QUALITY OF RABBIT MEAT AFTER APPLICATION OF BACTERIOCINOCGENIC AND PROBIOTIC STRAIN ENTEROCOCCUS FAECIUM CCM 4231 IN RABBITS

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ABSTRACT: Physico-chemical traits, amino acid composition and microbial status of rabbit meat were checked after rabbit feed supplementation by bacteriocinogenic and probiotic strain Enterococcus faecium CCM 4231 in this study. Significant increase of amino acids threonine and serine (p < 0.001) in Musculus longissimus dorsi of rabbits was noted after CCM 4231 supplementation. Leucine, histidine, glutamic acid, proline, alanine, tyrosine were also detected in higher concentrations in the samples from experimental group than in control samples. Changes in the physico-chemical properties of rabbit meat were not significant. Reductive effect of CCM 4231 strain against E. coli after 24 hour storage in refrigerator was observed. The increased amino acid content and no negative influence on meat quality and nutritional value during CCM 4231 supplementation suggest that the application of this new bacteriocinogenic strain with probiotic properties could be promising in rabbits breeding.

KEYWORDS: Amino acids, Bacteriocin, Probiotic, Rabbit meat

INTRODUCTION

Rabbit breeding for meat production represents an important branch of livestock production (Dalle Zotte, 2002). Rabbit meat is considered to be one of the healthiest meats because of its easily digestion and dietetic properties, e.g. high values of proteins (20-21%) and unsaturated fatty acids (oleic and linoleic; 60% of all fatty acids), potassium, phosphorus and magnesium and also low fat, cholesterol and sodium content (Bielanski et al., 2000; Dalle Zotte, 2002; Hermida et al., 2006). Moreover, the energy value (427-849 kJ/100 g of fresh meat) of rabbit meat is comparable to various commonly consumed varieties of red meat (Dalle Zotte, 2002). Therefore, the rabbit meat is very useful in human dietetics and recommended for consumption e.g. to persons with cardiovascular illnesses (Hu and Willett, 2002).

Studies about the physico-chemical traits (pH, colour, water holding capacity, texture, etc.) and sensory properties of rabbit meat, e.g. appaerance, texture, flavour and others, are well known and permanently complete (Pla et al., 1998; Hernández et al., 2000; Dalle Zotte, 2002; Polak et al., 2006). The mineral analysis in rabbit meat had been already presented (Hermida et al., 2006). The knowledge concerning the rabbit meat composition, particularly its protein and amino acid (AA) profile is only on the basic level and studies dealing with AA content in rabbit meat are rare (Matusevičius et al., 2006; Simonová et al., 2008a). The concentration of AA, especially of essential is the one of those components which mostly influence the meat quality on the nutritional level. The nutritional quality of meat is influenced by numerous other factors, mainly by its microbiological status. Although, studies about microbes and spoilage in red meat and poultry are known (Huffman, 2002), the knowledge about the microbiological profile of rabbit meat is limited (Badr, 2004; Rodríguez-Calleja et al., 2004; 2005; 2006). The origin and/or occurrence of bacteria in rabbit meat is related with the environment when these animals live as well as with cross-contamination during preslaughter (crating, transportation, holding conditions) and processing (skinning, evisceration, storage) operations.

Nowadays, improvement of animal healthy status and welfare as well as the quality of raw meat and/or meat products by natural antimicrobial compounds has an increasing effect. There are many studies concerning the use of probiotics, bacteriocins, organic acids and plant extracts in rabbit breeding on their growth, performance, microbial stability and enzymatic activity in gastrointestinal tract (Škřivanová and Marounek, 2002; Matusevičius et al., 2004; Lauková et al., 2006; Tayeb et al., 2007; Simonová et al., 2008b; Szabóová et al., 2008a,b). In recent years, naturally occurring
Preparation of Enterococcus faecium CCM4231 for inoculation

MATERIALS & METHODS

Enterococcus faecium CCM 4231 is bacteriocin producing strain with probiotic properties (Lauková et al., 1993), which was applied in different ecosystems, e.g. rumen fluid, milk products, cheeses, fermented meat products and its favourable effect on microbiological profile and/or quality of mentioned products (environments) was shown (Lauková and Czikková, 1998; Lauková et al., 1999a, b; Lauková et al., 2001). Moreover, strain *E. faecium* CCM 4231 was detected as able to synthesize conjugated linoleic acid from linoleic acid (not published data). In relation to these properties of CCM 4231 strain, we decided for its application in rabbit's ecosystem, mainly in connection with quality of rabbit meat.

The objectives of this study were to examine the effect of bacteriocinogenic and probiotic strain *E. faecium* CCM 4231 on physico-chemical traits, amino acid composition and microbial status of rabbit meat (chilled and stored 24 h at 4 °C until microbiological analysis) after rabbit feed supplementation by this strain.

