

EFFECT OF PROBIOTIC BIOPLUS 2B[®] ON PERFORMANCE OF GROWING RABBIT

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Summary. The aim of this work was to study the efficacy of probiotic BioPlus 2B[®] dietary inclusion on performance of growing rabbits in summer conditions. One hundred and twenty 35-days-old New Zealand White rabbits were weaned and randomly divided into two equal groups with respect to litter size and body weight. Group 1 was served as control and fed a commercial and antibiotic-free diet. The rabbits in Group 2 were fed diet supplemented with 400 mg/kg of BioPlus 2B[®]. The animals were assigned to the diets *ad libitum* until 77 days of age. The probiotic inclusion corresponded to 1.28x10⁶ colony forming units (CFU) per g feedstuff, i.e. 6.4x10⁵ CFU/g of *Bacillus licheniformis* and 6.4x10⁵ CFU/g of *Bacillus subtilis* after pelletization. During the experiment of feed nutrient digestibility the rabbits were fed limited amount of feed. Each rabbit was identified, fed individually, each obtained its own feeding and stationary watering container. During the investigation, individual rabbit's excrements were collected twice a day, frozen and kept until chemical analysis was carried out.

Rabbits were housed individually in wire-mesh flat-deck cages (30x61x28 cm) under controlled conditions. The inside temperature was 18-23°C, however, due to the hot summer, it could reach sometimes 23-26°C. The treatment did not affect 77d body weight and 35-77d daily weight gain (2427 g and 34.4 g/day in Group 1, while 2434 g and 34.4 g/day in Group 2, respectively). Feed efficiency was comparable for both groups. The supplementation had a beneficial effect on the sanitary risk (3.3% and 23.3% in Groups 1 and 2, respectively), primarily due to the lower mortality between 35 and 49 days of age (0% and 71% of all losses occurred in this period in Groups 1 and 2, respectively). With a dose of 400 mg/kg of BioPlus 2B[®], the morbidity in Group 2 was by 3% and the mortality rate by 17% lower (P<0.002) compared to Group 1, resulting in a 20% decrease (P<0.001) of sanitary risk (morbidity+mortality) during the fattening. In conclusion, the results from this study indicate that it could be advantageous to supplement the diet of growing rabbits with BioPlus 2B[®] in summer conditions, primarily aiming to reduce the sanitary risk during the fattening period.

Key words: probiotics, rabbit, *B. licheniformis*, *B. subtilis*, growth.

PROBIOTIKO „BIOPLUS 2B[®]“ POVEIKIS TRIUŠIŲ PRODUKTYVUMUI

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Santrauka. Šio darbo tikslas – ištirti probiotinio raciono priedo „BioPlus 2B[®]“ poveikį augančių triušių produktyvumui vasaros metu. Naujosios Zelandijos baltųjų veislės triušiai buvo nujunkyti ir analogų būdu paskirstyti į dvi grupes pagal vados dydį ir kūno svorį 35-ą amžiaus dieną. Bandomoji (I) grupė buvo šeriama pramoninės gamybos pašarais be antibiotikų. Bandomajai grupei (II) į pašarus buvo pridėdama 400 mg/kg probiotiko „BioPlus 2B[®]“. Iki 77 amžiaus dienos triušiai buvo šeriami *ad libitum*. Probiotinio priedo kiekis atitiko 1,28x10⁶ kolonijas formuojančių vienetų (CFU) vienam gramui pašaro, t. y. 6,4x10⁵ CFU/g *Bacillus licheniformis* ir 6,4x10⁵ CFU/g *Bacillus subtilis* po pašarų granuliavimo. Triušiai buvo laikomi individualiuose plokščiadugniuose narveliuose iš vielos tinklo (30x61x28 cm) reguliuojamomis aplinkos sąlygomis (16 šviesos:8 tamsos apšvietimo ciklas). Patalpos temperatūra buvo 18–23 °C, tačiau dėl vasaros karščių kartais galėjo siekti ir 23–26 °C. Probiotinis priedas nedarė įtakos 77 dienų triušių kūno svoriui bei 35–77 dienų triušių paros priesvoriui (2427 g ir 34,4 g parai II grupėje, kai I grupėje rodikliai buvo atitinkamai 2434 g ir 34,4 g parai). Pašaro efektyvumo rodikliai abiejose grupėse buvo panašūs. Priedas teigiamai paveikė sanitarinę riziką (3,3 proc.) pirmiausia dėl mažesnio 35–49 dienų triušių kritimo. Kai „BioPlus 2B[®]“ dozė buvo 400 mg/kg, palyginus su kontroline (I) grupe sergamumas buvo 3 proc., o kritimas 17 proc. mažesnis (p<0,002), todėl penėjimo metu sanitarinė rizika (sergamumas+kritimas) sumažėjo 20 proc. (p<0,001).

