THE EFFECT OF ADDING A MIXTURE OF PROBIOTIC STRAINS TO THE FEED OF RABBITS ON THEIR HEALTH AND PERFORMANCE STATUS

Nikolay Karapetkov
Lactina Ltd. – Bankya, Bulgaria
E-mail: nikolay.karapetkov@lactina-ltd.com

ABSTRACT

Six commercial probiotic strains (*Lactobacillus delbrueckii ssp. bulgaricus NBIMCC 8244, Lactobacillus acidophilus NBIMCC 8242, Lactobacillus helveticus NBIMCC 8269, Lactobacillus delbrueckii ssp. lactis NBIMCC 8250, Streptococcus thermophilus NBIMCC 8253 and Enterococcus faecium NBIMCC 8270*) were selected for a feeding trial with White New-Zealand rabbits. A study was conducted considering common farming practices in the European Union by targeting sensitive parameters in comparison to a negative control group. Characteristics like body weight (at the beginning and at the end of the trial), feed intake, mortality/morbidity and survival in the gastrointestinal (GI) tract were observed. A significantly higher bodyweight was achieved when using the probiotic mixture in the feed during the experiment with rabbits from birth to weaning at 35 days of age and from weaning to 77th day, compared with the control negative group without addition of commensals by 8.5% ($P < 0.05$). This body weight gain (BWG) was probably mainly a protein anabolism inducing lean meat formation and thus giving an additional benefit to farmers. The results suggest that the diversity of gut microbiota affects weight development of rabbits and that intentional manipulation of community structure may be useful for regulating energy balance in animals. The survival rates of freshly prepared cultures of the strains in simulated gastric and intestinal juice at pH 2.5 and 7 were tested and some of the strains showed good tolerance to these conditions. The use of lactobacilli probiotics, in view of the significant improvement of growth parameters in animals, should be carefully monitored, and the advantages of using these and new strains should be considered in a well-contemplated analysis.

Key words: Probiotics, rabbits, lactic acid bacteria, productivity, body weight gain, transit tolerance

Lactic acid bacteria (LAB) can be used in animal nutrition as probiotics and as a source of other feed additives like amino acids, enzymes and vitamins, which could confer health benefits and affect favorably the zoo technical performance of animals (Davis et al., 2008). The bacterial beneficial modes of action include regulation of intestinal microbial balance, enhancing the gastrointestinal barrier function (García-Lafuente et al., 2001), expression of bacteriocines (Du Toit et al., 2001), enzymatic activity inducing absorption and nutrition (de Lange et al, 2010), immunomodulatory effects (Pouwels et al., 1996) and prevention of pathogens to colonize and infect the mucosa. The use of live bacterial cultures in the animal industry, whether to improve resistance to specific pathogens or to enhance animal health, also improves production parameters (Kenny et al., 2011) like increasing the feed utilization efficiency (Shim et al., 2012), average daily weight gain through improvement of body weight gain (BWG) (Angelakis & Raoult, 2010; Zokaeifar et al., 2012), better digestibility of nutrients and improvement of gut health (Kim et al., 2012). However, the live weight gain hypothesis is not undisputed. Bernardeau & Vernoux (2013) suggested that BWG was found in only around 25% of published data, therefore it is not

a common phenomenon. When BWG was found in the literature it was in the range of 10% or lower. Significant weight gain in animals was reported to be species dependent, furthermore the administration of some lactic acid bacteria was even associated with weight loss (Million et al., 2012). Regardless, animal models of obesity have demonstrated an association between the alteration of the microbiota composition with the development of obesity (Musso et al., 2010, Liou et al., 2013).

The selection of strains for the present study was based on basic in vitro prerequisite criteria, related to identification, gut survival and colonization ability and safety but also parameters like feeding, compatibility with other additives, animal category, dose and used technology. The aim of this study was to determine the efficacy and safety of the strains and to assess the effect of the mixture of the six bacterial strains on rabbits on a normal diet.

MATERIALS AND METHODS

Bacterial strains:

Enterococcus faecium NBIMCC 8270, Lactobacillus acidophilus NBIMCC 8242, Lactobacillus helveticus NBIMCC 8269, Lactobacillus delbrueckii ssp. lactis NBIMCC 8250, Lactobacillus delbrueckii ssp. bulgaricus NBIMCC 8244 and *Streptococcus thermophilus NBIMCC 8253* are part of the culture collection of Lactina Ltd. (Bankya, Bulgaria), which were selected as a probiotic preparation in order to cover a wide range of possible probiotic effects. The total cell count of the six strains, which were incorporated in equal proportions was > 5.109 CFU/g.

Bile-salt tolerance:

In order to exert a beneficial effect, it is generally considered important that commensals remain viable during transit of the gastrointestinal tract (GIT) in sufficiently high concentrations either to colonize or to positively affect the host. In vitro determination of viability under conditions similar to those prevailing in the GIT was performed, according to Charteris et al., 1998. Simulated gastric juice was prepared by suspending pepsin (3 mg/ml; Sigma) sterile sodium chloride solution (0.5% w/v) and adjusting the pH to 2.5 with HCl (37%). Portions (0.2 ml) of washed cell suspensions of the strains in phosphate buffered saline (PBS pH 7.0), were inoculated in 1.0 ml of simulated gastric or pancreatic juice and 0.3 ml NaCl (0.5% w/v), mixed and incubated at 37 °C. Total viable counts (CFU/ml) were evaluated at 1, 60 and 120 min in cultures tested for gastric transit tolerance, and at 1, 120 and 240 min in tests for small intestinal transit tolerance. The initial viable count (CFU/ml) of the washed cell suspension from each strain tested was determined prior to the transit tolerance assay and was used to calculate loss of viability.

