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Endogenous Losses of Chemical Elements in the Digestive Tract and Their Correction

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Abstract

At present, the reduction of endogenous losses in the body of farm animals is considered as one of the ways to increase the efficiency of livestock production. Existing methods of reducing the endogenous losses imply feeding of animal with phytase, changing diet, etc.

The aim of our research was to study possible reduction of endogenous losses using the *Bifidobacterium longum* preparation. Two series of experimental studies were performed on a model of Wistar rats.

In the first experiment we had tested the methodology and estimated the size and composition of endogenous losses. The selected method made it possible to form a flow of irretrievably lost endogenous chemical elements (Co, Cr, Cu, Fe, etc.) from the body of animals. These elements comprised up to 80% of the pool. The used diet provided the most significant decrease in the pool of microelements: chromium by 72.4 %, iron by 72.1%, cobalt by 64.7%.

In the II experiment losses of chemical elements decreased selectively under the influence of *Bifidobacterium longum*. Feeding animals with bifidobacteria made it possible to reduce the loss of calcium by 3.9%, phosphorus by 17.6%, copper by 28%, zinc by 15.2%. Moreover, the pool of lead in the body increased by 16.1%. The inclusion of *Bifidobacterium* in the diet of animals maintains the strength of cortical bones and increases phosphorus content in the bones of the experimental animals by 19.4%. Works on the selection of microorganisms' strains decreasing endogenous losses of minerals are promising during the development of new probiotic preparations.

Keywords: chemical elements, probiotics, *Bifidobacterium longum*, endogenous losses

1. Introduction

A priori a set of substances sucked through the wall of a digestive tract consists of exogenous and endogenous component. The total mass of the endogenous component is comparable to the mass of exogenous substances. Thus, for 1 g of exogenous nitric of food body releases approximately 1 g of endogenous nitrogen into the digestive tract. Depending on the level of compliance with a set of nutrients the body needs, the difference between the mass of macro- and micronutrients of exogenous and endogenous origin can reach ten times in favor of fluctuating substances from the blood (Galperin YM & Lazarev PI, 1986, p. 340; Miroshnikov SA & Kvan OV & Deryabin DG, 2006, p. 142). Thus this decrease in endogenous losses of substances regarded as one of the ways to increase the efficiency of animal nutrition.

In particular, the loss of endogenous proteins and amino acids, (Mariscal-Landín G & Sève B & Collèaux Y & LeBreton Y., 1995, pp. 136-146; Hodgkinson SM & Souffrant WB & Moughan PJ, 2003, pp. 2525–2534) fat (Schulze H & van Leeuwen P &Verstegen MW & Huisman J & Souffrant WB & Ahrens F, 1994, pp. 2362–2368), minerals (Kim BG & Lee JW & Stein HH, 2012, pp. 289-295) etc.

Science has a certain experimental data on the influence of various factors on endogenous losses. The relationship between the level of phytic acid and endogenous losses of amino acids (Park CS & Oh SI & Kim

BG, 2013, pp. 186-192); cellulose content and endogenous losses of phosphorus (Son AR & Kim BG, 2015, pp. 369-73).

Naturally these studies were continued as a part of the development of measures to reduce the endogenous losses, including the introduction of additional phytase in the diet. (Cowieson AJ & Ravindran V, 2007, pp. 745-752) and etc.

The aim of our research was to study the endogenous losses of chemicals in animals because of non-mineral diet and to work out the ways to reduce their size by using probiotics.

2. Material and Methods

2.1 Animals

Investigations were carried out on the model of Wistar male rats and hens of final cross "Rhodonite". The experimental animals were kept in conditions of experimental-biological clinic (vivarium) of the Institute bioelementology of Orenburg State University, in accordance with the recommendations.

2.2 Ethics Comitet

The experimental studies were carried on animals out in accordance with the Regulations of the Russia (1987) and «The Guide for the Care and Use of Laboratory Animals (Nationak Academy Press Washington, DC 1996)."

2.3 Rations and Feed

The 1st experiment scheme suggested the formation of two groups (n = 20) 2-month animals by the vapor-analogues that at the end of the preparatory period (30 days.) were transferred to the regime of the basic reference period (21 days.), which provides maintenance of animals in group I (control) on a balanced diet according to the recommendations of the Institute of Nutrition (Kodentsova VM & Vrzhesinskaya OA & Spirichev VB & Shatnyuk LN, 2003, pp. 23-233), the individuals of Group II on non-mineral diet.

