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# APPLICATION OF PROBIOTICS AND PHYTOBIOTICS IN RABBITS NUTRITION

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*ABSTRACT.* The effect of probiotics and phytobiotics was tested in the experiment on rabbits of meaty line of the New Zealand White hybrid in feeding and fattening experiment. The experimental animals (96) were divided into three experimental and one control groups. In the first experimental group the complete granulated mixture was tested enriched with 15g plant extract XTRACT per 100 kg mixture. Additive commercial product XTRACT has three active substances - essential oil extracts from capsicum, cinnamaldehyd and oreganum. In the second one the animals were fed complete granulated mixture and culture of *Enterococcus faecium* EF 2019 strain ($1.0x10^9$ CFU/ ml; 500 µl/animal/day) was administered daily in drinking water for the period of 21 days. Inoculum of rifampicin marked like *Enterococcum faecium* EF 2019 strain was prepared according to Simonová et al. (2005). The third experimental group were fed like the first group (with the supplement of XTRACT) and culture of *Enterococcus faecium* EF 2019 strain ($1.0x10^9$ CFU/ ml; 500 µl/animal/day) was administered daily in drinking water for the period of 21 days. The diet fed in the control group did not contain any coccidiostat and rabbits were not inoculated. The experiment lasted for 42 days until the animals attained the slaughtering weight 2.5 kg. Body weight and feed consumption were registered weekly. Three animals from each group were slaughtered at 42$^{nd}$ day by cutting the jugular vein and the carotid artery after electroanaesthesia (90 V for 5 sec). The samples of *m. longissimus dorsi* (MLD) were collected immediately after death and stored 24 h at 4˚ C and then physico-chemical analysis were made. In the fattening experiment there growth rate was registered and feed consumption per unit of live weight was calculated (3.42 – 3.56 kg / kg). There were no significant differences between control group and experimental groups in nutrient utilization and nutrient digestibility. The results showed the favorable values of water holding capacity, the high content of total proteins (22.7 – 22.9 g per 100 g), and very favorable content of intramuscular fat (0.93 – 3.63 g per 100 g). Energetic value did not exceed the value of 417 - 426 kJ in 100 g MLD. The pH value of rabbit meat was 5.75 – 5.89 48 h post mortem. The supplementation of XTRACT to diets for rabbits stimulates animal growth and performance. It is very good stimulant for prevent infectious intestinal diseases, but it is not a healing agent. Application of *Enterococcus faecium* EF2019 strain showed the positive effect on physico-chemical trails of rabbit meat.

*Key words: rabbits, probiotics, phytobiotics, fattening, growth rate, meat quality*

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In the last 20 years, rabbit production has become an increasingly intensive system. Productivity, measured as reproduction rate, meat production or growth rate in the fattening period, is comparable to other intensively reared farm animals. On the other hand, feed efficiency, pathological conditions associated with high mortality, and considerations of meat quality have become limiting factors for the economic output of a farm unit (Ouhayoun, 1998). Growth- promoting antibiotics, more correctly antimicrobials, are compounds which can be safety added into animal feed to improve the efficiency of di-

gestion. This means that the animal grows well. Antibiotics licensed as growth promoting have been especially selected. This class of feed additives is being phased out in the European Union and they will be no longer used after the start of January 2006. This new situation has allowed for the investigation and use of feed additives such as acidifiers, probiotics, prebiotics and phytogenic substances (Fuller, 1989; Kamel, 2001; Simonová and Lauková, 2004; Simonová et al., 2005; Simon, 2005). Many plant extracts have been reported to improve animal performance and well-being, especially under dietary or other enteric stressors and as alternatives to growth- promoting antibiotics (Leitgeb et al., 2001). Plant extracts are a category of alternatives to in-feed antibiotics which are characterized in the literature not only by antimicrobial (Cowan, 1999, Dorman and Deans, 2000, Chrastinová et al., 2005), but by antioxidant (Botsoglou et al., 2004) and antitoxin effects, too (Azumi et al., 1997). In particular, the combination of plant extracts investigated here has been shown to have effects on digestive physiology and on microbial populations in the piglet (Manzanilla et al., 2004) and the broiler chicken (Jamroz et al., 2003).

The experimental animals (n=96) were divided into three experimental and one control groups. In each group were 24 rabbits from weaning (35-days old males of meaty line of the New Zealand White hybrids rabbits) to the slaughtering weight 2.5 kg. Rabbits were housed in all-wire two animals per cage. Environmental conditions were the following: temperature $18^0$C, relative humidity 60%, 12 hour light: 12 hour dark daily photoperiod cycle. Rabbits were fed ad libitum and they had free access to drinking water from nipple drinkers.