Preparation of the six probiotic strains for the animal trial:

The pure cultures of *Enterococcus faecium NBIMCC 8270, Lactobacillus acidophilus NBIMCC 8242, Lactobacillus helveticus NBIMCC 8269, Lactobacillus delbrueckii ssp. lactis NBIMCC 8250, Lactobacillus delbrueckii ssp. bulgaricus NBIMCC 8244* and *Streptococcus thermophilus NBIMCC 8253* were cultivated in skimmed milk in a 500 l online fermenter (3C, France) by the company Lactina Ltd. (Bankya, Bulgaria). An inoculum of 10 l in skimmed milk was transferred to 500 l sterilized inoculation medium (6 kg sucrose, 0.6 kg yeast extract, 0.2 kg monosodium glutamate). After fermentation and concentration, the cell biomass was freeze-dried reaching a cell count of 5.109 CFU/g.

Animal trial and diet. Experimental design:

The experiment involved the offspring of ten female rabbits of the breed White New-Zealand rabbit, which were aligned on age and insemination rate. Each group contained two female rabbits with little born rabbits, whose offspring formed the respective group in accordance with the design of the experiment (Table 1). The little rabbits born in the first group were 15, in the second group they were 14, in the third, fourth and fifth group there were 15. During the suckling period until weaning at age of 35 days there was no insemination of the animals. After weaning, the sex of the animals was determined for each group. The first group had eight males, seven fe-

male little rabbits, the second group had seven males, seven female little rabbits, the third group had seven males, and eight female young rabbits, the fourth group had eight males and seven female little rabbits, while in the fifth group had seven male and eight female little rabbits. In the experiment participated 74 young rabbits which after weaning were distributed in five groups for fattening; each group had three repetitions, so that the trials began from birth until slaughtering age each rabbit obtained probiotic preparation in accordance with the scheme of the experiment. Total duration of the trial was 77 days.

Breeding conditions:

The rabbit-breeding farm (Sanadinovo, Bulgaria) practiced a semi-intensive cycle of insemination, in which 8–9 inseminations were carried out per year. The female rabbits were inseminated 10–14 days after giving birth. The weaning of the little rabbits was done on the 35th day, and they were sorted in groups and accommodated in cells for fattening. The rabbit does were divided one by one in each cell, while the little rabbits for fattening – five of them in each cell. Each little rabbit had an area of 0.08–0.10 m^2 at its disposal, calculated based on the floor area.

Fodder and feeding:

During the entire experimental period the rabbits obtained a whole ration of combined fodder, whose composition included grain feed of wheat, maize, oats and barley; the proteins were supplied with the soya groats and sunflower groats, raw fodder such as Lucerne meal, side products such as wheat whole meal and some additives like whitening, salt and vitamin-microelements pre-mixture. The combined fodders were produced in the fodder factory, belonging to the rabbit-breeding farm. The fodder production line included a grinding mill, mixer of 300-kilogram capacity and granulating press. The first 300 kg of the basic mixture were produced without the addition of probiotics in the form of flour meal. Then a definite amount of the basic mixture was weighed and the feed additive was added to it in accordance with the experimental scheme, the mixture was homogenized in a mixer of capacity 100 kg and thereafter it was granulated. The data from the analyses of not granulated fodder showed that the content of raw fibrous material is 10.61% ± 0.13, for the granulated fodder the content is 9.94% ± 0.13. The concentration of the probiotic strains in the fodder intended for the experimental groups was 1.5.109 CFU/kg (300 g/t feed group), 2.5.109 CFU/kg (500 g/t feed group), 3.5.109 CFU/kg (700 g/t feed group) and 5.109 CFU/kg complete feeding stuff (1000 g/t feed group).

Controlled parameters:

Studies were designed to demonstrate the efficacy of the additive – normally of the lowest recommended dose – by targeting sensitive parameters in comparison to a negative control group.

In the course of the trial, the following indicators were observed:

- Survival of the rabbits – day by day and group by group;
- Reasons for the rabbits that had dropped off (if any);
- Body weight on the 1st, 7th, and on the 35th day at weaning, on the 56th and on the 77th day;

Table 1. Experimental scheme of first efficacy trial: five groups, each group has three repetitions, each repetition has five animals

Group	Combined fodder	Content of probiotic strains g/t fodder
First group – control group	Granulated combined fodder for rabbits	0
Second group – experimental group	Granulated combined fodder for rabbits	300
Third group – experimental group	Granulated combined fodder for rabbits	500
Fourth group – experimental group	Granulated combined fodder for rabbits	700
Fifth group – experimental group	Granulated combined fodder for rabbits	1000

- Body mass growth by period;
- Consumption of fodder;
- Fodder expenditure per unit of body mass growth;
- Content of microorganisms in the starter, the grower and in the finisher mixture;
- Content of raw protein and raw fibers in the basic mixture prior to and after the granulation.

Statistical analyses:

For mathematical processing of the obtained results and for establishing the reliability of the data for the differences between the groups we used IBM SPSS Statistics 22 and Origin Lab 8. The rabbits of each group were measured one by one individually. The statistical processing of the data on the body weights was done on the basis of the individual measurements of the weights of all the rabbits of the group. The statistical processing of the data on the consumption of the fodder and the utilization of the fodder was done on the basis of the average values of the data for each repetition of the group i.e. three repetitions in a group with all five groups.

RESULTS AND DISCUSSION. CHARACTERIZATION OF STRAINS

Bile-salt tolerance:

Bile-salt tolerance of the six strains was quantified according to Charteris et al. (1998). The results are shown in Figures 1. Bile-salt tolerance is important for strains to grow and survive in the upper small intestine. The in vitro tests in conditions similar to those prevailing in the upper parts of the GIT, proved a species-dependent tolerance to simulated gastric juice, containing pepsin (0.3% w/v) (pH 2.5) and a simulated small intestinal juice with bile salts (pH 7). The exposure to pH 2.5 was more destructive to all strains than the exposure to bile salts and only the strains *Streptococcus thermophilus NBIMCC 8253* and *Enterococcus faecium NBIMCC* 8270 were more resistant at acid conditions where all other isolates lost > 90% viability during simulated gastric transit. *Lactobacillus lactis NBIMCC 8250, Streptococcus thermophilus NBIMCC 8253* and *Enterococcus faecium NBIMCC 8270* showed high transit tolerance in 0,3% bile acid.