Animal diet of the Group II consisted of boiled polished rice (rice cooked in distilled water for 15 minutes, followed by removal of broth and washing), supplemented with vitamins A, D, C, K, E, B1, B2, B3, B4, B5, B6, Bc, B12, guided by the recommendations of the Institute of Nutrition (Kodentsova VM & Vrzhesinskaya OA & Spirichev VB & Shatnyuk LN, 2003, pp. 23-233). Watering was carried out with distilled water.

After two weeks of the experiment samples were taken with intervals of two hours and the elemental composition of the chime of duodenum of the experimental animals was investigated.

The 2d scheme of experience suggested the formation of three groups of five months of rats (n = 20) by pairs of analog which after a preparatory period (25 weeks), and the content in the diet, balanced on the recommendations of the Institute of Nutrition, was transferred to the main reference period (4 weeks). The experimental procedure was supposed to keep animals on non-mineral diet, with the difference that the animals of group II received additionally per os liquid probiotic comprising the strain *Bifidobacterium longum* (6,2 ml / kg body weight, 1 ml contains about 107 microbial bodies (Trushina EN & Mustafina DC & Nikitiuk DB etc, 2006, pp. 70-74).

2.4 Elemental Analysis

The elemental composition of biosubstrates of animal and feed was studied using the methods of atomic emission and mass spectrometry with inductively coupled argon plasma. The studies were conducted in the laboratory ANO "Center of biotic medicine" Moscow (accreditation certificate GSEN.RU.TSOA.311, Russia RU.0001.513118) (ICAP-Devices 9000 «Thermo Jarrell Ash, USA, Perkin Elmer Optima 2000DV, USA).

$$mloss= (mo-m1)-mintake \quad (1)$$

where:

m_{loss} – calculated value of endogenous losses of the chemical element, mg;

m_o- pool of chemical element in the body in the beginning of the experiment, mg;

m_1- pool of chemical element in the body in the end of the experiment, mg;

m_{intake} – intake of chemical element from the deficient diet, mg.

2.5. Statistical Analysis

The statistical analysis of the resulting material was carried out using the program «Statistica 10.0». The level of significance was considered significant at $p \leq 0.05$. [9] Calculation of the loss of endogenous chemical elements from the body of animals was made on the assumption that the digestibility of chemical elements from the

deficient diet was 100%.

3. Results

3.1 The Mineral Composition of Diets

Evaluation of the elemental composition of polished rice, prepared by the method mentioned above, revealed very low level of certain chemical elements (Table 1).

Table 1. Mineral elements in the rice used in the experiment *

Index	Recommended by the Institute of Nutrition	Studied diet
Macronutrients:	мкг/г	мкг/гол.сут
calcium	1430	2409,6
potassium	-	7216
magnesium	2197	5380
sodium	668,3	2084,4
phosphorus	7496	17120
Trace elements:		
- Vital:		
cobalt	0,3	0,128
chrome	0,45	1,35
copper	9,98	38,8
iron	131,4	86,8
iodine	-	1,32
manganese	126,1	131,2
selenium	0,48	1,46
zinc	79,8	213,2
- Conditionally vital:		
arsenic	0,38	1,88
nickel	1,99	2,57
tungsten	0,31	0,35
- Toxic and potentially toxic:		
silver	-	0,068
strontium	27,8	24,2
aluminum	-	20,8
cadmium	0,099	0,028
lead	-	0,11

Note: * Figure - Krasnodar (polished rice cooking for 15 minutes, followed by removal of broth and washing with distilled water).

3.2 The Estimated Value of the Loss of Endogenous Chemicals

In the 1st experiment the amount of endogenous losses of chemical elements from the body of the experimental animals is calculated.(Table. 2).

Table 2. The value of calculated loss of endogenous chemical elements from the body of animals (Experiment I)

Chemical element	The content in the animal at the end of experiment, mcg	The amount of endogenous losses		
		ug / animal	the initial mass,%	ug / kg / day
Ca	933347± 1024	391153	29,5	165347
P	703880±421	343528	32,8	145215
Co	2,4±0,2	4,4	64,7	1,85
Cr	7,6±2,1	20,2	72,4	8,54
Cu	127±7,45	92	42,0	38,9
Fe	4945± 395,00	12755	72,1	5392
Mn	99,3±7,9	54,4	35,4	23,0
Zn	3312±90,7	922	21,8	390

The content of chemical elements in the chyme of animals is much greater than their concentration in the used diet (Table 3).

