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Effects of different feed components and the treatment of the litter on the ammonia emission of equine urine

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Abstract – The main goal of our research was to study the effects of different feed components and the treatment of the litter on the NH_3 emission of equine urine. Four adult horses were used in the experiment. The basic diet consisted of 1500 g mashed oat, fed twice a day (morning and evening), and ad libitum hay. During the night the horses were kept separately in concrete floor boxes, the litter was wheat straw. The whole study was divided into 3 main periods. In the first period of the experiment the litter and the floor were treated with a probiotic supplement for two days in the evenings. In the second period of the experiment for 8 days the basic diet was supplemented with 40 ml of probiotic added to the oat. Before the third period of our study the horses were fed with their regular diet for two weeks. After that the oat was changed to a high-protein-value horse feed for 12 days. At the end of the second and third periods, the litter was treated again. Urine contaminated straw samples were collected after each period before and after the litter treatments from different places of the litter in the morning before the daily littering. From the samples, the dry matter content and the NH_3 emission were measured. Statistical analysis was carried out with linear modelling. According to our results, both the probiotic treatment of the feed and the high-protein-value horse feed decreased the urine NH_3 emission significantly ($p<0.05$). However, the NH_3 emission was only slightly influenced by the litter treatment. Only in the case of probiotic feed supplement could a decrease in emission be detected, but the result was not significant. There was no difference in the dry matter content of the samples. Based on our results, we can conclude that both the high-protein-value horse feed and the probiotic supplement applied in our experiment can be useful to decrease urine NH_3 emission by horses. The two-days litter treatment however seems to be too short to gain any positive effects.

Keywords – horses, probiotic supplement, high protein value horse feed, litter management, ammonia emission

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INTRODUCTION

Ammonia (NH_3) is a key air pollutant that can have significant effects on human and animal health and the environment. In agriculture, especially in the livestock sector, nitrogen is released into the environment in the form of ammonia. Much of the ammonia emissions from animal manure come from the hydrolysis of urea (Varel, 2006). Nitrogen compounds are converted to ammonia by microbial activity (Santoso, 1999), thus, ammonia emission results from the activity of aerobic or anaerobic bacteria (Zhang, 1991). Ammonia formed during the breakdown of unused amino acids absorbed from the intestine is excreted in the urine in the form of urea. The decomposition process of uric acid is carried out by the urease enzyme which catalyses the hydrolysis of urea into ammonia and carbon dioxide in the aqueous medium, allowing ammonia volatilization to the atmosphere (Such et al., 2021-a).

The highest ammonia emissions are in the livestock sector (Lekkerkerk, 1998). Within the sector, pig, poultry, and cattle farms are primarily responsible for this. (Dublecz, 2019) (Figure 1.). According to the latest Hungarian air pollutant inventory report, agriculture is responsible for 92% of the total national emissions, of which about 66% takes the animal production and the ammonia emitted from the manure (IIR, 2021). A small part of it comes from horse farms (less, than 2 %). The main reason for this is that there are fewer animals in horse farms in a given area than in other farms. The average protein requirement of horses is also lower than that of other animal species. Horses are mostly used for sport and work (Such et al., 2017). For high performance, the locomotor and respiratory systems need to be healthy. Because horses are most often kept in stables, the harmful effects of ammonia primarily affect horses and the people working alongside them. These effects slowly, over many years, result in serious, in many cases irreversible, diseases. Veterinary researchers have shown that ammonia levels in an average horse stall can exceed 200 parts per million (ppm) (The Horse.com, 2018). For humans, the U. S. Occupational Safety and Health Administration (OSHA) has set a 15-minute exposure limit for gaseous ammonia levels of only 35 ppm (Agency for Toxic Substances and Disease Registry, 2004). Ammonia inhalation, particularly at the high levels known to exist in the stalls, daily, results in inflammation and constriction (narrowing) of the airways and mucus accumulation. Initially, horses with ammonia-induced airway disease have decreased stamina and athletic prowess and tend to cough during exercise (Holcombe et al., 2006). Ammonia accumulating close to the ground in closed enclosures (boxes) poses a serious threat to resting, sleeping horses (Pratt et al., 2000; Fleming et al., 2009). Therefore, it is recommended to place the ventilation openings close to the ground. In recent years, the unique value of elite sport horses has risen significantly, by as much as several million euros (Horse & Hund, 2021). Especially these highest value horses spend most of their time in the stable. Respiratory damage caused by the inhalation of ammonia (Bodó, Hecker, 1992) can result in a significant loss of performance as well as a reduction in the life of the horse, causing severe economic damage to owners. Reducing the accumulation of ammonia is therefore very important in horse keeping.