Apibendrinant galima teigti, kad vasaros sąlygomis augančių triušių davinį naudinga būtų papildyti produktu „BioPlus 2B[®]“, pirmiausia siekiant sumažinti sanitarinę riziką penėjimo periodu.

Raktažodžiai: triušiai, probiotikas, *B. licheniformis*, *B. subtilis*, augimas.

Introduction. In relation to the importance of caecal microbial fermentation, the digestive process is very complex and fragile in rabbits. That is why rabbits are rather sensitive to enteric diseases and especially when they are exposed to negative impacts, e.g. weaning or heat stress, causing high losses. This problem can be avoided by antibiotics. However, because of the general intention to limit antibiotics in animal feed as growth promoter concerning side-effects, resistance and recent public perception about healthy food, new alternatives to antibiotics are needed (Marzo, 2001). Probiotics that contain yeast, live bacteria or bacterial spores can also prevent enteric diseases of rabbits. Instead of growth promoters with antibiotics that kill some of the rabbit's own gastrointestinal flora, probiotics promote gut colonisation and stabilize eubiosis by competitive growth against harmful microorganisms, reducing the intestinal pH with production of lactic acid and encouraging digestion by producing enzymes and vitamins. These functions strengthen the animal's own non-specific immune defence (Fortun-Lamothe and Drouet-Viard, 2002). Dietary administered probiotic bacteria decreased the frequency of *E. coli* translocation (Lee et al., 2000) and were effective in preventing the growth of *E. coli* O157:H7 in the intestine of neonatal rabbits (Tachikawa et al., 1998). Hamrany et al., (2000) found a dose-dependent positive effect of a probiotic bacterium on *E. coli* occurrence in the caecum and small intestine in young rabbits.

Term "probiotics" in translation from Greek means "for life". In many authors', scientists' opinion, probiotics – living microorganisms or their fermented products, which decompose undesirable food matter, increase digestion of feed. Therefore such probiotal preparations and their compositions added to feed "widen" the system of fermented digestion. Enzymes are the most important factors of digestion. These are protein molecules, which can catalyse and improve acceleration of feed digestion. Enzymes secretion takes place mainly in stomach, liver and small intestines. The development of enzymes' secretion system is not a rapid process for an animal, even with wholly developed digestion system it has to pass an adaptation stage. For this reason young, small animal being in a stressful state is more sensitive to the illnesses of digestion process; the animal grows slowly (Fuller, 1989; Fotso et al., 2000).

It is obvious that addition of probiotic BioPlus 2B[®] to feed has a significant synergetic effect. Composed of three natural components – yeast extracts, enzymes and

useful bacteria, it can maximally increase feed efficiency (Konecka, Jazierski, 1997). In the scientific papers by different authors we also looked for the connection between the level of feeding and obtaining rabbit production of high quality; the data that partially correspond to the results of our research (Fortun – Lamothe et al., 1999; Fernandez – Carmona et al., 1996; Rymkiewicz, Lewczuk 1999; Lebas F, 2000). Several studies reported the beneficial effect of dietary addition of bacteria (Szabó-Lacza et al., 1988, Jensen and Jensen, 1989, Vörös and Vörös, 1998, Hamrany et al., 2000), bacteria+yeast (Aquilar et al., 1999), bacteria+yeast+enzyme (Duperray, 1991, Tawfeek et al., 1991, Vörös and Gaál, 1992, GipperT et al., 1992 and 1996, Maertens et al., 1994, Abdel-Samee, 1995, Kamra et al., 1996., Kermauner and Štruklec, 1997) and bacteria+herb extract (Goby et al., 2000, El-Adawy et al., 2002, Zanaty, 2002) on health status and zootechnical traits in rabbits. Probiotic BioPlus 2B[®] (Chr. Hansen A/S Hørsholm, Denmark) consists of *Bacillus licheniformis* and *Bacillus subtilis*. The advantage of these spore-forming bacteria is that they are able to survive the pelletization process (Bosch, 1995) and the transit through the stomach, they germinate in the intestine and use a large number of sugars (carbohydrates) for their growth and they produce a range of relevant digestive enzymes (amylase, protease, lipase).