Lactobacillus bulgaricus NBIMCC 8244 and *L. helveticus NBIMCC 8269* were able to grow at pH 3 and 4 and may thus be regarded as acid tolerant (results not shown). Acid-tolerant strains have an advantage in surviving the low pH conditions in the stomach (pH 2.0 in extreme cases), where hydrochloric and gastric acids are secreted. *Streptococcus thermophilus NBIMCC 8253* and *Enterococcus faecium NBIMCC 8270* did not grow at pH 3.0; speculatively these strains might survive passage of the stomach, although in lower numbers than the other two strains.

Feeding trial rabbits:

The average body weight of the little rabbits on the first day was within the limits of 58.5 g for the fifth group and up to 64.3 g for the fourth group. The body weight of the newly born rabbits in the first group was within these limits too – 60.7 g, second group – 60.6 g and third group – 59.8 g. Table 2 represents the data on the body weight increase of the rabbits during different growing periods and group by group.

The groups were well aligned on this indicator, while the difference between the separate groups was not statistically significant. The individual measurements of the little rabbits at the age of seven days showed average body weight 150.2 g, 140.2 g, 150.5 g and 153.6 g respectively for the control group and the experimental groups (300, 500, 700 and 1000 g probiotics/t feed). Considering these values, it is not yet possible to find a correlation between the body weight increase of the little rabbits and the tested probiotic, because they were still suckling and they did not consume any fodder yet.

Fifteen days after the start of the trial the little rabbits started to accept the fodder mixture too, but they continued suckling from their mothers. At weaning, on day 35, the body weights of the animals were measured. The data shows lowest body weight of the rabbits, belonging to the control group, which did not receive the tested probiotic – 770.7 g. The body weight of the rabbits belonging to the group with lowest dose of added commensals – 300 g/t of fodder was 3.5% higher, however the increase was nonsignificant.

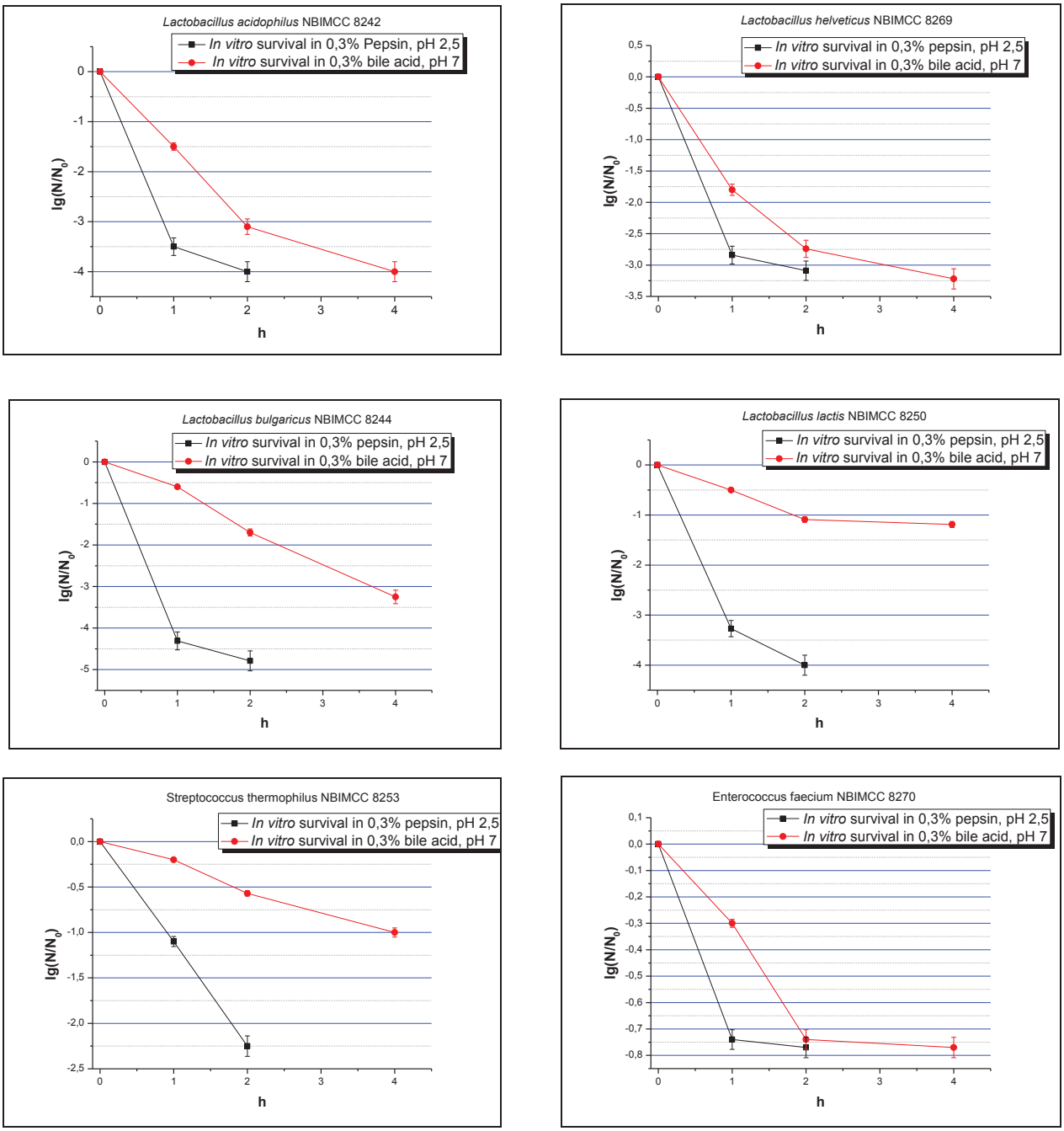

Fig. 1. *In vitro* survival in the conditions of GIT of *Lactobacillus acidophilus* NBIMCC 8242 in 0.3% Pepsin, pH 2.5, at start, in one and two hours and in 0.3% bile acid, pH 7, at start, in two and four hours. Data are expressed as mean ± SEM