In the first experimental group the complete granulated mixture was tested enriched with 15g plant extract XTRACT per 100 kg mixture. Additive commercial product XTRACT has three active substances - essential oil extracts from capsicum, cinnamaldehyd and oreganum.

In the second one the animal were fed complete granulated mixture and culture of *Enterococcus faecium* EF 2019 strain ($1.0x10^9$ CFU/ ml; 500 µl/animal/day) was administered daily in drinking water for the period of 21 days. Inoculums of rifampicin marked like *Enterococcus faecium* EF 2019 strain was prepared according to Simonová et al. (2005).

The third experimental group were fed like the first group (with the supplement of XTRACT) and culture of *Enterococcus faecium* EF 2019 strain ($1.0x10^9$ CFU/ ml; 500 µl/animal/day) was administered daily in drinking water for the period of 21 days.

The diet fed in the control group did not contain any coccidiostat and rabbits were not inoculated. The experiment lasted for 42 days until the animals attained the slaughtering weight 2.5 kg. Body weight and feed consumption were registered weekly. Three animals from each group were slaughtered at $42^{nd}$ day by cutting *v. jugularis* and the carotid artery after electroanaesthesia (90 V for 5 sec). The samples of *m. longissimus dorsi* (MLD) were homogenized collected immediately after death and stored 24 h at $4^0$C and then physico-chemical analysis were made. The ultimate pH at 48 h post mortem was determined by Radelkis OP -109 with a combined electrode penetrating 3 mm into the MLD. Protein and fat content were estimated using an INTRATEC 1265 spectroscop and expressed in g/100 g; from these values the gross energy value of samples of meat was calculated:

Energy value (kJ/100g) = (16.75 * total protein content + 37.68 * total fat content).

The samples of individual feeds and complete granulated mixture were analyzed for the content of nutrients (Table I) according to STN 46 7092. The content of DE and ME was calculated by the equation of Wiseman et al. (1992).

*Table 1.* ***Ingredients and chemical composition of feed mixture***

| Ingredients | g/kg feed | Chemical analysis | g/kg feed |
|---|---|---|---|
| Dehydrated alfalfa meal | 400 | Dry matter | 885.4 |
| Dehydrated sugar beet pulp | 100 | Crude protein | 164.2 |
| Oats | 130 | Crude fibre | 171.7 |
| Wheat middlings | 60 | Fat | 33.0 |
| Dry malting gems | 50 | Nitrogen-free extract | 446.9 |
| Sunflower meal | 140 | Organic matter | 815.8 |
| Monocalcium phosphate | 6 | Starch | 139.4 |
| Sodium chloride | 3 | Ash | 69.6 |
| Limestone, pulverized | 9 | Calcium | 9.3 |
| Soybean meal (extruded) | 75 | Phosphorus | 6.9 |
| Vitamin premix* | 1 | Lysine | 7.5 |
| Carob meal | 25 | Methionine+cystine | 6.5 |
| DL- methionine + wheat flour | 1 | ME (MJ/kg) | 10 |

*Provided per kg of diet: vit. A 12000 IU; vit. $D_3$ 2500; vit. E 20 mg; vit. $B_1$ 1.5 mg; $B_2$ 7.5 mg; $B_6$ 4.5 mg; vit. $B_{12}$ 30 μg; vit. K 3 mg ; nicotinic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg; choline chloride 450 mg.

Ceacum digesta were collected on 21 day and 42 day of the trial. Digesta was analyzed for pH and VFA (molar production of acetate, propionate, butyrate, valerate, capronate), ammonia-N concentrations and content of nutrients.

Body weight and feed consumption were registered weekly, the amount of wasted pellet continuously and feed conversion ratio values were calculated. The significance of differences was evaluated by the t-test.

The experimental diets were balanced on nutrients content and energy value (Table 1). Average initial and final body weight, daily weight gain, daily feed intake per rabbit, and the calculated feed conversion ratio for the experimental period are presented in Table 2 and Fig. 1 and 2.

