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Effect of yucca and probiotic supplements on ammonia levels in rabbit farms

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Abstract: Rabbit breeding is a highly intensive production industry. Rabbit meat is characterized by specific taste and qualities. Due to its composition, it is considered to bring many health benefits to the people. The microclimate in rabbit farms is critical to animal health, productivity and welfare. In order to achieve optimal production from rabbits, suitable environmental conditions must be created by controlling temperature, humidity, lighting, noise, air quality, water and food. In recent decades, the use of biologically active supplements in different branches of animal husbandry has increased. Biologically active supplements have natural origin, are safe and have proven positive effects on farm animals. They are easy to grow, and are also a renewable and sustainable source of beneficial ingredients in animal nutrition. Such supplements are probiotics, ferments and medicinal herbs.

Biologically active substances are known to restore and maintain the balance of intestinal microflora, to improve the functions of immune system and to improve the overall health of farm animals. Another desirable effect of the diet supplements, such as the medicinal plant *Yucca schidigera* supplementation and different types of probiotics, is the ability to reduce ammonia levels in the produced manure. Diet supplements are easily applicable, by adding the extracts to the fodder or bedding of farm animals.

Keywords: ammonia levels; yucca; probiotics; rabbit farms

INTRODUCTION

Agriculture is an industry, whose development is essential for the people. One of its subsectors, which is characterized by highly intensive production, is rabbit breeding. In order to achieve maximum economic efficiency, it is necessary for farmers to have knowledge about the physiology, nutrition and diseases of this type of farm animals.

Special attention should also be paid to rooms and equipment, and last but not least, to the possibilities for the realization of the production. In general, the New Zealand White, Californian rabbit breeds and the Tsika hybrid, which has become increasingly popular in the last 15-20 years, are the most widespread rabbit breeds worldwide. These are the so-called „broiler“ type, which is characterized by high precocity, strong constitution, resistance to diseases, high litter size, as well as very good meat qualities and good slaughter yield (National Agricultural Advisory Service, General Directorate „Agriculture advisory“, 2022).

Rabbit meat is characterized by exceptional nutritional quality. It is a diet meat with low levels of fat, cholesterol and sodium. It contains proteins with high biological value and high levels of potassium, phosphorus, selenium, iron and vitamin B12. The introduction of natural biologically active ingredients to the rabbit fodder improves the quality of rabbit meat (Pogány Simonová et al., 2022).

Despite the good climatic conditions and the breeding traditions, the development of rabbit breeding is poorly developed in Bulgaria, due to the presence of number of difficulties. Most important problems are the poor professional qualification of rabbit breeders, outdated equipment, high production costs that often exceed purchase prices, lack of stable export markets and partners, lack of direct subsidies for rabbit farmers, insufficiently developed scientific potential to ensure the sector, as well as insufficient management of the Bulgarian market.

The breeding conditions are extremely important for farm animal's welfare. Creating a suitable and comfortable environment directly affects animals health, growth and productivity. Improving the microclimate in livestock facilities can be achieved through various methods, such as maintaining a constant temperature, provide enough/good ventilation to ensure good air quality, maintaining good lighting (regime) and providing good bedding. Nowadays automated systems allow maintaining of appropriate microclimate parameters with minimal human labor and high energy efficiency (Amber et al., 2004, Flores-Velázquez et al., 2017).

Rabbits are particularly sensitive to high concentrations of ammonia in the air, which significantly increases the death-rate (Colina et al., 2001). Studies confirm that increase in the cases of pneumonia in rabbit farms is directly related to the increase of ammonia concentration in the air. In animals raised in such conditions different damages to the respiratory, nervous, digestive, immune and reproductive systems were observed. (Cui et al., 2021a; Cui et al., 2021b; Flores-Velázquez et al., 2017; Dyavolova et al., 2013; Moneva et al., 2016). The acceptable level of ammonia in the air is 0.005 mg/l, acceptable level of carbon dioxide - 0.1%. (Order No. 44 of April 20, 2006 On the veterinary medical requirements for livestock facilities).

Ammonia gas emissions in rabbit farms can be successfully reduced through use of natural biologically active supplements. Supplements with plant origin incorporated in the feed can easily be implemented in animal breeding. They are safe and have proven positive effects in all farm animals. Plants from which the supplements are derived are easy to grow which makes them a renewable and sustainable source of beneficial ingredients for animal nutrition. The plant *Yucca schidigera* is able to reduce the concentration of ammonia in the air of barns and the odor of ammonia from manure (Adegbeye et al., 2019).