Table 3. The chyme elemental composition of the laboratory animals, mkg/g

	Before feeding	In 4 hours	In 6 hours	In 8 hours
Ca	161,00±24,0	97,83±9,8	129,00±13,0	96,20±9,6
Co	0,03±0,009	0,02±0,003	0,03±0,005	0,03±0,004
Cr	0,63±0,1	0,10±0,012	0,36±0,04	0,39±0,047
Cu	6,38±1,0	1,38±0,1	1,97±0,2	1,77±0,2
Fe	45,48±13,6	8,06±2,0	12,16±2,4	22,16±4,4
Mn	3,01±0,5	2,81±0,3	6,51±0,7	3,09±0,3
P	470,00±117,0	434,00±65,0	606,00±91,0	643,00±97,0
Zn	9,11±1,4	7,29±0,7	7,16±0,7	5,89±0,6

In the 2d experiment similar results were obtained with the difference that the introduction of the culture of the Bifidobacterium longum was accompanied by a selective decrease in loss of the chemical elements (Table 4).

Table 4. The value of calculated loss of endogenous chemical elements from the body of animals (Experiment II)

Chemical element	Group			
	I		II	
	amount in the body at the time of completion of the experiment	losses for the period of experience	amount in the body at the time of completion of the experiment	losses for the period of experience
Ca, мг	1578±170,4	567	1640±95,1	505
P, мг	1175±58,4	522	1382±10,5**	315
Cu, мкг	188±3,1	163	243±9,4***	108
Zn, мкг	7007±205	2070	8074±314*	1003
Pb, мкг	9,4±0,1	2,1	8,1±0,4*	3,4

Note: * p≤0,05; * p≤0,01; * p≤0,001.

The inclusion of Bifidobacterium longum into the animal diet was accompanied by the increased bone compression force limit when degradation occurs (Table 5).

Table 5. Maximum compression force when destruction of tubular bones of animals starts, H (II experiment)

Value	The period of the experiment		
	start	ending group	
		I	II
Min	12,0	10,0	12,0
Max	25,4	23,0	27,4
M±m	18,7±2,86	16,5±0,5	19,7±0,78*

Feeding of Bifidobacterium longum was associated with the increase phosphorus in bones of animals of group II up to 51.5% (P <0.01) as compared to group I (Table 6).

Table 6. The ammount of calcium and phosphorus in bones of the test animals at the end of the experiment,% of DM (Experiment II)

Element	Group	
	I	II
Calcium	18,8±1,7	19,04±0,56
Phosphorus	3,3±0,46	5,0±0,04**

Note: a - the calculation of the content of elements produced on a low-fat tissue.

The study found the selective action of bacteria Bifidobacterium longum in the endogenous losses of minerals, expressed in a more intensive excretion of animals of group II of a number of heavy metals and stabilize the level of certain essential elements (Table 7).

Table 7. The ammount of elements in the experimental animals at the end of experiment, mg (experiment II)

Group	Cu	Zn	Pb	Ni	Mo	Sn	Ag	Mn
I	0,089±	5,0±	0,009±	0,004±	0,009±	5,12±	0,002±	0,012±
	0,003	0,25	0,0001	0,001	0,001	0,45	0,000	0,001
II	0,254±	8,2±	0,008±	0,002±	0,006±	4,682±	0,001±	0,045±
	0,009***	0,47**	0,0004	0,001	0,0001*	0,099	0,000	0,005**

Note : *** - P<0,001; ** - P<0,01; * - P<0,05

The study revealed the selective effect of bacteria Bifidobacterium longum on endogenous losses of minerals which was expressed in a more intensive excretion of a number heavy metals from animals of group II and stabilization of the level of certain essential elements (Table 8).