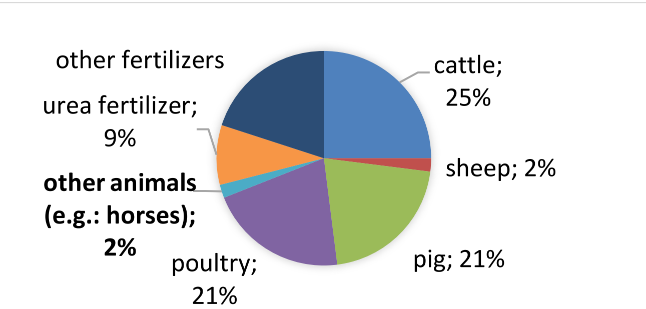

Figure 1. Composition of ammonia emissions from Hungarian agriculture (2013) Source: Dublecz (2019).

To reduce ammonia emissions, one of the (most important) options that can lead to animal health and performance, the best possible use of its capabilities, is the right amount and quality of protein in feed rations, including the amino acid composition most similar to the animal's needs (Mahan and Howes, 1995). Among our farm animals, the horse’s protein requirement is the lowest. Meyer found in 1987 that a maximum of 2g of protein per kg of body weight is ideal. Higher amounts increase water requirements, and ammonia accumulating in the blood increases the risk of enterotoxaemia. An increase in nitrogen excretion involves more energy consumption, and the ammonia content of the stable air also will be higher. It poses a serious health risk to horses. The urea content of the urine increases during feeding with a higher protein content diet (Weir et al., 2017). Horse feeds based on the defined age, health status and work performed are very helpful in feeding the required amount of protein. In these feeds, proteins are formulated based on recommendations and research, and the amino acid composition of the selected ingredients is also taken into account. Today, most essential amino acids are available in crystalline form. These supplements allow the feed to contain the amino acid composition most similar to the animal's needs. It is important to follow the recommended amount when feeding. The first limiting amino acids for horses are lysine and threonine (Graham-Thiers and Kronfeld, 2005). To improve the digestion of horses, it is recommended to feed the daily portion of grain in several portions (Frape, 2011).

Another option is to reduce ammonia emissions and improve the digestibility of the diet by feeding certain feed supplements. From these, natural ingredients, probiotics, prebiotics, enzyme supplements, and other herbal preparations are recently the most important (Wenk, 2000, Bartos et al., 2015). Probiotics are defined by the World Health Organization as “microorganisms” administered in a live form and in adequate amounts to improve the health of the host. Probiotics can kill pathogenic microorganisms with the help of their antimicrobial compounds such as bacteriocins and organic acids. They improve the stability of the gastrointestinal microbial environment, thereby preventing the binding and colonization of pathogens, stimulating intestinal-induced immune responses, improving the digestion and absorption of nutrients (Such et al., 2021-b). Vladár's (2011) experiment with horses also proved this. As a result of the experiment of Such et al. (2017), it can be concluded that some probiotics can be successfully used in the feeding of horses to improve digestibility.

Bacteria in the environment break down urea into ammonia, so the quality of the litter is also can be very important. According to Fleming et al. (2008), wheat straw litter is the most unfavourable, and straw pellets have the best ammonia-binding effect. In addition, so-called littermates have become more common, which may allow less ammonia to be generated. Pratt et al. (2000) measured 25% less ammonia in a month treated with an ammonia-absorbing compound compared to the control (untreated) condition. The mechanism of action of litter managers is to keep the litter dry, to create a microflora adequate to the beneficial microorganisms, to displace harmful or pathogenic microbes, to displace urease-producing bacteria, to reduce rot processes thus reducing the rate of ammonia and other gas production.

The main goal of our research was to study the effects of different feed components (a commercially available probiotic supplement developed for animals and a high-protein-value horse feed) and the probiotic treatment of the litter on the NH_3 content of equine urine and the ammonia emissions.