Probiotic supplements are a modern way of improving and maintaining the health status of animals and an economically effective way to increase productivity and reproductivity of farm animals. Probiotics are preparations of beneficial microflora that suppress pathogenic gut bacteria and improve overall health; other probiotics do not contain living microorganisms, but create opitimal conditions for development of beneficial microflora. The mechanism of action of the probiotics is to colonize the gut with beneficial microflora or to create a favorable conditions for the beneficial bacteria (Mancini et al., 2021).

Ammonia levels in rabbit farm

Although ammonia is a toxic molecule, intoxication is often overlooked in the diagnosis of diseases. Under intensive production, rabbits produce great amount of waste (Amber et al., 2004). Ammonia, carbon dioxide, hydrogen sulfide, methane, etc. are formed in unventilated rooms during the decomposition of feces, urine, bedding and feed residues. Their higher concentrations are poisonous to animals. The problem is less common in private farms where rabbits are raised in good hygienic conditions. Prevention of ammonia intoxication could be achieved by regular cleaning of cages, housing and good aeration (Flores-Velázquez et al., 2017).

Flores-Velázquez et al. (2017) emphasized that adequate ventilation is necessary to maintain air quality, remove excess heat and control humidity. The authors studied a total of 96 rabbits. They concluded that ventilation holes located 0.2 m above the ground and outlet holes 1.2 m above ground level improved air circulation in the area below the rabbit cages, leading to a reduction in ammonia concentration.

There are different stages of ammonia intoxication, depending on the duration of exposure and concentration of ammonia in the air. At the very beginning, typical symptoms associated with the disease in upper respiratory tract are observed: secretion from the nose and eyes, swollen eyelids and irritation of corneal surface of the eye, sneezing and snoring, rarely high body temperature.

The effect of ammonia inhalation on respiratory rate was investigated by Mayan et al. (1972). New Zealand White rabbits were exposed to 50 ppm and 100 ppm ammonia for 2.5 to 3 hours which significantly reduced respiration rate by 32.3% and 34.0%, respectively, compared to the animals in the control group. In the animals exposed to the higher ammonia concentration, an increase in urea nitrogen in the blood from 19.4 to 24.6 mg/100 ml ($P<.005$) and in CO_2 in the blood - from 14.3 to 18.9 meq/liter of plasma was found.

Ammonia is a heavy gas, which is toxic in high concentration. It is formed by bacteria that decompose the waste, urine and excrement under warm conditions. Ammonia intoxication is therefore more likely to occur in the summer than in the winter. Ammonia production was found to occur most rapidly under conditions of high humidity. Because it is an irritant gas reaching the epithelium of trachea or bronchi, at high concentrations ammonia can paralyze cilia, alter mucosal flow, and, in severe exposures, destroy the superficial layers of the epithelial lining (Camble and Clough 1976).

Matsumoto (1989) observed the effect of ammonia on the rapidly adapting pulmonary stretch receptor (RAR). The observations were made in spontaneously breathing rabbits, before and after their treatment with isoprenaline or atropine. The results showed that Inhalation of vapor from 15% ammonia solution increased the activity of RARs and this increased activity was not significantly altered by isoprenaline (50 μg/kg) or atropine (0.3 mg/kg). It was concluded that the ammonia-induced RAR stimulation results in a direct action of the endings.

High concentrations of ammonia vapors can radically alter the "normal" histological parameters of the trachea of apparently healthy animals. Hyperplasia of the tracheal epithelium depends on the amount of ammonia and the length of time the animals are exposed to it (Camble and Clough 1976).

Publications by Bulgarian authors also proved the negative influence of ammonia. According to Dyavolova et al. (2013a) rabbits typically excrete large amount of ammonia through urine. High concentrations of ammonia affect hematological parameters and inhibit oxygen binding to hemoglobin through influence on pH (Dyavolova et al.. 2013a; Dyavolova et al.. 2015; Moneva et al., 2016).

Prolonged exposure of rabbits to ammonia resulted in decline of basal plasma cortisol level in spring and increase of rectal temperature in summer. The rabbits had higher hematocrit value in winter and higher leukocyte count in summer (Dyavolova et al., 2013b).

Dyavolova et al., (2013a) investigated the effect of ammonia on some hematological indicators and the possibility of reducing its effect by adding pyridoxine. Pyridoxine stimulates hemoglobin production (Cartwright et al., 1944). Dyavolova et al. (2013a) found that high ammonia concentrations caused elevated levels of WBC and hematocrit values. These effects of ammonia intoxication were prevented by supplementation of pyridoxine. It was also observed that exposure to high air ammonia levels caused adrenal hypertrophy (Dyavolova et al., 2014).