Table 8. Content of elements in the test animals at the end of experiment, mg (experiment II)

Group	Cu	Zn	Pb	Ni	Mo	Sn	Ag	Mn
I	0,089±	5,0±	0,009±	0,004±	0,009±	5,12±	0,002±	0,012±
	0,003	0,25	0,0001	0,0005	0,001	0,45	0,000	0,001
II	0,254±	8,2±	0,008±	0,002±	0,006±	4,682±	0,001±	0,045±
	0,009***	0,47**	0,0004	0,0002*	0,0001*	0,099	0,000	0,005**

Note: *** - P<0,001; ** - P<0,01; * - P<0,05

4. Discussion

The problem of optimization of the value of endogenous nutrient losses by farm animals (Almeida FN & Stein HH, 2011, pp. 617-622; Kong C & Adeola O, 2014, 917-925) has recently been solved by the introduction to the diet of enzyme preparations (Cowieson AJ & Ravindran V, 2007, pp. 745-752) through the reduction of the individual substances in the diet and feed (Schulze H & Van Leeuwen P & Verstegen MW et al., 1994, 2362-2368; Cowieson AJ & Ravindran V, 2007, pp. 745-752; González-Vega JC & Walk CL & Liu Y et al., 2013, 4807-4816). At the same time it seems reasonable to decline the size of the loss of endogenous substances through the use of probiotics. It is proved by the data on the production by bifidobacteria and lactobacilli of proteolytic enzymes degrading phytate (Haros M & Carlsson NG & Almgren A et al., 2009).

The objective of our study was to research the value of endogenous losses of chemical elements from the body and the possibility of correction using the preparation Bifidobacterium longum. In the first experiment the method of forming a significant flow of endogenous losses was tested. Based on the data presented in other researches (Halperin YM & Lazarev PI, 1986, p. 304) it was hypothesized that keeping of the animals on deficient diet will provide a significant release of endogenous agents during enteral homeostasis. It is known that the less diet is balanced the more weight of endogenous substances in the intestine chyme. It is logical considering that the biological meaning of all functions of the digestive system is the formation of blood plasma (Smirnov MA & Subbotin VV, 2001, pp. 17-22). However a significant mass of substances released from the blood into the gastrointestinal tract is not absorbed and irretrievably lost.

Due to technical difficulties in estimating the loss of endogenous chemical elements there was made the assumption that comprehensibility of chemical elements from the deficient diet was 100%.

In the first experiment it was determined that chosen method allows to create the flow of irretrievably lost endogenous chemical elements (Co, Cr, Cu, Fe, etc.) from the body of animals reaching 80% of the total pool (four weeks of observation). Moreover it was observed in a broad range from 21.8 to 72.1% compared to the beginning of the experiment (Table 2). In absolute terms the greatest losses were losses of macronutrients - calcium and phosphorus: 343,5-391,1 mg for one animal over the period of the experiment. However these values were only a third of the total content of these substances in the body. The most significant decrease was noted for the pool of trace elements: chromium by 72.4%, iron by 72.1%, cobalt by 64.7%. Amino acids were

characterized by the heterogeneity and high variability of the composition of endogenous losses (Reis de Souza TC & Barreyro AA & Mariscal-Landín G, 2013, p. 4-36).

The duodenal chyme of animals had higher content of chemical elements in comparison with the diet. In particular the concentration of cobalt in the chyme was 0.02-0.03; of chromium 0.1-0.39; of iron 8.16-22.16 mc /g which exceeded analogical level in the diet by 6-7 times for cobalt, for chromium by 2.9-10.6 times, for iron by 3.7-10.1 times.

Therefore an incomplete intestinal absorption of endogenous substances led to elements depletion of the body. During experiment II similar results were obtained. Introduction of culture Bifidobacterium longum was accompanied by a selective decrease in the loss of chemical elements (Table 3). In particular at the end of experiment the content of calcium in the organism of animals of group II exceeded those in group I by 3.9%, of phosphorus by 17.6% ($R \leq 0.01$), of copper by 28.5% ($R \leq 0{,}001$), of zinc 15.2% ($p \leq 0.05$). The only exception was lead which level in the body tissues of animals of Group II decreased compared to control by 16.1% ($p \leq 0.05$). It was mentioned above that bacterial flora has an important role in the absorption of essential trace elements in the intestine (Lopez HW & Levrat MA & Guy C et al., 1999, pp.; Balamurugan R & Mary RR & Chittaranjan S et al., 2010). Improved absorption of minerals in the colon (Macfarlane S & Macfarlane GT & Cummings JH, 2006), increase of solubility of the mineral-protein complexes (Yeung CK & Glahn R & Welch RM et al., 2005) were based on this effect. An important influencing factor of intestinal microflora and bifidobacteria, in particular the availability of minerals in the intestine, is their ability to degrade phytate (Haros M & Carlsson NG & Almgren A et al., 2009.). This property is used for optimization of iron metabolism by stimulating the growth of bifidobacteria using probiotics (Tako E & Glahn RP & Knez M et al., 2014). Importance of phytate-degrading effect of bifidobacteria was confirmed in our research by the increase of phosphorus in the bones of animals - by 51.5% ($P < 0.01$) compared to control level (Table 6)