*Table 2.* ***Performance of rabbits in response to dietary supplementation of probiotics and phytobiotics*** $(\overline{X} \pm SD)$

| Performance parameters | XTRACT | EF 2019 | EF 2019 + XTRACT | CONTROL |
|---|---|---|---|---|
| Number of animals | 24 | 24 | 24 | 24 |
| Initial weight, g | 968.3 ± 94.8 | 977.1 ± 97.0 | 962.5 ± 89.2 | 964.2 ± 109.2 |
| Live weight on 56th day of age, g | 1910.4 ± 213.6 | 1850.0 ± 151.8 | 1897.8 ± 194.3 | 1810.4 ± 207.6 |
| Final weight, g | 2460.0 ± 195.4 | 2621.8 ± 103.5 | 2573.0 ± 163.6 | 2465.0 ± 180.4 |
| Daily gain, g | 40.5 | 40.1 | 42.2 | 38.3 |
| Mortality (n) | 1 | 2 | 2 | 1 |
| Feed conversion ratio between 35th and 56th day (g/g) | 2.99 | 2.71 | 2.82 | 2.81 |
| Feed consumptiom per kg gain | 3.56 | 3.47 | 3.42 | 3.32 |

There were observed no significant effect of treatments on studied parameters. Feed consumption per kg of gain was the lowest in group with the supplement of culture of *Enterococcus faecium* EF 2019 strain ($1.0x10^9$ CFU/ ml; 500 μl/animal/day) administered daily in drinking water for the period of 21 days. We observed positive effect of 15 g of XTRACT supplementation on health of rabbits. Supplementation with XTRACT

promoted also a small increase in feed intake and growth rate, without effect on feed efficiency or the whole-tract digestibility of OM (data not shown). This higher intake could be related to an improvement in health status of the animals or to an enhancement on feed acceptance (Wenk, 2005).

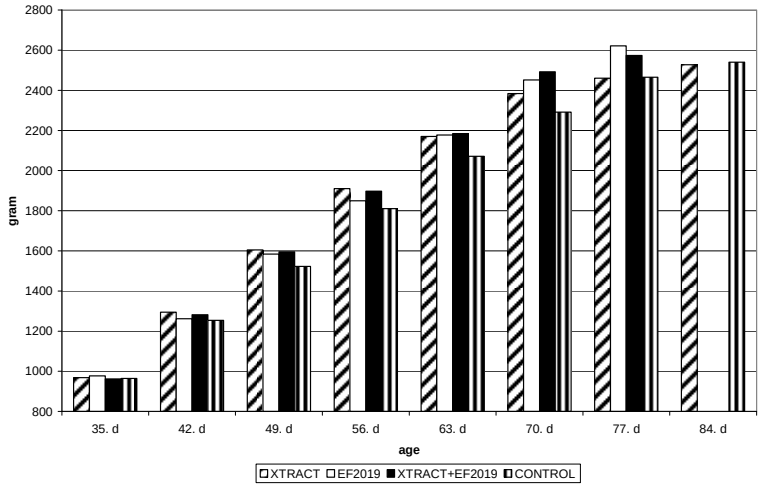


*Fig. 1. Growth of live weight in rabbits*

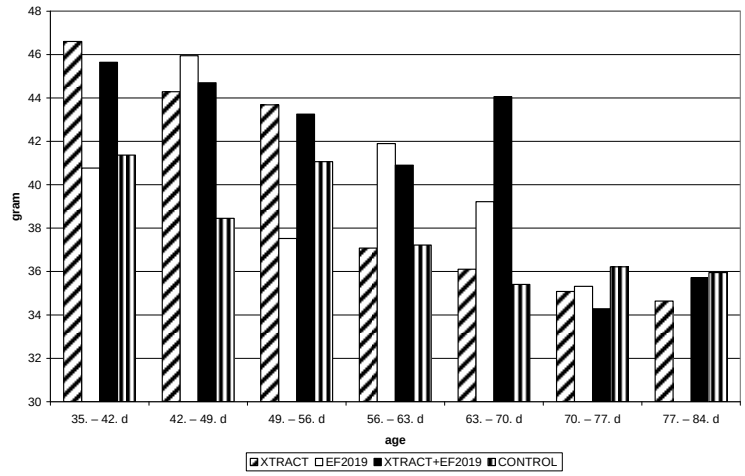


*Fig. 2. Average of daily gain from 35 to 84 days in rabbits*

Our results are in agreement with the results by Manzanilla et al. (2004).

The pH of the *m. longissimus dorsi* 48 h post mortem was not significantly affected by strain *Enterococcus faecium* EF 2019, even pH was reduced in experimental group. After probiotic treatment, the reduction of fat content was observed in experimental samples; whereas, opposite effect of EF 2019 strain on protein content was recorded, in comparison to control value (Table 3). The energy content was also increased ($P<0.05$). On the other hand, higher fat and lower protein content were described by Piles et al. (2000) and Simonová et al. (2006) when the effect of selection for growth rate on carcass composition was tested.