MATERIALS AND METHODS

Table 1. Layout of the experiment: sc-sample collection (morning); lt-litter treatment (Greenman Animal) (evening); ps-probiotic supplement (Greenman Probio); hf-horse feed (Pavo 18Plus)

Days 1 and 2	Day 3	Day 4	Day 5	Day 6-12	Day 13	Day 14	Day 15
sc	lt	*sc*, lt	*sc*	ps	*sc*, ps	*sc*, ps+lt	*sc*, ps+lt
Day16	**Day 17-30**	**Day 31-32**	**Day 33-40**	**Day 41**	**Day 42**	**Day 43**	**Day 44**
sc	break	hf+ oat 1:1	hf	*sc*, hf	*sc*, hf+lt	*sc*, hf+lt	*sc*

The experiment (Table 1.):
Four adult horses (averages weight 500 kg) were used in the experiment. The basic diet consisted of 1500 g mashed oat, fed twice a day (morning and evening), and ad libitum high-quality mixed grass hay (on average 1.5 kg/100kg BW). During the night the horses were kept separately in concrete floor boxes (3x4 m), the litter was wheat straw. The whole study was divided into 3 main periods. In the first period of the experiment the litter and the floor were treated with a probiotic supplement (Greenman Animal), according to the manufacturer's recommendations, mixed with water in a ratio of 1: 1 (on average 1 ml/m2) for two days in the evenings. In the second period of the experiment for 8 days the basic diet was supplemented with 40 ml of probiotic (Greenman probio) added to the oat. Before the third period of our study, the horses were fed with their regular diet for two weeks. After that, the oat was changed to 1500 g high-protein-value horse feed (Pavo 18Plus) for 12 days. At the end of the second and third periods, the litter was treated again. Urine contaminated straw samples were collected at the beginning of the experiment and after each period, before and after the litter treatments in two consecutive days (2x4 samples/sampling). Sampling was made from different places of litter in the morning before the daily littering.[1] From the samples, the dry matter content and the NH3 emission were measured. The average crude protein consumption[2] was 650g / day when feeding oats and 690g / day when feeding horse feed, which corresponds to the animal's maintenance need.

Composition of the products used in the experiment

Probiotic Litter Treatment (Greenman Animal): water, probiotic technology (*Bacillus* sp., *Bifidobacterium* sp., *Lactobacillus* sp., *Lactococcus* sp., *Rhodopseudomonas* sp., S*accharomyces* sp.), bio sugar cane molasses.

Composition and nutrient content of the Probiotic supplement (Greenman ProBio): chlorine-free water, bio sugar cane molasses, lactobacillus culture (*Lactobacillus casei* ATCC 7469, *Lactobacillus plantarum* ATCC 8014)> 3x105 CFU/ml, yeast (*Saccharomyces cerevisiae* IFO0203) <1x106 CFU/ml, zeolite and sea salt.

Nutrient content: crude protein 0.15%; crude ash 0.5%; sugar 2%; calcium 460 μg/g; magnesium 190 μg/g; phosphorus 40 μg/g.

Composition and nutrient content of the horse feed (Pavo 18Plus).

Nutrient content: DE 12.1 MJ/kg, digestible protein 13%, crude protein 16%, crude fat 7.5%, crude fibre 15%, acid soluble ash 7.7%, sugar 5%, starch 17%, Lysine 7.5g, methionine 2.1g.

Mineral content: calcium 0.8 %, phosphorus 0.5 %, sodium 0.4 %, potassium 1.2 %, magnesium 0.5 %, copper 55 mg, iron 180 mg, zinc 215 mg, manganese 160 mg, selenium 0.6 mg, iodine 1.0 mg.

Vitamin content (per kg): vitamin A 23.400 NE, vitamin D3 3000 NE, vitamin NE 460 mg, vitamin B1 25 mg, vitamin B2 30 mg, vitamin B6 16 mg, vitamin B12 250 μg, choline 250 mg, D-biotin 600 μg, folic acid 14 mg.

Composition: soy hulls, spelt lucerne, soybean extraction meal (vapour heated), wheat bran, puffed barley, molasses, oat bran, rapeseed meal, wheat, soybean oil, apple pomace, linseed, corn gluten feed, sodium chloride, pea flakes, carrot flakes, magnesium oxide, sunflower meal, chicory, linseed oil.

Measurements

Samples were sealed in double nylon bags and stored at -20ºC until the start of the assays. After release, 15 grams of each sample was weighed and wrapped in aluminum foil, making sure that the foil packages and the openings formed on the foil were nearly the same sizes. Measurement was carried out at the Laboratory of the Hungarian University of Agriculture and Life Sciences (Georgikon Campus, Keszthely, Hungary). Samples wrapped in foil were placed in five-litre plastic

[1] Only with the manure or urine contaminated litter are removed.