The animals in the third (500 g probiotics/t feed) and fourth group (700 g probiotic/t feed) grew practically at the same pace, outperforming the control group by 15% ($P < 0.01$). The most obvious effect of the addition of the probiotic mixture was observed in the fifth group where the highest dose of the probiotic was added – 1000 g per ton fodder. The results show a positive effect of the probiotic mixture at doses of 500 (2.5.109 CFU/kg feed), 700 (3.5.109 CFU/kg feed) and 1000 g (5.109 CFU/kg feed) per ton of fodder for weaned rabbits at 35 days of age. During the fat-

Table 2. Body weight of the rabbits, g

Groups Indices	Control group 1	300 g probiotic/t feed group 2	500 g probiotic/t feed group 3	700 g/t probiotic/t feed group 4	1000 g/t probiotic/t feed group 5
1st day					
X ± Sd	60.7 ± 4.62	60.0 ± 6.73	59.8 ± 9.31	64.3 ± 17.16	58.5 ± 7.73
SEM	1.19	1.80	2.40	4.43	1.99
Vc %	7.61	11.22	15.57	26.67	13.21
Reliability*	NS				
7th day					
X ± Sd	150.2 ± 15.16	140.2 ± 15.40	150.5 ± 24.61	150.9 ± 18.38	153.6 ± 14.46
SEM	3.91	4.12	6.35	4.74	3.73
Vc %	10.09	10.98	16.35	12.18	9.41
Reliability*	NS				
35th day					
X ± Sd	770.7 ± 87.54	797.9 ± 70.73	898.0 ± 118.15	884.0 ± 103.84	980.7 ± 96.25
SEM	22.6	18.9	30.51	26.81	24.85
Vc %	11.36	8.86	13.16	11.75	9.81
Reliability**	1:3**; 1:4**; 1:5***; 2:3*; 2:4*; 2:5***; 3:5*; 4:5*				
56th day					
X ± Sd	1430.7 ± 228	1569.3 ± 250	1516.7 ± 424	1635.3 ± 312	1696.7 ± 291
SEM	58.9	66.9	109.5	80.8	75.2
Vc %	15.94	15.97	27.95	19.12	17.16
Reliability*	1:4*;1:5**				
77th day					
X ± Sd	2177.1 ± 305	2241.3 ± 229	2333.3 ± 282	2402.7 ± 289	2401.3 ± 190
SEM	81.6	63.6	72.9	74.80	49.1
Vc %	14.00	10.21	12.08	12.03	7.49
Reliability*	1:4*; 1:5*; 2:5*				

$P < 0.05^{*}$; $P < 0.01^{**}$; $P < 0.001^{***}$; NS – no reliability. The figures denote the number of groups

tening period from 35th to 56th day the animals reached body weight between 1430.7 and 1696.7 g (Figure 2). Significant was only the difference between the control and the fourth and fifth and the control group, which overweight the group without feed additive by 14.3% and 18.6% respectively ($P < 0.05$).

At the end of the experimental period, on the 77th day the rabbits reached body weight as follows: 2177.1 g, 2241.3 g, 2333.3 g and 2401.3 g. (Figure 3). The higher body weight of the rabbits from the fourth and fifth group (700 g and 1000 g additive per ton feed) was significantly higher ($P < 0.05$) in comparison with the control group. The results for these two groups were practically the same – 10% higher body weight.

Figures 4 and Figure 5 show the average daily growth of rabbits by groups on the 56th and 77th days. The rabbits of the control group, which did not receive probiotic had the lowest average daily growth, and the highest – those of the fourth and fifth groups. For the period from the 1st until the 56th day the average daily growth of rabbits by groups is as follows: 24.46 g, 26.95 g, 26.01 g, 28.05 g and 29.25 g. For the entire experimental period from birth to the 77th day, the average daily growth by groups was as follows: 27.48 g, 28.33 g, 29.52 g, 30.37 g and 30.42 g. The results indicate the same trend as in the first period – 3.6 % higher growth at the lowest dose compared with the control group, 7.4% higher growth at a dose of 500 g per ton of fodder probiotic mixture and 10.5% higher growth rate in the fourth and fifth groups.

Feed consumption and conversion:

Table 3 presents data for the total quantity of consumed fodder by groups throughout the experimental period, the daily consumption of one

rabbit and the consumption of fodder per unit of growth. Mathematical processing was done on the basis of the average figures of the three repetitions for each group. From birth until the 77th day the rabbits were consumed total: the first group – 136.62 kg fodder, the second – 123.57 kg, the third – 135.6 kg, the fourth – 135.57 kg and the fifth group – 130.20 kg. For the period