The aim of this study was to investigate the efficacy of dietary inclusion of probiotic BioPlus 2B[®] on the performance of growing rabbits under summer conditions.

Materials and Methods. The study was carried out in a rabbit farm, between June and July 2005. Rabbits were kept individually in wire net flatdeck cages (30x61x28 cm) in a closed and air temperature controlled building (18-23°C) with forced ventilation and regulated photoperiod (16L:8D). However, in the hot summer, the inside temperature reached 23-26°C from June 15.

Involving 120 New Zealand White rabbits born in one reproduction cycle, the animals were divided into two groups considering litter size at birth and 35 days body weight at weaning. A commercial and antibiotic-free diet (I), with 16% crude protein, 2% crude fat, 15.5% crude fibre and 10.3 MJ/kg DE, was fed in the control group. Group II received the I diet supplemented with 400 mg/kg of probiotic BioPlus 2B[®]. Feeds were provided *ad libitum* until 77 days of age. The data of feeding experiments is presented in Table 1. Rabbits in the control group were fed the same diet without probiotics.

Table 1. Design of feeding experiments, (n=18)

Age, day	Group	The amount of mixture of probiotic preparations, mg/kg	Metabolic energy, (MJ)
35-77	I (experimental)	–	10,3
35-77	II (experimental)	400	10,3

The probiotic inclusion corresponded to 1.28×10^6 colony forming units (CFU) per g feedstuff, i.e. 6.4×10^5 CFU/g of *Bacillus licheniformis* CH 200 and 6.4×10^5

CFU/g of *Bacillus subtilis* CH 201 after pelletization (diet was exposed to 75°C for 45 sec. under pelletizing).

Individual body weight and feed intake were weekly

measured when morbidity (ill but survived rabbits) and mortality were also registered. Daily weigh gain, feed conversion and sanitary risk (morbidity+mortality) were calculated from the data. Estimating the effect of feeding, data of body weight, daily weight gain, feed intake and feed efficiency were subjected to analysis of variance, while data of mortality, morbidity and sanitary risk were analyzed using Chi-square test of STATGRAPHIC package 6.0 (1992).

During the experiment of feed nutrient digestibility the rabbits were fed limited amount of feed. Each rabbit was identified, fed individually, each obtained its own feeding and stationary watering container. During the investigation, individual rabbit's excrements were collected twice a day, were frozen and kept until chemical analysis was carried out.

Analysis of the basic chemical composition of meat included the determination of percentage of dry matter, crude protein, fat, ash, soluble protein and protein after its precipitation (Budslawski and Drabent, 1972). Meat physicochemical properties, i.e. pH (a pH-meter manufactured by the "Radiometer" company, with an electrode GK 23311C), colour brightness (spectrometer at a wavelength of 560 nm), amino acids (Moore and Stein, 1963) and water-holding capacity (Grau and Hamm, 1952) were also determined. The results were analyzed statistically, taking into consideration arithmetic means (\bar{x}), standard deviation (s), coefficients of variation (v) and coefficients of simple correlation (r). The differences between the means for groups were determined by an analysis of variance in a non-orthogonal design. A computer program Statistica 7.0 was applied to create a database and for statistical calculations (Statistica für Windows™ 1995).