[2] Calculated on the average crude protein content of the daily ration (average 7.5 kg hay; 1.5 kg oat or horse feed).

balloons and kept at room temperature for one hour. Measurement of the amount of ammonia was done with a Draeger X-am 5600 apparat, similar, to the method of Santoso et al. (1999). No further measurements were taken, the 1-hour measurements were close to the maximum measurement range for several groups of samples.

To determine the dry matter content, 5 g of the sample was weighed into a previously measured jar. For each sample, two parallel measurements were performed. The samples were dried in a drying case at 130 ˚C for two hours (to constant weight), then removed and left to cool in exsiccator. The weight of the samples was measured together with the jar and the dry matter content of the samples was calculated.

Statistical analysis:

Statistical analysis was carried out with linear modelling. In the model, the outcome variable was ammonia emission. Litter treatment, horse feed, and probiotic were the explanatory variables as 0/1 dummy variables. The significance ($p<0.05$) of the explanatory variables was examined by t-test. Analysis was made with SPSS version 25.0.

RESULTS

The probiotic and horse feed were readily consumed by the animals, and no feed refusal occurred.

The litter treatment did not significantly affect the rate of ammonia emissions. (Figure 2.).

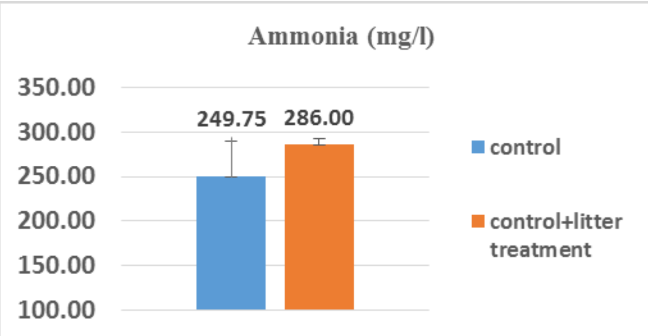

Figure 2. Urinary NH_3 emissions as a result of the litter treatment.

When replacing oats with a diet containing high biological value protein, the amount of ammonia emission was significantly reduced compared to the control values. However, no significant difference was found in the treatment of the litter in this case either (Figure 3.).

Probiotic supplementation, like horse feed, resulted in a significant reduction in urinary ammonia emissions. When used in combination with the litter treatment, a further reduction tendency was observed, although the difference was not statistically significant (Figure 4.).

Both feeding high-protein-value horse feed and probiotic supplementation resulted in significantly lower ammonia emission than the control diet. The litter treatment caused a slightly declining tendency only in the case of probiotic supplementation. Performing the study with four horses, presumably due to the relatively high standard deviation observed for a small number of items, the result was not statistically verifiable (Table 2.)
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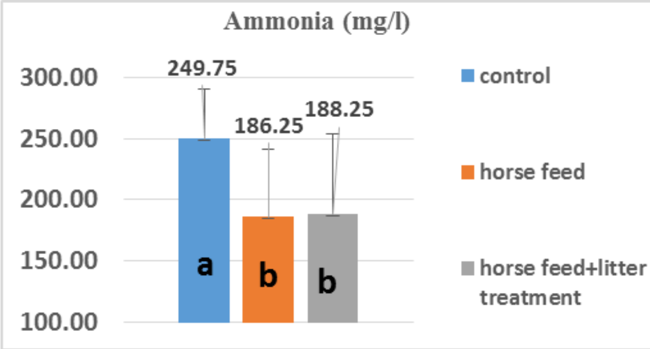

Figure 3. Urinary NH_3 emissions as a result of horse feed and litter treatment. [ab] Averages with different letters differ significantly ($p<0.05$).

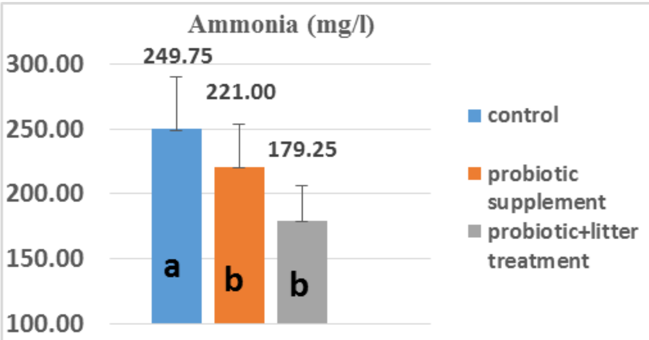

Figure 4. Urinary NH_3 emissions as a result of probiotic supp-lement and litter treatment. [ab] Averages with different letters differ significantly ($p<0.05$).