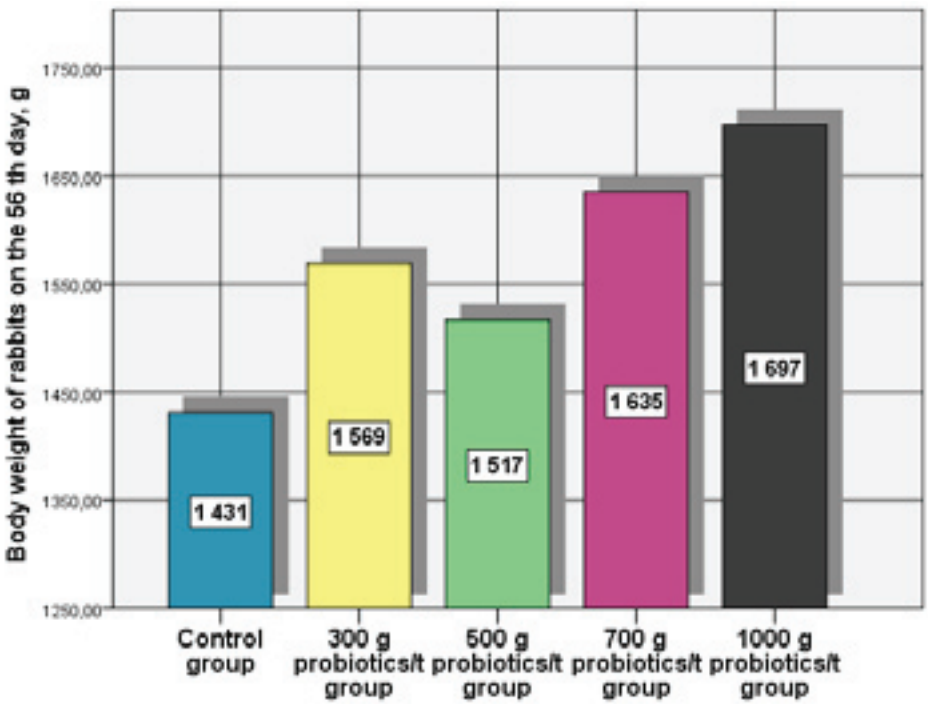

Fig. 2. Body weight of rabbits on the 56 th day, grams

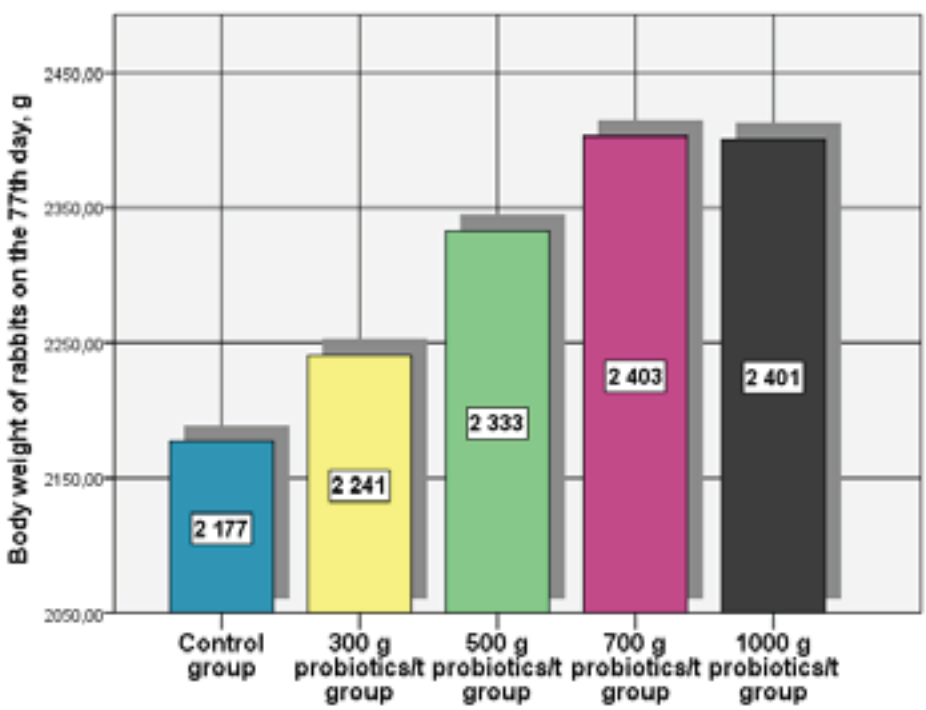

Fig. 3. Body weight of rabbits on the 77th day, grams

from the 1st to 77th day i.e. from birth to the end of the experiment, the average daily consumption of fodder for a rabbit was between 113 and 118 g. As a percentage of the control group the figure was respectively 100%, 90.5%, 99.3%, 99.3% and 95.3%. During the experiment, the consumption of fodder per 1 kg weight growth increase by groups were as follow: 3.232 kg,