The pathway hypothalamic-pituitary-ovarian (HPO) in rabbits as a response mechanism to ammonia exposure was investigated by Cui et al. (2021a) at Hebei Agricultural University (China). For this purpose, 90 female rabbits of the IRA breed at two months age were divided into 3 groups, and were exposed to different concentrations of ammonia - 3 ppm, 30 and 50 ppm. A relative increase in the rate of cell apoptosis was observed in the group exposed to 50 ppm - 8.15%, 0.79% and 0.092% in hypothalamus, pituitary gland and ovaries compared to the control group. The levels of the apoptosis-related proteins Bax, Bcl-2, Caspase-3 and Caspase-9 in the ovaries were also examined. The expression

levels of Bax and Caspase-9 were significantly increased ($P < 0.05$), while Bcl-2 was significantly decreased ($P < 0.05$) (Cui et al. 2021a). The authors concluded that ammonia caused a decrease in the number of primary and secondary follicles and an increase in the number of atretic follicles, indicating inequality of ovarian development and function. These results indicated that inhalation of ammonia is likely to damage the morphological structure of the hypothalamus, pituitary gland, and ovaries, causing disturbed hormone secretion and enhanced cell apoptosis (Cui et al. 2021a).

Another study by Cui et al. (2021b) aimed to evaluate the effect of ammonia exposure on growth, lipid metabolism and microbial flora in the cecum of rabbits. A total of 150 female rabbits (35 days old) were used, randomly divided into three groups. They were placed at 0 ppm (control group, CG), 10 ppm (LAC – low ammonia concentration) and 30 ppm ammonia (HAC – High ammonia concentration) respectively for a period of 28 days.

Estimating the effects of ammonia concentration, they found a reduction of average daily weight gain in the LAC group and HAC group compared to the CG. Also, the structural integrity of the cecum was corrupted and the thickness of the mucosa and serosa were significantly reduced in LAC and HAC.

The level of acetate, butyrate and propionate was reduced in the HAC group. As the concentration of ammonia increased, lipid metabolism deteriorated and the number of potentially harmful bacteria increased, reducing the abundance of beneficial bacteria and thus damaging the structural integrity of the cecum and reducing the concentration of Short Chain Fatty Acids (SCFA) (Cui et al . 2021b).

Many studies indicated the harmful effects of ammonia on the rabbit's health, which required the need of finding affordable and easily applicable methods to reduce ithe concentration in the air and rabbit manure. The implementation of food supplements as Yucca and Probiotics have a proven effect on reducing the levels of harmful gases in rabbit farms.

Yucca supplementation

One of the plant species that has proven positive effect on various aspects of health of farm animals is *Yucca schidigera*. Mojave yucca (*Yucca schidigera*) is a blooming plant originated from the desert regions of the United States, Mexico and the Caribbean Islands (Abdelsalam and Fathi, 2023). It is characterized by very high content of saponins and phenolic compounds with an antioxidant effect. Saponins are surface-active glycosides that exist in various forms and structures in plants. They are consisted of a fat-soluble nucleus that contains a steroid or triterpenoid structure and possess amphiphilic/hydrophilic and lipophilic properties (Adegbeye et al., 2019). The main phenolic compounds are trans-3,4',5-trihydroxystilbene (trans-resveratrol), trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene and yucaols A and C (Chrenkova et al., 2012). Because of this chemical composition the plant possess anti-inflammatory and anti-arthritic properties (Cheeke et al., 2006). Included as a supplement to the feed, this herb contributes for increasing the productive and reproductive characteristics of rabbits (Ashour et al., 2014). The authors investigated the effect of supplementation of *Yucca schidigera* extract (YE) to the diets of growing rabbits. Authors studied growth performance. Carcass characteristics, serum biochemistry and liver oxidative status. A total of 80 New Zealand White (NZW) rabbits at age of 5 weeks were randomly assigned to four treatments with four repetitions. The three experimental groups received different amounts of YE, respectively 200, 400 and 600 g kg^{-1} YE added to the basal diet. Rabbits which were fed with diet supplemented with YE did not have affected growth performance, but had improved immunity responses. Animals which were fed with diet supplemented with phytogenic additive had lower levels of ammonia in blood, Malondialdehyde (MDA) in the liver and increased hepatic antioxidant activities.