Table 2. Urinary NH_3 emissions as a result of the treatments. [ab] Averages with different letters differ significantly ($p<0.05$).

	NH_3 emission mg/l (means +/- dispersion)
control (oat + hay)	249.75 +/- 40.2 [a]
litter treatment	285.00 +/- 6.7 [a]
probiotic	221.00 +/- 32.4 [b]
probiotic and litter treatment	179.25 +/- 26.9 [b]
horse feed	186.25 +/- 55.4 [b]
horse feed and litter treatment	188.25 +/- 65.8 [b]

The dry matter content varied between 31-40% in each group. No statistically significant differences were found between the values obtained. This confirms that the dry matter content of the samples did not affect the results obtained with ammonia emissions.

DISCUSSION

In the case of feeding oats and ad libitum grass hay, the litter manager did not affect the amount of ammonia emissions. One reason for this is presumably that due to the weaker protein utilization, the amount of nitrogen excreted in the urine was particularly high, which could no longer be substantially compensated by the two-day litter treatment, and the decrease in urease-producing microbes was not sufficient.

From the results of the experiment, it can be concluded that feeding horse feed can significantly reduce the amount of ammonia excreted in the urine. This is mainly because these forage feeds not only contain the right amount of protein, but these proteins have the amino acid set that best meets the needs of the animal. This significantly improves protein utilization. On the other hand, high-value horse feeds contain fermentable carbohydrates, like molasses, which can by mammals reduce ammonia emission in the intestines, because ammonia formed in the post-small intestinal tract is converted in a greater proportion to bacterial protein. As a result, less ammonia is absorbed from the caeca and recirculated to the liver, which reduces the synthesis of uric acid (Aarnink and Versteggen, 2007)

Supplementary feeding of the probiotic developed for animals had a beneficial effect on the amount of ammonia measured in the urine, due to the fact that the probiotics can significantly improve the digestibility of nutrients, including protein (Anadón et al., 2006). This is also supported by the results of a previous experiment with the probiotic used in our current research (Such et al., 2017). A decreasing trend in the amount of ammonia can be detected when the probiotic is fed with the litter treatment at the same time. Among the beneficial effects of probiotics is that they help maintain the health of the gut flora, and the litter manager presumably contributed to the more efficient growth of beneficial bacteria entering the litter with the manure. Although the effect could not be statistically verified, mainly due to the small number of elements, it may be recommended to use probiotic supplementation and litter treatment together to achieve a better result.

The applied litter manager product has been used for a long time in the stables. Although there are no specific measurement results, our observations show that using the product according to the manufacturer's recommendations has significantly improved air quality in the applied area. The previously mild smell of ammonia, which had been experienced regularly, disappeared after a few days, and the well-being of the horses also improved. When the product was mixed into the drinking water daily, the mild cough symptoms of the horses also moderated after a few days (observation after one week use the litter manager). It can therefore be assumed that the beneficial effects of the litter manager can be felt after a longer period (min. 1 week) of application in the case of basic diet or horse feed, too. When the product is fed together with the applied probiotic supplement, by a higher number of elements the reduction in ammonia emissions is most likely to be statistically justified even after two days of treatment.

In the case of horses, the ammonia emissions have a lower environmental impact compared to other animal species, where production takes place on farms with large numbers of animals. The danger to horses and the people working around them is even greater. It is important to mention that careful feeding is not enough to eliminate this, it is definitely recommended to make the housing conditions as ideal as possible for both horses and people. The structure of the stable is extremely important, especially in terms of ventilation. It is important to avoid keeping horses in completely enclosed boxes for most of the day. It may be advisable to paddle the horses throughout the year so they spend less of their time in the stable box, resulting in a reduction in the ammonia load. Feeding protein with more accurately meeting the amino acid requirements of horses (high-protein-value horse feed) or possibly using a probiotic supplement developed for horses, using the adequate litter. Fleming et al. (2008) proposed straw pellet instead of wheat straw and probiotic litter management (min. 1 week) are highly recommended to maintain our horse's health and long-term balanced performance.

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