The results demonstrated that introduction of Bifidumbacterium longum in the diet of animals associated with mineral-deficient diet ensures the retention of tubular bones strength of animals (Table 5). Test animals treated with Bifidobacterium longum had a high compressive strength of tubular bones = 19.7 N, 19.4% ($P < 0.05$) which exceeded such indicator for group I and was not significantly different from that for the rats at the beginning of experiment.

Intake of strain Bifidobacterium longum led to the increase of copper level by 2.9 times ($P<0{,}001$) and zinc level by 1.7 times ($P<0{,}01$) compared to the control.

There was a significant decrease of molybdenum by 1.5 times ($P < 0.05$) comparing with the group I. The manganese content in the body of the test animals increased by 3.8 times ($P < 0.01$) comparing with the group I.

Feeding by Bifidobacterium longum was accompanied by a decrease in the level of heavy metals in the organism of rats: lead – by 11.1%, nickel - by 2 times; tin – by 8.6% and silver by 50.0% comparing with the group I. This may be the result of bonding of toxic elements by bacteria (Shuhong, Y., Meiping, Z., & Hong, et al., 2014).

5. Conclusion

Thus the performed study allowed to demonstrate the prospect of work on the correction of endogenous losses of chemicals from the body. The selective effect of Bifidobacterium longum on the elemental status may be used to develop a new generation of probiotics correcting mineral metabolism in animals and humans.

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References

Aliev, A. A. (1985). Contemporary view about digestion. Enteral homeostasis and plasma-forming function of the digestive system. Role of gastrointestinal tract in the intermediate metabolism. Collection of studies All-Russian Scientific Research Institute of Animal Physiology, Biochemistry and Feeding V. XXX. Borovsk, 3-10.

Almeida, F. N., & Stein, H. H. (2011). Standardized total tract digestibility of phosphorus in blood products fed to weanling pigs. *Rev. Colomb Cienc Pecu., 24*, 617-622.

Cowieson, A. J., & Ravindran, V. (2007). Effect pf phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. *Br. J. Nutr., 98*(4), 745-52.

Eide, D. J. (2014). Bacillithiol, A new role in buffering intracellular zinc. *Mol. Microbial, 94*(4), 743-6.

http://dx.doi.org/10.1111/mmi.12793

Galperin, Y. M., & Lazarev, P. I. (1986). Digestion and homeostasi.s M .: Science. p. 304.

Gonzales-Vega, J. C., Walk, C. L., Liu, Y., & Stein, H. H. (2013). Endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. *J. anim. Sci., 91*(10), 4807-16.

He, Y., Chen, Z., Liu, X., Wang, C., & Lu, W. (2014). Influenceof trace elements mixture on bacterial diversity and fermentation characteristics of liquid diet fermented with probiotics under air-tight condition. *PLoS One., 9*(12), 114218. http://dx.doi.org/ 10.1371/journal.pone.0114218

Helmann, J. D. (2014). Specificity of metal sensing: iron and manganese homeostasis in Bacillus subtilis. *J. Biol. Chem., 289*(41), 28112-20. Http://dx.doi.org/10.1074/jbc.R114.587071

Hodgkinson, S. M, Souffrant, W. B., & Moughan, P. J. (2003). Comparison of the enzyme-hydrolyzed casein, guanidination, and isotope dilution methods for determining ileal endogenous protein flow in the growing rat and pig. *J Anim Sci., 4*, 2525–2534.

Kim, B. G, Lee, J. W., & Stein, H. H. (2012). Energy concentration and phosphorus digestibility in whey powder, whey permeate, and low-ash whey permeate fed to weanling pigs. *J Anim Sci., 90*, 289–295.