In other studies, the positive influence on digestion and nutrient retention has also been confirmed (Wang et al., 2023). A significant improvement in live weight, growth and feed utili-

zation was also found (Abaza and El-Said, 2005; Abdelsalam and Fathi, 2023). A positive effect of the yucca extract was observed on slaughter parameters of rabbits, intestinal morphology (Abdelsalam and Fathi, 2023; Wang et al., 2023), biochemical and morphological parameters of the blood, as well as on immunological status of the animals (Abdelsalam and Fathi, 2023). In addition to the stated effects, *Yucca schidigera* extract has shown to reduce the ammonia concentration in the air of barns and the ammonia smell of the manure. In ruminants, yucca can reduce emissions of methane, nitrous oxide, reduce nitrogen excretion in urine and feces (Adegbeye et al., 2019). Saponin is the main bioactive component of yucca extract and is present in steroid form. This component reduces the level of free ammonia by physically binding to it (Sahoo et al., 2015). Plant saponin extract was used to manipulate rumen fermentation and was observed to inhibit methanogens and methane production, reduce protozoa as well as ammonia emissions (Adegbeye et al., 2019).

According to research data by various authors summarized in the review article by Adegbeye et al. (2019) supplementation of *Yucca schidigera* extract in concentration of 250 mg/kg in feed for New Zealand White rabbits reduced nitrogen in feces and urine and increased nitrogen balance by 10.23%. In a ration containing urea, the same amount of the herb reduced the ammonia nitrogen content in the cecum by 33%. This effect may be due to the inhibition of the activity of the enzyme urease, which reduced the rate of conversion of urea to ammonia, or modification of the microflora of the colon.

Chrenkova et al. (2012) also confirmed the effects of yucca (*Yucca schidigera*) powder extract added to the food of rabbits on nutrient digestibility and qualitative parameters in caecum were monitored. Yucca Dry extract of 5g/100 kg was added to the feed of the animals of the first experimental group. The animals from the second experimental group had diet enriched with yucca dry extract of 20g/100 kg. In animals receiving the extract in a dose of 5g/100kg, digestibility of protein and fat improved.

Chrenkova et al. (2012) concluded that effect of yucca extract addition is manifested with reduced levels of ammonia in rabbit's farms which confirmed the results of other authors mentioned above.

Probiotic as a food supplement

The use of antibiotics for growth promotion purposes was banned in the European Union from 01.01.2006, and the use of sub-therapeutic doses of medically important antibiotics in animal feed and water to promote growth and improve food efficiency became illegal. Recommendations were given to stimulate and improve the health of animals and their resistance by using probiotics, ferments, medicinal herbs and minerals, which was regulated by Regulation (EC) No. 1831/2003 of the European parliament and of the council /22.08.2003/ This process highlights the necessity of introducing probiotics as a food ad in all branches of animal husbandry.

Probiotics have proven to be effective in increasing growth and meat quality, significantly improving health due to the improvement due to improving stomach microflora, stimulation of the immune system, reduction of mortality and diseases from bacterial infections (Adli et al., 2023; Amber et al. 2004, Mancini, S. & G. Paci, 2021). Using probiotics from the first days of the animal's life, contributed to a healthy intestinal microflora, which ensured normal digestion and high resistance to infectious agents (Sharma et al., 2016). Scientists pointed out that intake of probiotics does not lead to side effects, and at the same time, such therapy was accessible, cheap and effective for all farm animals. The rabbit is a monogastric hindgut fermenter, as via caecotrophy its digestive physiology allows it to obtain proteins and vitamins. Therefore, their intake of probiotics is essential for their good development (Mancini et al., 2021). In their study In their study Mancini et al. (2021), through Meta-analysis, concluded that probiotics are increasing production performance and blood parameters of domesticated rabbits. : This conclusion of the cited above authors is important, because rabbits have unique physiology (monogastric herbivore) and functioning of their

intestinal tract needs delicate balance. Dimova et al. (2017) reported similar results. They determined statistically significant effect of probiotic "ZOOVIT" on fertility and reproductive capacity of farm animals. Further studies are needed to establish the optimal doses of "ZOOVIT" in rabbit does, growing rabbits and rabbits for fattening.

Sharma et al. (2016) also stated that use of probiotics has many potential benefits, such as modified host metabolism, immune stimulation, anti-inflammatory action, exclusion and reduction of pathogene bacteria in the intestinal tract, reduced bacterial contamination on processed broiler carcasses, enhanced nutrient absorption and performance and ultimately decreased human health risk.