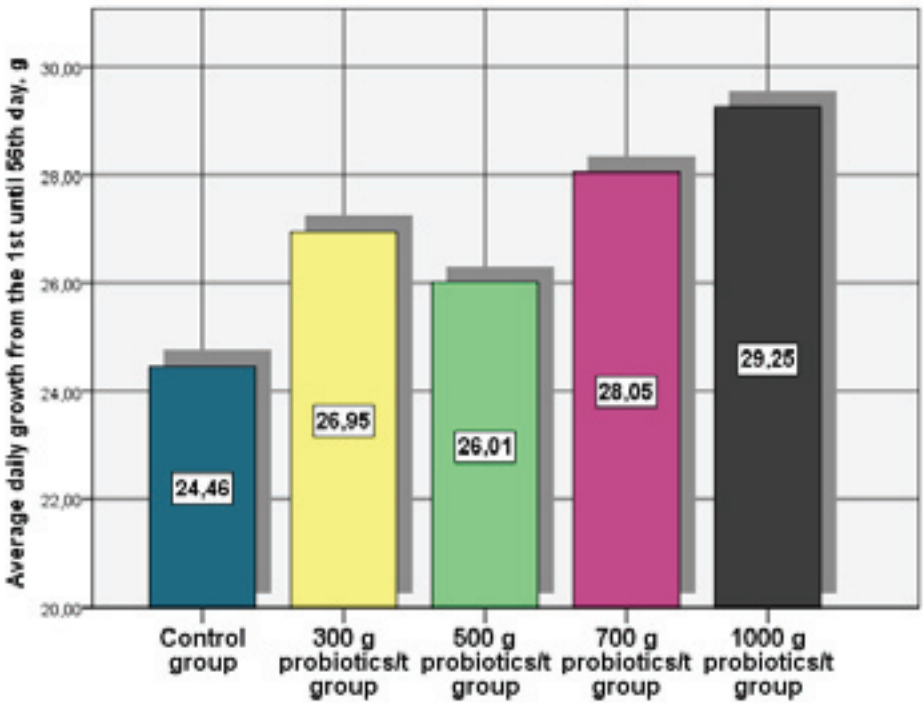

Fig. 4. Average daily growth from the 1st until 56th day, grams

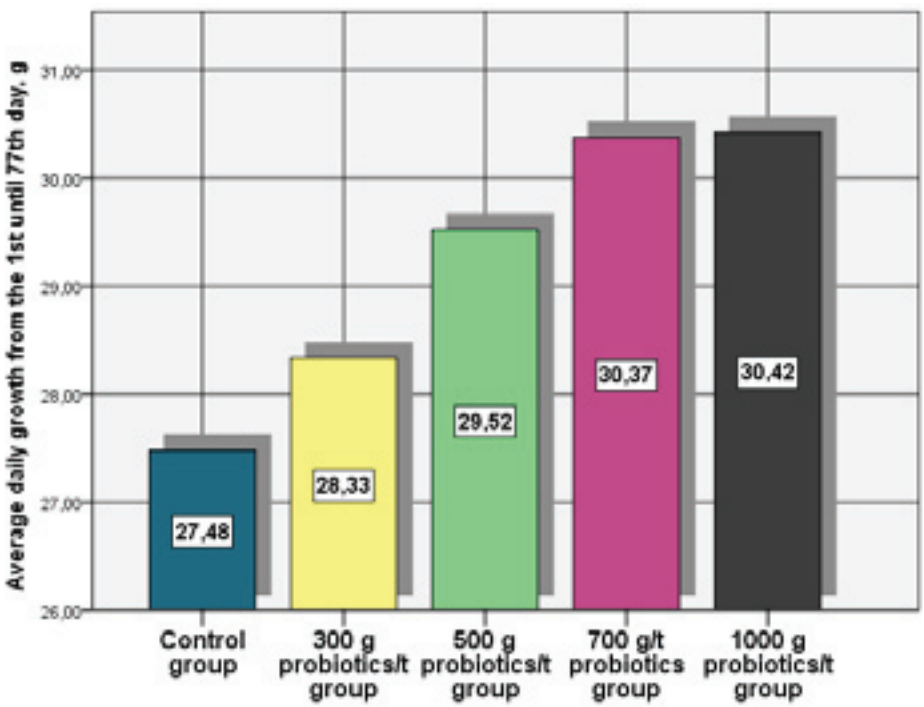

Fig. 5. Average daily growth from the 1st until 77th day, grams

3.022 kg, 2.924 kg, 2.836 kg and 2.724 kg. The feed utilization in all experimental groups compared to the control group was better by 6.5% to 15.7% (Figure 6).

Study on meat quality:

After completion of the experiment, four male rabbits from four groups (control, 500 g/t feed, 700 g/t feed and 1000 g/t feed) with bodyweight close to the average for the group were slaughtered. The veterinary examination and analysis covered carcass, cuts of the body parts and internal organs: heart, liver, lungs, spleen, muscular and glandular stomach, digestive tract. Results from this carcass analysis are presented in Table 4.

Significant differences in performance between the studied groups were observed. The yield of the carcass was virtually identical in all

Table 3. Consumption of fodder and fodder conversion

Groups Indices	Control group	300 g probiotics/t group	500 g/t probiotics group	700 g/t probiotics group	1000 g/t probiotics group
Consumed Fodder, total, kg	136.62	123.57	135.60	135.57	130.20
Consumed fodder, total 1–77 day, g/rabbit/day	118 + 10.59	107 + 6.72	117 + 6.74	117 + 5.31	113 + 11.39
% to group 1	100.0	90.7	99.5	99.5	95.8
Fodder for 1 kg body weight increase, %	3.232 + 0.13	3.022 + 0.10	2.924 + 0.13	2.836 + 0.07	2.724 + 0.12
% to 1st group	100	93.5	90.5	87.7	84.3
Reliability	1:3*; 1:4**; 1:5**;				

P < 0.05; P < 0.01***

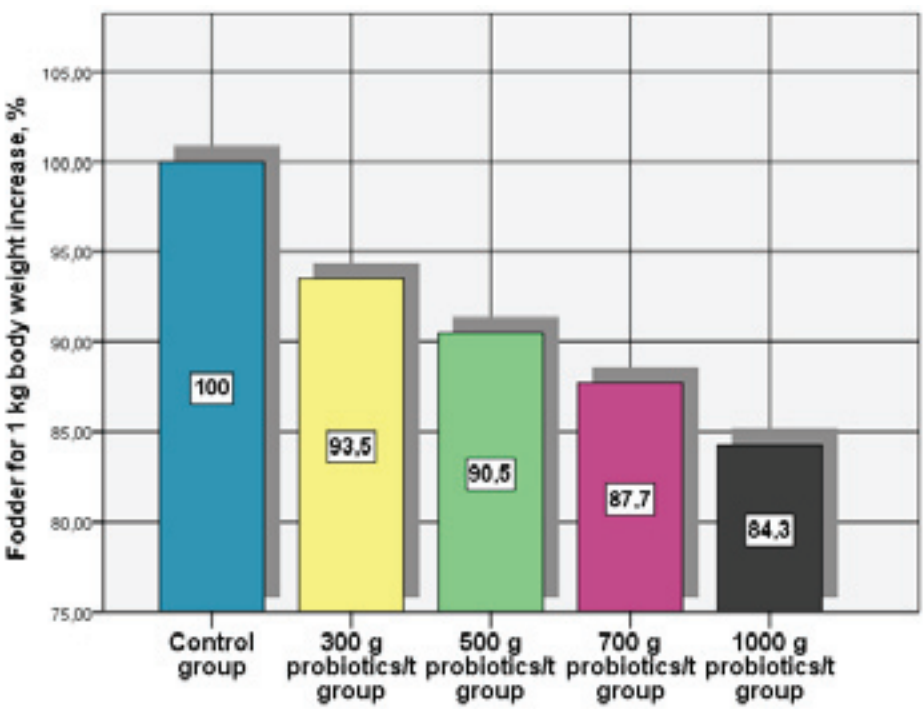

Fig. 6. Fodder per 1 kg body weight increase, %

Table 4. Results of the rabbits' carcass analysis

Indexes	Control group	500 g probiotics/t feed group	700 g probiotics/t feed group	1000 g probiotics/t feed group
Corps, g	1310 ± 114	1395 ± 126	1408 ± 153	1473 ± 118
Corps yield rate, %	52.2	52.6	54.4	52.6
Testicles, g	5.85 ± 2.09	5.45 ± 2.01	7.73 ± 2.22	7.05 ± 1.34
Kidneys, g	16.52 ± 2.98	16.62 ± 2.79	19.05 ± 4.59	19.67 ± 4.56
Lung, g	14.3 ± 3.41	14.45 ± 1.74	17.87 ± 1.33	15.90 ± 2.01
Liver, g	76.13 ± 14.71	82.52 ± 9.15	74.10 ± 10.54	82.80 ± 3.57
Heart, g	7.33 ± 1.38	7.85 ± 0.45	8.47 ± 1.49	8.25 ± 1.05
Spleen, g	2.97 ± 0.39	2.00 ± 0.22	2.27 ± 0.56	2.25 ± 0.70
Stomach, g	24.05 ± 5.98	26.17 ± 2.84	24.65 ± 0.62	27.10 ± 3.34