Results and Discussion. Addition of BioPlus 2B® did not affect significantly the body weight and daily weight gain (DWG) of rabbits between 35 and 77 days of age. The research results showed that at the end of the research 2 months old New Zealand rabbits, fed with composed feed containing BioPlus 2B® probiotic, were by 310 g or 18 % ($p>0,05$) heavier than rabbits in tested group fed with composed feed without an additive.

In summer, heat-stressed rabbits have higher rectal

temperature and respiratory rate, while their feed intake and consequently, their DWG decrease (Abdel-Samee, 1995, Kamra et al., 1996). Conversely to our result, Abdel-Samee (1995) reported that the adverse effect of heat stress could be alleviated by probiotics resulting in a 10% improvement in DWG (26.3-26.9 vs 24.3 g/d). However, Gippert et al. (1992) found only a 3% increase in DWG in poorer conditions (commercial unit and in summer: 29.5 vs 28.7) but an 8% improvement in more favourable facilities (experimental farm: 31.2 vs 29.3 g/d) with probiotic inclusion compared to the control group. In other study of Gippert et al. (1996), the DWG of individually kept rabbits was improved with probiotic inclusion (36.8 vs 34.8 g/d). Maertens et al. (1994) reported also a relatively better DWG (43.4 vs 42.3 g/d) and larger 70d weight of rabbits (2418 vs 2387 g) under optimal housing conditions (18°C, 3 rabbits/m²), whereas small and not significant differences were detected in DWG (41.8 vs 41.7 g/d) and final weight (2350 vs 2348 g) under less proper housing (14 animals/m²) in favour of the probiotic treatment. Probiotics did not affect the DWG (33.2 and 32.7 g/d) of rabbits compared to the control with stocking density of 6 rabbits/cage (Aguilar et al., 1999). Similarly to Bonanno et al. (1999), feed efficiency tended ($P=0.249$) to improve by 1.5% with the treatment (3.38 vs 3.43) that can be connected with the relatively lower ($P=0.280$) feed intake and better digestibility of the nutrients. In other studies the improvement of feed efficiency was due to a higher apparent digestibility of crude fibre and protein when probiotics and enzymes were provided together (Gippert et al., 1992, Kamra et al., 1996, Kermauner and Štruklec, 1997). In contrast to our result, Abdel-Samee (1995) reported higher, 8% improvement in feed conversion in young rabbits that were treated with different probiotics in summer period (3.9-4.1 vs 4.3 in the control group).

Fertile and suckled does, provided with tested feed, gave birth to bigger, more vital juvenile and had larger litter size. The increase of weight of rabbits during a day and night can be explained. The research on nutrient's portion digestion showed that used preparation improved their digestion. The addition mainly influenced on green protein and crude fibre digestion (Figure 1).

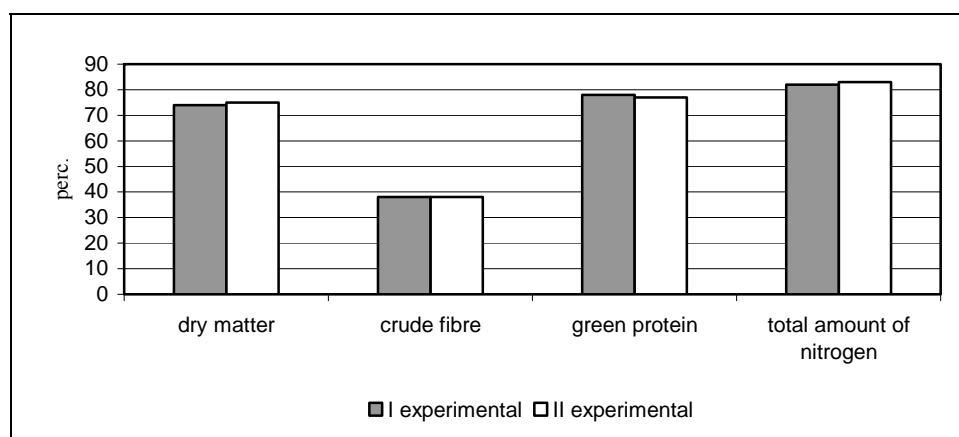


Figure 1. The digestion coefficient (in percents) of feed

After the rabbit meat test (the back muscles were chemically analysed) it was affirmed that addition of probiotic didn't influence the chemical composition of meat. The morphological composition of meat of tested slaughtered group of rabbits was relatively better developed; the carcass had longer back muscles and

higher leg weight (Figure 2).