The addition of probiotics to the fodder of farm animals also has an effect on reducing the released ammonia. Dietary probiotic has been found to decrease urease activity and subsequently reduce ammonia production in small intestinal contents of chicks, laying hen (Amber et al., 2004; Yeo and Kim, 1997) and rats (Amber et al., 2004; Kim and Kim, 1992).

Amber et al. (2004) tested eighty one growing New Zealand White rabbits at age of 35 days. The team investigated the effect of feeding diets containing yucca extract or probiotic on growth performance, nitrogen utilization, digestibility, blood parameters, caecal microbial activity and relative revenue. Rabbits were randomly divided into 3 groups and were fed three diets: without supplementation (control), with 250 mg/kg *Yucca schidigera* extract or with 0.5 mg/kg Lact-A-Bac (Probiotic) from 5 to 13 weeks of age. Nitrogen balance improved for rabbits, which were fed diets containing yucca extract and probiotic by 24 and 16.2%, respectively, compared to the rabbits fed with control diet. Improvement of nitrogen utilization could be as a result of decreasing ammonia concentrations in faeces and urine, as well as binding of ammonia by the yucca saponin molecules(Sliwinski et al., 2002), and also reducing urease activity in the gastrointestinal tract by dietary probiotics (Yeo and Kim, 1997; Duffy and Brooks, 1998). Probiotics affect the intestinal flora, replacing pathogenic urease-producing species with non-pathogenic neurease-producing lactobacilli**.** The above results showed that the supplementation of yucca extract and probiotic to diets could had a positive effect on the long term.

It could be concluded that adding yucca extract and probiotics to the rabbit's diet decrease urea and ammonia levels in the blood and caecum. This may be beneficial for overall health of rabbits and humans since there are reduced ammonia emissions in the rabbitry. By adding some probiotic strains to animal bedding ammonia levels can be reduced. Kim et al. (2021) studied the effect and mechanism of action of *Saccharomyces boulardii* (SB) in reducing the ammonia emission from livestock manure. Bu using qPCR analysis they followed the proliferation of anaerobic bacteria of *Proteus mirabilis*, which increased the manure pH through ammonia production. Significant decrease in pH and proliferation of *P. mirabilis* was found in SB group compared to control ($p < 0.05$). The SB group also reduced the amount of ammonia emitted from manure by 25% for 35 days. These results suggested that SB contributes to reducing ammonia emission from manure by lowering pH and inhibiting HAB as well as removing ammonia–nitrogen. The authors suggested that SB as a microbiological agent could reduce ammonia emission and also improve manure quality as a fertilizer.

Ammonia greenhouse gas emissions from rabbit farms can also be reduced by feed additives derived from municipal bio-waste (Biagini et al. 2021). Such method was the use of a water-soluble biopolymer (*Saccharomyces boulardii*) derived from waste materials in the treatment of urban lawns. The study included 35-day-old rabbits that were fed conventional diet (control) and a test diet with 0.05–1% *Saccharomyces boulardii* included. Urine and feces were examined. The animals fed 0.25% SB diet produced significantly lower emissions ($p<0.05$): for example, 30% less ammonia (NH_3), 25% less methane (CH_4), 9 % less nitrogen oxide (N_2O) and 8 % less carbon dioxide (CO_2) than the control group (Biagini et al. 2021).

By applying modern methods of molecular genetics, such as sequencing and cloning, Koger

and Kempen (2006) investigated the possibility of creating engineered microorganisms. They have to be adapted to the differences in enzyme activity in the gastrointestinal tract of each farm animal species. According to authors, microorganisms can be engineered to be efficient ammonia traps. Use of these microorganisms as probiotics or in biofiltration units may result in improved animal health and weight gain, as well as increased farm profitability. Positive effects on human health may also be achieved. The environmental benefits include reduction of ammonia and odor levels in the air and a decreased potential for ground water nitrification.

All references in the present review proved that probiotics had multiple positive effects on animal health, as well as on reducing ammonia concentrations in farms.

CONCLUSION

Supplements, such as Yucca extract and probiotics significantly influence the digestion of the provided fodder and nitrogen uptake. This leads to decreased levels of urea and ammonia in the blood and cecum of the rabbits, a process directly related to reduction of ammonia concentrations in the air. Good ventilation is also an essential factor for decreasing ammonia levels in the air; it provides optimal microclimate in the rabbit's rooms and improves rabbits health.

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