groups 52–54%, however the absolute weight values of the various internal organs (hearts, lungs, kidneys, testicles) in all groups were non identical – the organs of the animals in the experimental groups with supplementation of probiotics to the feed outperform the animals in the control group in terms of weight. This indicates that modulation of gut microbiota plays important role in animal's health and production.

Pathological-anatomical examination:

Test animals were routinely monitored for visual evidence of clinical effects, performance characteristics, product quality and for other parameters likely to be related to the biological properties of the bacterial additive. Critical endpoints known from the toxicological studies in laboratory animals were considered. No adverse effects could be detected during the efficacy trials. The pathologic-anatomical examination of the rabbits (Table 5) showed normally developed organisms without any visible pathogenic changes.

Health status of the rabbits:

During the test period two rabbits dropped out of the control group before they could reach the age of seven days, one bunny from the group fed with the recommended optimal dose (500 g/t feed) and one from the 700 g/t feed group, immediately after birth. From the fifth group 1000 g/t feed) there are no dropped out animals during the experiment.

DISCUSSION

The stress caused by incubation in acidic conditions, assayed on the six LAB strains with the best ability to grow in presence of bile salts, was observed on Enterococcus faecium NBIMCC 8270, which suffered a major injury during the first two hours, however showing noticeable adaptability in unfavorable conditions after the second hour of incubation. In all other strains exposure to acidic environment caused significant loss of activity. Since bacterial transit tolerance to gastric and bile acids is considered one of the crucial selection criteria for probiotic strains (Succi et al., 2005; Saarela et al., 2000), we determined the survival capacities of the strains strain. The investigation was performed using simulated bile acid and pepsin as an in vitro model for intestinal conditions.

The data are strongly indicative of a meaningful stimulation of the growth and development of the little rabbits compared to the control group without additive. The difference between the control group and the groups with the highest doses probiotic/t feed is statistically reliable ($P < 0.01$). During this period the results are also influenced by the factor milk giving ability of the mother and as the little rabbits start consuming fodder after the 12–15th day from their birth, the obtained results should not be explained only

Table 5. Summary of the rabbits' carcass analysis

Parameter	Observation
External examining of the rabbits	Fur is preserved. Not observed shaggy and breaches in the skin integrity. Traces of contamination, due to digestive disorders lack at the anal part.
Pathologic-anatomical findings	There were no observed subcutaneous bleedings.
Muscularity	It is pale pink and with no-subcutaneous fat
Internal abdominal organs	The abdominal cavity was without increased fluid. The internal abdominal organs were developed normally
Liver	It was with brown red color, preserved lobules, each with sharp edges. The visceral part of the gall was with normal size. Some of the gallbladders were filled with bile juices with dark green color
Stomach	The mucosa was without redness, bleedings and with numerous folds
Small intestine	They were with moist serosa, preserved motility and elasticity. The mucosa of the small intestine was without hyperemia and bleedings. In the lumen, there was a presence of a small amount of mucus and food with a thick pale greenish color and without any gasses
Colon	On the serous membrane, well-defined bands of Peyer's patches can be seen, mucous were without bleedings. The lumen of the intestines was filled with greenish paste content
Rectum	There were well-formed faeces
Mesenteric lymph nodes	There were enlarged and not swollen, with milky pale yellowish color, with no bleedings and with smooth cut surface
Mesentery	It was without oedema with clear and visible blood vessels
Spleen	It was normal, without bleedings or infarctions, with preserved sharp edges
Kidneys	They had normal size, not enlarged, with chocolate brown color, at cut on the papillae are not observed bleedings. In the renal pelvis were observed no gallstones
Bladder	There was presence of a small amount of yellowish liquid, the internal surface was without any bleedings
Lungs	They had a pale pink color, with no bleedings, in some with aspirated blood
Heart	It had brown red color, flexible, well-defined blood vessels with minimal pericardial fluid. Pericardium, myocardium and endocardium were without bleedings

on the basis of the effect of the tested feed additive. At the age of 77 days the rabbits, which had obtained probiotics at a dose of 700 and 1000 g/t fodder reached virtually the same body weight – 10% higher compared to the control group ($P < 0.05$). This BWG is likely to be a rather protein anabolism inducing lean meat formation rather than a fatty weight gain and is therefore compliant with consumer health. It has been demonstrated that in all the experimental groups the fodder consumed for 1 kg body weight was lower by 6% to 16% compared to the control group. The best feed utilization was observed in the 700 and 1000 g probiotics/t feed groups (3.5.109 CFU/kg and 5.109 CFU/kg complete feeding stuff respectively). The results suggest that intentional manipulation of community structure of the gut microbiota may be useful for regulating energy balance in animals.

The findings of this study are in alignment with the observations of Zeng et al. (2015), who reported that gut microbiota is also associated with the growth of rex rabbits. Bosi and Trevisi (2010) also claimed that in animals, ingestion of a supplemented diet containing selected microorganisms presented as probiotics makes it possible to counteract some of the negative effects of stress and leads to a compensatory BWG, proving that health benefits and zoo technical benefits are closely related. On the other hand,

analysis of the health status of the rabbits during the feed trial revealed that the use of live bacterial cultures in the animal industry not only improves growth but could also enhance the survival and thus reduce mortality among them. This is in agreement with results obtained by Alexopoulos et al. (2001) who concluded that administration of Bacillus cereus spores in dams during the end of pregnancy and during lactation, as well as to their offspring during suckling and the flat-deck period is beneficial for the life expectancy and performance of the piglets. Hypothetical modes of action include production of vitamins, modulation of the intestinal microbiota or anti-inflammatory properties (Cousin et al., 2012), greater crude protein retention (Shim et al., 2012), protection against Shiga toxin-producing E. coli O157:H7 (Ogawa et al., 2001), competitive exclusion, induction of digestive enzymes stimulate the natural digestive enzyme activity of the host (Zokaeifar et al., 2012).