MATERIALS & METHODS

Preparation of Enterococcus faecium CCM4231 for inoculation

To differ *E. faecium* CCM4231 from the other enterococci rifampicin-marked strain was prepared; it was subsequently cultivated using Todd-Hewitt agar (Imuna, Šarišské Michal´any, Slovakia) enriched with rifampicin (100 μg/ml) at 37°C. To prepare the inoculum, rifampicin-marked strain of *E. faecium* CCM4231 was cultivated in broth at 37°C for 18 h. Cells were harvested after centrifugation (2000 g, 30 min) and culture sediment was resuspended in saline buffer (0.85%, pH 7.0) to concentration 10^8 cfu/ml. The solution was kept at 4°C.

Experiment schedule and diet

Forty eight (48) five-week-old Hy-plus breed rabbits of male sex were used. All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals and the trials were agreed by the Ethical Commission of Institute of Animal Physiology in Košice.

Rabbits in the farm were divided into 1 experimental (EG) and 1 control group (CG) of 24 rabbits in each. The experiment lasted for 42 days. Rabbits were kept in standard cages, 2 animals per cage. The rabbits fed the commercial granulated diet for growing rabbits (ANPRO.FEED, VKZ Bučany, Slovakia) and had access to water ad libitum. The chemical composition of the diet was as follows: dry matter, 884 g/kg; crude protein, 173 g/kg; crude fibre, 147 g/kg; fat, 34 g/kg; ash, 71 g/kg; organic compounds, 813 g/kg; starch, 139 g/kg; calcium, 8 g/kg; phosphorus, 5 g/kg; magnesium, 0.9 g/kg; sodium, 1.4 g/kg; potassium, 9.6 g/kg; iron, 289.6 mg/kg; zinc, 0.6 mg/kg. Every day, at the same time in the morning, the rabbits administered an overnight culture of CCM 4231 strain added into the drinking water (500 μl/animal/day). Rabbits in CG were not inoculated and fed not supplemented commercial diet.

Slaughtering and sampling

Three animals from each group were slaughtered at days 21 and 42; they were stunned by electronarcosis (90 V for 5 s), immediately hung by the hind legs at the processing line and quickly bled by cutting the jugular veins and the carotid arteries. After the bleeding, the *Musculus longissimus dorsi* (MLD) were separated by removing skin, fat and connective tissue, chilled and stored 24 h at 4°C until physico-chemical analysis; the *M. biceps femoris* (MBF) muscles were taken from the left side of the carcasses and chilled and stored 24 h at 4 °C until microbiological analysis.

Physico-chemical, amino acids and microbiological analyses

The ultimate pH was determined 48 h post mortem (p.m.) by a Radelkis OP-109 with a combined electrode penetrating 3 mm into the MLD.

Colour measurements were taken on the carcass surface of the MLD. Colour was recorded using a MINISCAN XE plus which gives the average of three measurements of lightness (*L*), redness (*a*) and yellowness (*b*) coordinates.

Total water, protein and fat contents were estimated using an INFRATEC 1265 spectroscop and expressed in g/100g; from these values, the energy value was calculated [EC(kJ/100g) = 16.75 x Protein content + 37.68 x Fat content].

The ash content was determined by mineralisation of samples at 550°C according to STN 570185. Water holding capacity (WHC) was determined by compress method at constant pressure (Hásek and Palanská, 1976). Amino acids were determined in fat-free samples by ion-exchange chromatography (free amino acids) and by liquid chromatography (total amino acids) after acid hydrolysis in 6M HCl. Sulphur amino acids were hydrolyzed with hydrogen peroxide and formic acid. An Amino Acid Analyzer AAA 400 (Ingos a.s., Prague, Czech Republic) was used to separate amino acids.

Bacteria from meat samples (MBF) were selected by a Radelkis OP-109 with a combined electrode penetrating 3 mm into the MLD. Bacterial counts expressed in colony forming units (log 10 cfu/g) were determined by plating the diluted samples on a standard microbiological method using the appropriate dilutions in Buffered Peptone Water (Biomark, Pune, India). Bacterial counts expressed in colony forming units (log 10 cfu/g) were determined by plating the diluted samples on following media (ISO): Kanamycin Esculin Azide agar (Biomark) for enterococci, Violet Red Bile Glucose agar (Biomark) for *Escherichia coli*, Mannitol Salt agar (Becton & Dickinson, Cockeysville, USA) for coagulase-negative staphylococci (CNS), Baird-Parker agar enriched with Egg Yolk Tellurite supplement (Becton & Dickinson) for coagulase-positive staphylococci (CPS) and *Staphylococcus aureus* and...
RESULTS AND DISCUSSION