Kodentsova, V. M., Vrzhesinskaya, O. A., Spirichev, V. B., & Shatnyuk, L. N. (2010). Validation of the level of food fortification with vitamins and minerals. *Nutrition., 79*(1), 23-33.

Kong, C., & Adeola, O. (2014). Evalution of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian Australas *J. Anim. Sci., 27*, 917-925.

Lee, K. Y., & Heo, T. R. (2000). Survival of Bifidobacterium longum immobilized in calcium alginate beads in simulated gastric juices and bilesalt solution. *Appl Environ Microbiol, 66*(2), 869-73.

Ma, Z., Chandrangsu, P., Helmann, T. C., Romsang, A., Gaballa, A., & Helmann, J. D. (2014). Bacillithiol is a major bufferof the labile zinc pool in Bacillus subtilis. Mol. *Microbiol, 94*(4), 756-70. Http://dx.doi.org/10.1111/mmi.12794

Mariscal-Landín, G., Sève, B., Collèaux, Y., & LeBreton, Y. (1995). Endogenous amino nitrogen collected from pigs with end to end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. *J Nutr., 4*, 136–146.

Miroshnikov S. A., Kvan O. V., & Deryabin D. G. (2006). Ambiguity of the probiotics effect on the exchange of toxic elements in the body of laying hens. *Vestnik of the Orenburg State University, 2*, 142.

Moter, V., & Stein, H. H. (2004). Effect of feed intake on endogenous losses and amino acid and energy digestibility by growing pigs. *J. Anim. Sci., 82*, 3518-3525.

Oda, T., Seto, Y., & Hashiba, H. (1998). Supplementation of Bifidobacterium longum to a high-fat, low-calcium diet lowers cytolytic activity of fecal water in rats injected with 1,2-dimethylhydrazine dihydrochloride. *J. Nutr. Sci. Vitaminol (Tokyo), 44*(1), 187-94.

Park, C. S, Oh, S. I., & Kim, B. G. (2013). Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fednitrogen-free diets. *Rev Colomb Cienc Pecu, 26*, 186-192.

Plohushko, E. N., Larionov, L. P., & Litus, N. V. et al. (2002). Effect of lactobacilli with potential to develope new probiotics on nonspecific resistance of the organism. Immunology of Ural. *Materials of conference of Ural Immunologists, 2*, 13-14.

Reis de Souza, T. C., Barreyro, A. A., & Mariscal-Landín, G. (2013). Estimation of endogenous protein and amino acid ileal losses in weaned piglets by regression analysis using diets with graded levels of casein. *J Anim Sci Biotechnol, 22, 4*(1), 36. http://dx.doi.org/ 10.1186/2049-1891-4-36

Rodriguez, F., Lillington, J., Johnson, S., Timmel, C. R., Lea, S. M., Berks, B. C. (2014). Crystal structure of the Bacillus subtilis phosphodiesterase PhoD reveals an iron and calcium-containing active site. *J. Biol. Chem. 289*(45), 30889-99. Http://dx.doi.org/10.1074/jbc.M114.604892

Schulze, H., van Leeuwen, P., Verstegen, M. W., Huisman, J., Souffrant, W. B., & Ahrens, F. (1994). Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. *J Anim Sci., 72*, 2362–2368.

Sizentsov, A. N., Vishnyakov, A. I., & Novikov, A. E. (2011). Environmental aspects of the accumulation of lead and zinc by probiotic preparations based on Bacillus subtilis. *Vestnik of the Orenburg State University, 4*, 7.

Smirnov, M. A., & Subbotin, V. V. (2001). Normal activity of animals microflora and its correction by probiotics. *Veterinary Science, 11*, 17-22.

Son, A. R, Shin, S. Y., & Kim, B. G. (2013). Standardized total tract digestibility of phosphorus in copra expellers, palm kernel expellers, and cassava root fed to growing pigs. *Asian Australas J Anim Sci., 26*, 1609–1613.

Son, A. R., & Kim, B. G. (2015). Effects of dietary cellulose on the Basal endogenous loss of phosphorus in growing pigs. *Asian-Australas J. Anim. Sci., 28*(3), 369-73.

Trushina, E. N., Mustafina, D. C., Nikitiuk, D. B., Podbeltsev D. A., Mozgovaya, I. N., & Vustina, T. F. (2006). Immune stimulating effect of oral intake of bifidobacteria of different strains. *Nutrition, 75*(5), 70-74.