The meat of New Zealand rabbits from the control group did not differ significantly from the experimental groups in terms of protein value (tryptophan and oxyprolin) (Figure 3).

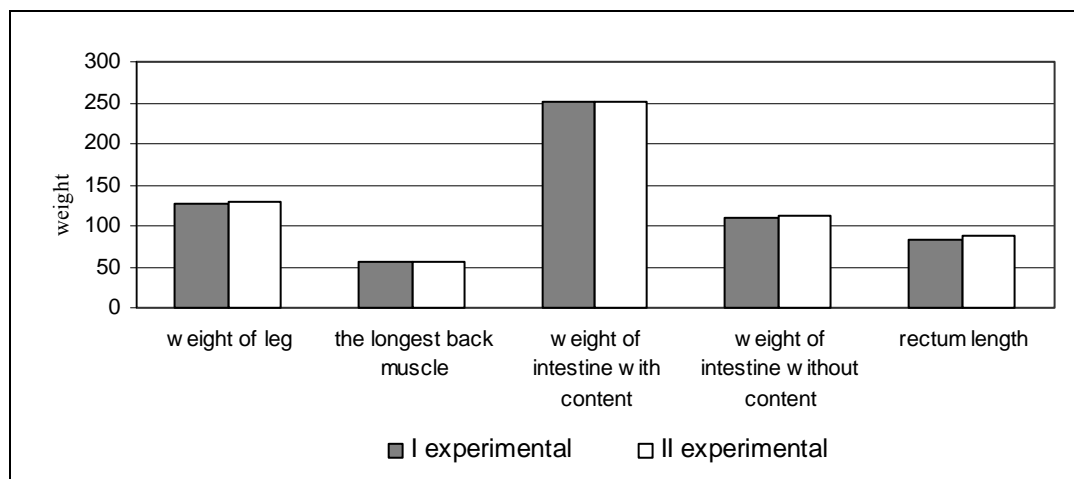


Figure 2. New Zealand rabbits controlled slaughter data

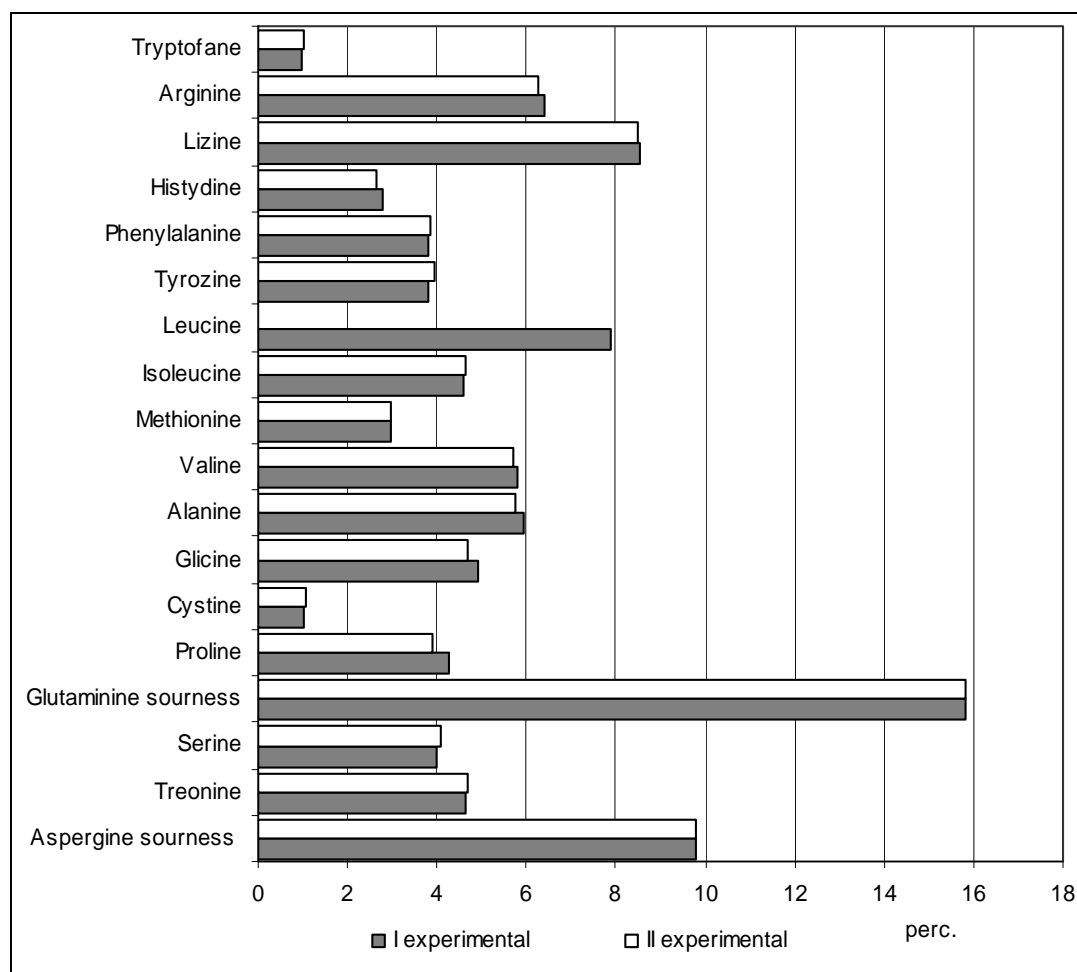


Figure 3. The influence of probiotic on the amount of amino acids in the samples of meat of rabbits

From the papers of other authors we noticed that the correlation between the level of feeding and high quality rabbit production and their data partially corresponded with the results of our investigation (Konecka, Jazierski, 1997; Rymkewicz, Lewczuk, 1999).

When probiotics are used aiming to replace the antibiotics in the diet, the zootechnical traits are scarcely improved but with promoting the development and maintenance of the caecal flora they result in good health status of the animals. BioPlus 2B[®] inclusion exerted a beneficial effect on the sanitary status of the rabbits, especially due to the lower mortality and primarily

between 35 and 49 days of age (0% and 71% of all losses occurred in this period in the II and I groups, respectively.). In the II group, morbidity was by 3% and the mortality rate by 17% lower ($P < 0.002$) compared to the I group, resulting in a 20% decrease ($P < 0.001$) in sanitary risk (morbidity+mortality) during the fattening. Other studies (Hollister et al., 1990, Tawfeek and Elhindawy, 1991, Abdel-Samee 1995, Hamrany et al., 2000) also reported a 2 to 16% better viability of rabbits with probiotic bacteria inclusion. Effect of probiotic supplementation on performance traits and sanitary risk is presented in Figure 4.



Figure 4. Effect of probiotic supplementation on performance traits and sanitary risk in rabbits

In piglets, in a low health-status arm, providing 106-107 viable spores of *B. licheniformis* (LSP 122) per g of feed exhibited a better weight gain and feed conversion with reduced incidence and severity of post-weaning diarrhea syndrome (PWDS) caused by enterotoxigenic *E. coli* strains (Kyriakis et al., 1999).

Conclusions

1. On the basis of our results could be advantageous to supplement the diet of young rabbits with BioPlus 2B[®] in summer conditions, aiming to reduce the mortality and sanitary risk during the fattening. Nevertheless, further investigations are needed to assess the efficacy of BioPlus 2B[®] inclusion in different environmental and housing conditions in rabbits.

2. The analyses of the chemical composition of meat indicated that the feed supplement did not have any influence on amount of dry matter and ash. In the meat of the latter investigated groups the amount of protein increased by 1.8% and 4.1% and fat slightly by 0.2 and 0.7% in comparison with the control group. The change of these indices influences and has a tendency to improve the quality of the rabbit meat.

3. One could state that preparation BioPlus 2B[®] used in the combined feed for rabbits did not have a negative influence on the chemical composition of the meat. The obtained differences of the data between the groups are statistically unreliable.

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