Physico-chemical properties of rabbit meat are presented in Table 1a. Addition of *E. faecium* CCM4231 (EG) did not significantly affect the physico-chemical composition of meat; higher values of yellowness (*b*ʹ), ash and water holding capacity (WHC) were detected compared to control animals. Dal Bosco et al. (2001) presented lower pH values in fresh rabbit meat after vitamin E addition as well as in control group (5.55; 5.42) than it was observed by us (EG: 5.61; CG: 5.68). On the other hand, lower pH values in rabbit meat measured after 48 hours by us in both, EG and CG than it was reported by other authors after 24 hours (Dalle Zotte and Ouhayoun, 1998; Lamberrini et al., 2006; Polak et al., 2006). Decreased of pH value in EG could be explained by depletion of glycogen reserve in muscles during storing and refrigeration. On the other hand, low pH is also important because of its bacteriostatic effect on meats. Moreover, pH is closely related to colour parameters (lightness, redness, yellowness) due to influence the muscle texture and the oxidation of pigments. It is also known, that lightness is negatively correlated to pH value, e.g. the lower pH, the higher lightness. While several authors confirmed this negative correlation (Dalle Zotte and Ouhayoun, 1998; Dal Bosco et al., 2001), our results were contradictory to those; it means that rabbit meat possessed lower muscle lightness, even though it did not has higher pH. The authors mentioned above presented also positive correlation between pH and red colour of meat, in accordance to our results. In our study, higher values of yellowness in both groups were measured compared to data presented by Dalle Zotte and Ouhayoun (1998) and Polak et al. (2006).

**TABLE 1a. Biochemical and biophysical composition of rabbits meat**

<table>
<thead>
<tr>
<th>N=3</th>
<th>EG - CCM 4231</th>
<th>CG</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH48</td>
<td>5.61 ± 0.04</td>
<td>5.68 ± 0.13</td>
</tr>
<tr>
<td>L* (lightness)</td>
<td>46.31 ± 3.45</td>
<td>49.15 ± 0.84</td>
</tr>
<tr>
<td>a* (redness)</td>
<td>2.58 ± 2.22</td>
<td>3.93 ± 1.84</td>
</tr>
<tr>
<td>b* (yellowness)</td>
<td>8.68 ± 0.67</td>
<td>8.57 ± 0.16</td>
</tr>
<tr>
<td>Water content (g/100g)</td>
<td>76.17 ± 0.15</td>
<td>75.97 ± 0.15</td>
</tr>
<tr>
<td>Protein content (g/100g)</td>
<td>21.60 ± 0.10</td>
<td>21.63 ± 0.15</td>
</tr>
<tr>
<td>Fat content (g/100g)</td>
<td>1.20 ± 0.10</td>
<td>1.40 ± 0.20</td>
</tr>
<tr>
<td>Energy value (KJ/100g)</td>
<td>407.02 ± 33.27</td>
<td>415.11 ± 6.17</td>
</tr>
<tr>
<td>WHC (g/100g)</td>
<td>32.82 ± 2.58</td>
<td>33.30 ± 2.94</td>
</tr>
<tr>
<td>Ash (g/100g)</td>
<td>1.033 ± 0.058</td>
<td>1.000 ± 0.001</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD. EG - experimental group, CG - control group, WHC - water holding capacity.
acid, we detected higher level of all AAs in rabbits compared to ostrich, chicken and beef (Sales and Hayes, 1996). Our findings provided basic and primary knowledge about the AA content of rabbit meat; these components also indicate its high biological and nutritional value.

### TABLE 2. Total counts of checked bacteria in rabbit meat (log10 cfu/g)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>N=3 Day 21</th>
<th>N=3 Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus sp.</td>
<td>1.93 ± 0.66</td>
<td>1.19 ± 0.16</td>
</tr>
<tr>
<td>CNS</td>
<td>2.81 ± 0.74</td>
<td>2.71 ± 0.66</td>
</tr>
<tr>
<td>CPS</td>
<td>4.16 ± 0.23</td>
<td>4.09 ± 0.68</td>
</tr>
<tr>
<td>S. aureus</td>
<td>4.77*</td>
<td>3.11*</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt; 1.00</td>
<td>1.13 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.30*</td>
</tr>
</tbody>
</table>

At day 21, colonies of *Proteus vulgaris* were not determined. sp. - species, CNS - coagulase-negative staphylococci, CPS - coagulase-positive staphylococci, ND - not determined, a-occurrence only in one sample, * - P<0.05

The quality of rabbit meat is also depending on its microbiological profile; the occurrence of bacteria in meat closely influences the safety and healthy of raw meat and/or meat products. Data concerning the microbial status of rabbit meat after 24 h storage at 4 °C temperature are presented in Table 2. At the end of the CCM 4231 application (day 21), *E. coli* bacteria were detected in lower counts in EG than in CG; *Staphylococcus aureus* occurred only individually, e.i. in one sample from three tested in both groups. At day 42 (the end of the experiment), the reduction of all tested bacteria – except the coagulase-positive staphylococci was observed in both, EG and CG. The counts of *E. coli* varied in the range from < 1.00 to 2.32; these data are in accordance with those presented by Rodríguez-Calleja et al. (2005; 0.49 – 4.00 cfu/g) and were lower than data showed by Badr (2004; 4.79 cfu/g).

On the basis of our results we can summarized that *E. faecium* CCM 4231 strain did not have a negative influence on the physico-chemical composition of meat; moreover, the concentration of threonine (essential amino acid) and serine significantly increased. The counts of tested bacteria, mainly of *E. coli* were also detected in low level in group administering CCM 4231 strain after 24 h storage in the refrigerator. We can concluded that improvement of rabbit meat quality and nutritional value by this bacteriocinogenic strain with probiotic properties is promising. Of course, detail studies are in progress and needed to spread the recent knowledge.

### ACKNOWLEDGEMENT

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### REFERENCES