*Table 3.* ***Physical and chemical parameters of meat quality (m. longissimus dorsi) in rabbits 24 hours post mortem***

| Parameters in m. l. d (n=3) | XTRACT $(\overline{X} \pm SD)$ | EF 2019 $(\overline{X} \pm SD)$ | EF 2019 + XTRACT $(\overline{X} \pm SD)$ | ***Control*** $(\overline{X} \pm SD)$ |
|---|---|---|---|---|
| Water content (g/100 g) | 75.2 ± 0.2 | 74.9 ±0.3 | 75.2 ±0.4 | 75.2 ± 0.2 |
| Total proteins (g/100 g) | 22.7 ±0.1 | 22.9 ± 0.4 | 22.8 ±0.4 | 22.7 ± 0.4 |
| Total fat (g/100 g) | 1.1 ± 0.1 | 1.13 ± 0.1 | 0.9 ± 0.1 | 1.03 ± 4.6 |
| Energy value (KJ/100 g) | 421.7 ± 5.5 | 426.3 ± 4.0 | 417.1±6.9 | 419.2±0.4 |
| pH 48 | 5.82 ± 0.03 | 5.75 ± 0.08 | 5.88 ±0.05 | 5.89 ±0.08 |
| Water holding capacity (g/100 g) | 29.96 ± 1.4$^{+}$ | 34.15 ± 4.5 | 33.93 ±1.2 | 34.58 ±4.6 |
| Ash content (g/100 g) | 1.03 ± 0.1 | 1.03 ± 0.1 | 1.07 ± 0.1 | 1.1± 0.1 |

+P< 0.05, significantly different to control

The rabbits were slaughtered before morning feeding and fermentation processes in the ceacum were observed. Molar percentage of observed VFA indicate that most intensive processes in the ceacum of rabbits were in control group than in other ones (Table 4). The similar results were described by Chrastinová et al. (2005). The supplement of XTRACT effected ratio of individual VFA, mainly acetic acid and propionic acid. Concentration of ammonia-N was the lowest in the rabbits fed diets with an overnight culture of *E. faecium* EF2019 administered for 21 day. It means that degradation of crude protein in caecum is inhibited. Lower concentration of ammonia-N effected a decline of pH value in the intestine and a suppression of potential pathogens such as *E. coli* or *Clostridium spp*.

*Table 4.* ***Metabolites (mmol/l00ml) in the caecal digesta of rabbits (n=3)***

| Additive | XTRACT (a) | EF2019 (b) | XTRACT+ EF2019 (c) | Control (d) |
|---|---|---|---|---|
| | | age 56 day | | |
| PH | 5.90±0.33 | 5.64±0.30 | 5.71±0.08 | 5.92±0.11 |
| N-$NH_3$ (mmol/l) | 17.65$^{b}$± 9.58 | 12.50 ±0.33 | 17.87$^{b}$±2.42 | 22.58$^{aBc}$±5.44 |
| Acetic a. | 4.698$^{b}$±0.32 | 3.152 ±1.39 | 5.124$^{B}$±0.61 | 4.076 ±1.86 |
| Propionic a. | 0.382$^{b}$±0.03 | 0.278 ±0.06 | 0.356±0.075 | 0.378±0.20 |
| Butyric a. | 1.636$^{bd}$±0,23 | 1.238 ±0,54 | 1.853$^{bc}$±0,08 | 1.139 ±0,51 |
| Other VFA | 0.176 ±0.02 | 0.181±0.01 | 0.157±0.02 | 0.141±0.02 |
| Total VFA | 6.893$^{b}$±0.58 | 4.849 ±1.96 | 7.490$^{b}$±0.77 | 5.735 ±2.45 |
| | | age 77 day | | |
| Acetic a. | 5.233±0.93 | 4.507 ±0.54 | 5.416 ±0.33 | 6.10b ±1.69 |
| Propionic a. | 0.415 ±0.04 | 0.456 ±0.07 | 0.412±0.03 | 0.62abc±0.26 |
| Butyric a. | 1.672±0.20 | 1.733 ±0.41 | 1.825±0.16 | 1.86 ±0.27 |
| Other VFA | 0.183 ±0.03 | 0.207±0.02 | 0.188±0.03 | 0.20±0.04 |
| Total VFA | 7.50±1.16 | 6.903 ±0.95 | 7.842±0.36 | 8.791 ±2.16 |

Means in the same row with different letters differ significantly (a, b, c. d: P<0.05; A, B: P<0.01)

So, the live weight growth, feed conversion and health of fattening rabbits were compared with control group fed feed mixture without additive. The best results were obtained with 15 g plant supplement XTRACT in 100 kg of mixture. The supplement decreased mortality of rabbits on the farm, positively influenced fermentative processes

in caecum of rabbits, shortened the period of rabbits fattening and non significantly effected rate of growth (P>0.05)

The results present the positive effect of *Enterococcus faecium* EF 2019 strain on determined physico-chemical composition of rabbit meat.

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