

## Hungarian University of Agriculture and Life Sciences

## Szent István Campus

## Agricultural Biotechnology (Animal) MSc Course

## THE EFFECT OF SEPARATION TECHNOLOGY ON ANIMAL MANURE ON ANTIBIOTIC RESISTANT BACTERIA AND HORMONAL ACTIVITY

Primary Supervisor:

Dr. Edit Kaszab

associate professor

Author:

Nur Hafizah Binti Mohd Yusoff

DDXJSA

Institute/Department:

Institute of Aquaculture and Environmental Safety, Department of Environmental Safety

Gödöllő 2023 With of the rapid expansion of agricultural production, side effects to the environment such as the increase of animal wastes, worsen soil acidity and limited animal wastes management are dramatically increasing. Several solutions have been implemented to reduce the impact of animal wastes to the environment and public health such as composting and bedding recovery systems. Composting is considered as one of the possible methods for the management of organic waste such as sewage sludge, or manure due to its capability to reduce the number of microorganisms that present in the waste products. Moreover, the additional of the organic matter to the farmland soil can improves the structure of the land and health of the soil as it can retain the moisturisation and providing beneficial nutrients to the plantation. Another option is the usage as bedding for the animals promoting their welfare.

However, extensive use of compost may cause increase the accumulation of bacterial growth especially inside the soil residues. This can gradually lead to the antibiotic resistance phenomenal to the soil microorganisms. Antibiotic resistance bacteria are not a new problem to the agriculture sector but in fact become one of the biggest challenges to this industry as it become more resistant to the antibiotic treatment and may cause adverse effects to the human health. Antibiotic resistance is a condition where bacterial strains are no longer affected by applied antibiotic, neither undergoes bacteriostatic nor bactericidal. If these problems left untreated, it may cause the health treatment become more limited and jeopardized to the environment. Hence, it is important to monitor antibiotic resistance genes for better control of the antibiotic resistance bacteria (ARB).

In this study, we examined a newly developed system known as Bedding Recovery Unit (BRU) that was introduced as another initiative for better management of animal liquid manure. This technology uses hydraulic systems to separate liquid and solid wastes. Liquid manure from this treatment will be supplied for irrigation of plantation while solid manures are used as bedding inside the farm. The use of compost as the bedding systems not only cost-effective, but also environmentally friendly as it can reduce the wastes material as well as lower the pollution caused by the usage of synthetic bedding such as rubber mats or straw. It is also more comfortable for the dairy cow and reduce the irritation on the body. The treated solid wastes using BRU systems also claimed to be able reduce or remove the emergence of pathogenic microorganisms due to the intensive composting process and hence make it safer to the farm animals and environment.

However, the activity of the system in the reduction of antibiotic resistant (AR) bacteria, antibiotic resistance genes (ARGs), or in the reduction of hormonal (ED) activity has not been investigated in details. In this study, we collected manure samples from two different dairy

farms located in Hungary, prior and after BRU treatment, to evaluate the effectiveness of this system in the removal of AR bacteria, ARGs and ED effect. Samples were analysed in the laboratory of the Institute of Aquaculture and Environmental Safety with traditional cultivation and molecular biological methods; ED effect was determined using a *Saccharomyces* bioreporter assay. In addition, antibiotic resistance test was done on five different bacterial isolates to study the antibiotic resistance on the chosen strains.

Based on our results, the colistin, carbapenem resistant microorganisms were fully removed in one of the BRU-treated samples where the BRU system was operating properly, however the hormonal activity after BRU treatment trended to be higher. In acetone extracted form, all raw and treated samples shown notable estrogenic activity with the maximum intensification between 374-659%, (where the estrogenic activity of the positive control E2 was measured to be 1175%). In case of BRU-treated samples (extracted with acetone), the androgenic activity was also significant with a maximum intensification of 120 and 101%, respectively (where the positive control DHT's intensification was 269%). At the same time, water extracts of the examined samples did not show significant hormonal activity. Samples with hormonal activity showed as notable difference in the composition of cultivable bacteria with dominance of Comamonas species, such as C. testosteronii and C. kerstersii. Further investigation should be proposed to identify whether the hormonal activity is originating from naturally produced hormones or influenced by the endocrine disruptors (ED). Based on antibiotic resistance profiling, one of the detected species originating from raw manure, Empedobacter stercoris was resistant to the examined Aminoglycosides (gentamicin, amikacin) and to colistin, and at the same time, it was only moderately sensitive to imipenem, a Carbapenem agent, which verifies the presence of ARBs in untreated manure and highlight the importance of manure treatment.