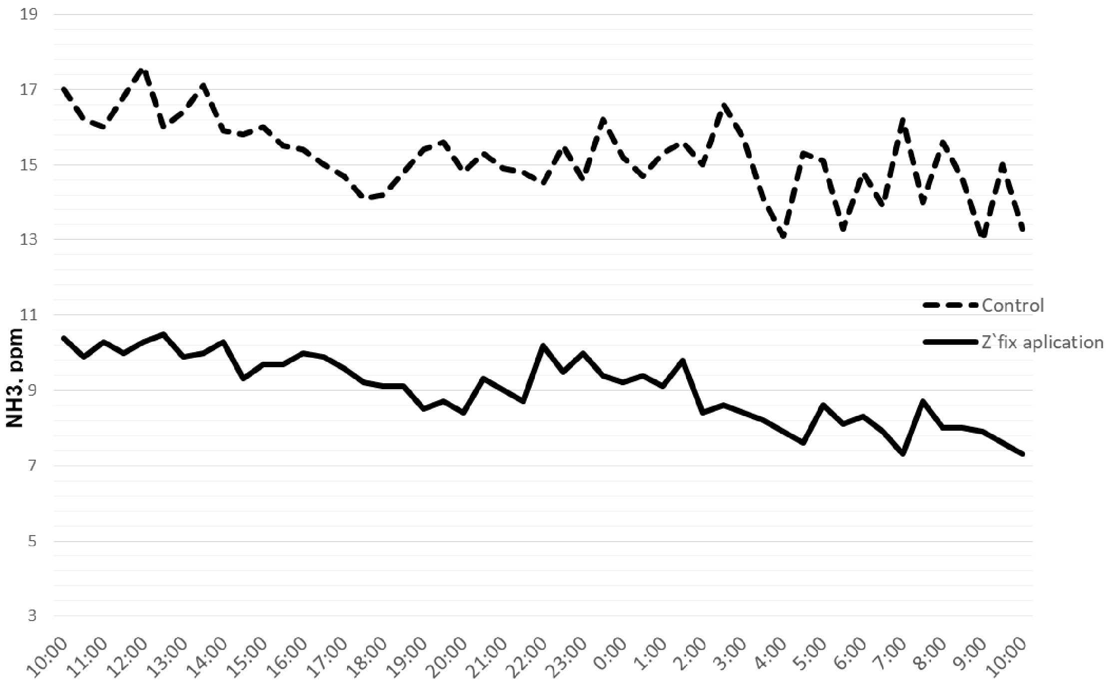


Fig. 1. **Practical monitoring of 24 h $NH_3$ emissions in barn (with Z`fix agent application and control; means of 10 monitoring at five points during monitored period)**

With the use of the Z`fix agent, a daily consumption of straw decreased by up to 30 % to the level of 6.3-7.7 kg·LU$^{-1}$ compared to the control level of 8.0-15.0 kg·LU$^{-1}$. During each monitoring period (150-180 days) with Z`fix agent treatment, improved cleanliness of animals, better value of welfare and bedding stability were observed. Gilhespy et al. [5] found significant difference only in ammonia emissions between the straw levels of 4.0 kg·LU$^{-1}$ and of 2.0 kg·LU$^{-1}$, whereby emission increased by 39 % in response to halving the straw use.

The composting process of pig manure production is one way to reduce loses of nutrients and to eliminate undesirable organisms in manure. Our results confirmed that the manure produced by composting (field dunghill) and the combination of manure production (barn) with the Z`fix agent application have positive influence on the fermentation process, on manifestation of desirable microorganisms, and on nutrient loses (dunghill). Armstrong *et al.* [6] tested the application of composted pig bedding litter. Bacteria in the composted manure with the agent Z'fix application (app. 12.4 log 16S rDNA.g$^{-1}$) were more than 3-fold more abundant as compared to the values (app. 12.2 log 16S rDNA.g$^{-1}$) in the control after 36-day fermentation. Fungal biomass in the manure with the agent Z'fix application (app. 8.8 log 18S rDNA.g$^{-1}$) was more than 5-fold higher as compared to the values (app. 8.1 log 18S rDNA.g$^{-1}$) of the control in the respective state of fermentation.

The amount of both bacterial and fungal biomass (determined as a number of copies of 16S rDNA and 18S rDNA per gram of dry biomass) was significantly higher in the manure with the agent Z'fix application as compared to manure without agent application (control). A possible explanation may be a significantly lower temperature (40-45 ºC) during the fermentation process of the composting manure with the agent Z`fix in comparison with the temperature range (50-60 ºC) during the fermentation without agent application. It was evidenced that the temperature was an important selective factor for the development of microbial (e.g. actinobacterial) populations in composts [7] and high temperature could result in the absence of the mesophilic microbial community in manure [8].

In the experiment, the value of the composted pig manure calculated under methodological patterns set by [8] attained the level of 27.02 EUR.t$^{-1}$ (Z`fix agent application) and the level of 21.92 EUR.t$^{-1}$ (control). The total amount of the available N by its 65 % utilization (medium soils) can be increased by 1.63 kg·t$^{-1}$ $N_{tot}$ in the case of the composted pig manure with the agent Z`fix application. With the usual manure dose (20 tons per 1 ha), the saving in N represents a level of 32.6 kg applied in the form of mineral fertilizers. When applied for winter rape, this value represents the increase of hectare yield by 0.4 tons.

## Conclusions

1. Based on the findings (2018) focused on pig manure production (straw bedding), the Z`fix agent application had a positive influence ($P < 0.05$) on the pig manure quality, on the course of the fermentation process, on the barn conditions (animal welfare, humidity, ammonia production and others), and on daily consumption of straw (reduction by 30 % to 7.7 kg·LU$^{-1}$).
2. With the use of the Z`fix agent compared to the control, increased contents of the following compounds were found: N-$NH_4^+$ (by 54 %), N (by 37 %), $K_2O$ (by 10 %), $P_2O_5$ (by 46 %). On the other hand, the C:N ratio decreased from 23:3 to 21:1.
3. In the barn, a significant decrease of the maximum levels of mean ammonia emissions (by 38 %) and of maximum value of humidity (by 10 %) were determined compared to the control.
4. Compared to the control, the pH value increased from 8.7 to 9.1, whereas the maximum values of temperatures reached by the pig manure fermentation process decreased on the other hand from 30-42 ºC to 20-27 ºC (straw bedding), resp. from 50-60 ºC to 40-45 ºC (composting).
5. During manure production, fermentation and composting processes, the amount of both bacterial and fungal biomass indicated putative higher biological activity with the agent Z`fix application.
6. The total available amount of N by its 65 % utilization (medium soils) can be increased by 1.63 kg·t$^{-1}$ $N_{tot}$ in the case of the composted pig manure with the agent Z`fix application.
7. In agreement with the requirements posed by the Gothenburg Protocol, the application of the Z`fix agent with its contribution to ammonia emission reduction and pig manure production enhancement is desirable.

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