CONCLUSIONS

The main point identified here concerning the action of beneficial microorganisms added to feed on farm animals is that BWG is significant after reaching certain concentration of the bacteria in the feed. Data collected during this experiment are in support of the existence of a link between probiotics and weight development in animals and the possibility that beneficial microorganisms with probiotic effects may be helpful on animals. Considering the body weight probiotic effect of the LAB strains and their transit tolerance, the need for further studies is apparent including involvement of a larger number of experimental animals in order to substantiate a health benefit during long-term use of probiotics and understand the modes of action and effectiveness of various probiotic strains in animals.

ACKNOWLEDGEMENTS

This work was financially supported by Lactina Ltd. (Bankya, Bulgaria).

REFERENCES

Alexopoulos, C., Karagiannidis, A., Kritas, S. K., Boscos, C., Georgoulakis, I. E., & Kyriakis, S. C. (2001). Field evaluation of a bioregulator containing live Bacillus cereus spores on health status and performance of sows and their litters. *Transboundary and Emerging Diseases*, *48*(3), 137-145.

Angelakis, E., & Raoult, D. (2010). The increase of Lactobacillus species in the gut flora of newborn broiler chicks and ducks is associated with weight gain. *PLoS One*, *5*(5), e10463.

Bernardeau, M., & Vernoux, J. P. (2013). Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. *Clinical Microbiology and Infection*, *19*(4), 321-330.

Bosi, P., & Trevisi, P. (2010). New topics and limits related to the use of beneficial microbes in pig feeding. *Beneficial microbes*, *1*(4), 447-454.

Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (1998). Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *Journal of applied microbiology*, *84*(5), 759-768.

Cousin, F. J., Foligné, B., Deutsch, S. M., Massart, S., Parayre, S., Le Loir, Y., & Jan, G. (2012). Assessment of the probiotic potential of a dairy product fermented by Propionibacterium freudenreichii in piglets. *Journal of agricultural and food chemistry*, *60*(32), 7917-7927.

Davis, M. E., Parrott, T., Brown, D. C., De Rodas, B. Z., Johnson, Z. B., Maxwell, C. V., & Rehberger, T. (2008). Effect of a Bacillus-based direct-fed microbial feed supplement on growth performance and pen cleaning characteristics of growing-finishing pigs. *Journal of animal science*, *86*(6), 1459-1467.

De Lange, C. F. M., Pluske, J., Gong, J., & Nyachoti, C. M. (2010). Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livestock Science*, *134*(1), 124-134.

Toit, M. D., Dicks, L. M. T., & Holzapfel, W. H. (2003). Identification of heterofermentative lactobacilli isolated from pig faeces by numerical analysis of total soluble cell protein patterns and RAPD-PCR. *Letters in applied microbiology*, *37*(1), 12-16.

Garcia-Lafuente, A., Antolin, M., Guarner, F., Crespo, E., & Malagelada, J. R. (2001). Modulation of colonic barrier function by the composition of the commensal flora in the rat. *Gut*, *48*(4), 503-507.

Kim, J. S., Ingale, S. L., Kim, Y. W., Kim, K. H., Sen, S., Ryu, M. H., & Chae, B. J. (2012). Effect of supple-

mentation of multi-microbe probiotic product on growth performance, apparent digestibility, cecal microbiota and small intestinal morphology of broilers. *Journal of animal physiology and animal nutrition*, *96*(4), 618-626.

Liou, A. P., Paziuk, M., Luevano, J. M., Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Science translational medicine*, *5*(178), 178ra41-178ra41.

Million, M., Angelakis, E., Paul, M., Armougom, F., Leibovici, L., & Raoult, D. (2012). Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. *Microbial pathogenesis*, *53*(2), 100-108.

Musso, G., Gambino, R., & Cassader, M. (2010). Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded?. *Diabetes care*, *33*(10), 2277-2284.

Ogawa, M., Shimizu, K., Nomoto, K., Takahashi, M., Watanuki, M., Tanaka, R., & Takeda, Y. (2001). Protective Effect of Lactobacillus casei Strain Shirota on Shiga Toxin-Producing Escherichia coliO157: H7 Infection in Infant Rabbits. *Infection and immunity*, *69*(2), 1101-1108.

Pouwels, P. H., Leer, R. J., & Boersma, W. J. (1996). The potential of Lactobacillus as a carrier for oral immunization: development and preliminary characterization of vector systems for targeted delivery of antigens. *Journal of biotechnology*, *44*(1-3), 183-192.

Saarela, M., Mogensen, G., Fonden, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. *Journal of biotechnology*, *84*(3), 197-215.

Shim, Y. H., Ingale, S. L., Kim, J. S., Kim, K. H., Seo, D. K., Lee, S. C., & Kwon, I. K. (2012). A multi-microbe probiotic formulation processed at low and high drying temperatures: effects on growth performance, nutrient retention and caecal microbiology of broilers. *British poultry science*, *53*(4), 482-490.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., Pacifico, S., & Coppola, R. (2005). Bile salt and acid tolerance of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese. *FEMS microbiology letters*, *244*(1), 129-137.

Zeng, B., Han, S., Wang, P., Wen, B., Jian, W., Guo, W., & Yang, M. (2015). The bacterial communities associated with fecal types and body weight of rex rabbits. *Scientific reports*, *5*, 9342.

Zokaeifar, H., Balcázar, J. L., Saad, C. R., Kamarudin, M. S., Sijam, K., Arshad, A., & Nejat, N. (2012). Effects of Bacillus subtilis on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, Litopenaeus vannamei. *Fish & shellfish immunology*, *33*(4), 683-689.