Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 2, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

EFFECTS OF DIRECT-FEED MICROBIAL *BACILLUS* SUPPLEMENTATION ON PIGLET'S MICROBIOTA

Mihaela DUMITRU*, Mihaela HĂBEANU, Nicoleta LEFTER

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

*Corresponding author email: mihaela.dumitru22@yahoo.com

Abstract

Direct-fed microbial (DFM) supplementation in piglet's nutrition may offer high benefits to the young animals by diminishing the bacteria pathogens from the gastrointestinal tract and environment. In this study, we evaluated the efficiency of Bacillus subtilis ATCC 6051a (BS, 1.6 x 10^9 CFU/mL) on the piglet's microbiota in the weaning crisis. A total of 60 piglets, 30 days ± 3 days of age, were allotted in 3 homogeneous groups (C, E1 and E2, 10 piglets/6 pens, 2 replicates/group) supplemented with BS 1% (E1), respectively 3% (E2)/kg feed. At the end of the trial (46 ± 3 d-old), 6 piglets were slaughtered (2 piglets/group) and intestinal content (ileum and cecum) were collected for evaluating the microbiota and intestinal pH values. The piglet's faces were collected for microbial analysis on 1-d, 8d, and 16 d. The numbers of lactic acid bacteria (LAB), Coliforms bacteria, Enterococcus spp., Clostridium spp. and Bacillus spp. from intestinal content were modified at the addition of BS, whereas, from faeces samples, the microbiota was insignificant (P ≥ 0.05). The addition of BS 1% and 3% decreased the numbers of Escherichia coli biotype β-hemolytic from piglet's intestinal content (P ≤ 0.05) and faces vs. C group. Salmonella spp. was not present. The intestinal pH from the ileum and cecum segment was observed to be lower in E1 + BS 1%, while in E2 + BS 3% the pH was higher vs. C group. In conclusion, 1% supplementation of Bacillus subtilis ATCC 6051a in piglets feed had a positive effect during the post-weaning period on endogenous microbiota, fecal microbial count and intestinal pH evolution.

Key words*: probiotic, piglets health status, intestinal microbiota, intestinal pH.*

INTRODUCTION

Direct-fed microbials (DFM) present the capacity to modify gastrointestinal microflora, morphology and immunity after weaning (Mingmongkolchai and Panbangred, 2018).

The feed addition of lactic acid bacteria, yeast and *Bacillus* as DFM (Liu et al., 2018) can bring a number of benefits to animals microbial flora as balancing the health status and maintaining the intestinal ecosystem (Dumitru et al., 2019). Generally, the bacteria used as source of probiotic products are part of the intestinal (commensal) flora of the host (Scharek et al., 2007) and as live microorganisms, can improve the intestinal microbial stability of young animals (He et al., 2017), avoiding's the disadvantage of antibiotics use (Isolauri et al., 2004). According to Casula and Cutting (2002), the presence of *Bacillus* spores and their capacity to resist under exhibits gastrointestinal conditions (thermostability and the capacity to tolerate the low pH values and bile salts), make them to germinate in significantly numbers along to the animal gut. Furthermore, Bacilli as Gram-positive bacteria are present in substantial numbers in agricultural soils and other environment conditions (Cornea et al., 2003), being responsible for the enzymatic process by enhanced animal digestion.

Addition of *Bacillus* as DFM to piglets' diets may improve gut health, by modifying the microflora, thus controlling and protect from pathogenic bacteria, increase nutrient digestibility and feed efficiency, and also, to perform the growth performance of piglets. Due to their stability, *Bacillus* are ideally suitable to produce a variety of enzymes which intensify the digestion process (Merchan et al., 2011). Supplementation piglets' diet with various strains of *Bacillus* involve positive results on body weight gain, feed conversion ratios, lower mortality with a reduction of diarrhea incidence in weaning crisis (Taras et al., 2005). It was reported that *Bacillus subtilis* (*BS*) improves the animal health status by stabilizes the gastrointestinal tract after weaning (Liu et al., 2017). Currently, many of the researches into

probiotics have directed on the protection against pathogens. Scharek et al. (2007) reported that administration of *Bacillus* spp. diminished the intestinal counts of enterotoxigenic *Escherichia coli*, diarrhoea incidence, and morbidity in weaned piglets. Furthermore, Du et al. (2019) confirmed that oral feeding with *BS* in concentration of 1.5 x 10^{10} CFU/mL was able to protect the newborn piglets by inhibiting the pathogenic *E. coli* which is responsible for infection, severe intestinal disorders and death. Their stability to form endospores, make *Bacillus* species to remain viable at higher temperatures during animal feed pelleting and stable for long-term storage (Baker et al., 2013). The aim of the current study was to evaluate the effects of dietary *Bacillus subtilis* ATCC 6051a as source of probiotic, by inclusion in piglets diets, on microbiota from intestinal content (ileum and cecum), faecal and the evolution of pH values.

MATERIALS AND METHODS

The experimental protocol was approved by the ethic guideline Research Committee of INCDBNA Balotesti, Romania.

Bacterial strain, culture medium and growth conditions

Bacillus subtilis ATCC 6051a (*BS*), a bacterial strain used as DFM was purchased from the American Tissue Culture Collection (ATCC) in the form of freeze-dried. The probiotic properties of *BS* were analyzed *in vitro* and presented in a previous study (Dumitru et al., 2019). The bacterial strain was incubated aerobically in the nutrient medium, in a shaker-incubator (200 rev min^{-1}) at 37°C for 24 h. The strain concentration used in this study was 1.6 x 10^9 CFU per mL g^{-1} feed.

Piglets, experimental treatments and diets

A total of 60 piglets Topigs hybrid [♀ Large White × Hybrid (Large White × Pietrain) × ♂ Talent, mainly Duroc] with average body weight (BW) of 8.41 ± 0.92 kg. (30 d ± 3 d of ages) where used in a 16-d experiment. Piglets were randomly allotted to 3 groups distributed in 10 piglets/6 pens, two replicates per group: negative control (C) and 2 experimental groups with the addition of *Bacillus subtilis* (*BS*) in a dose of 1% (E1+*BS* 1%), respectively 3% (E2+*BS* 3%). The concentration of *BS* was 1.6 x 10^9 UFC/mL g^{-1} feed. The probiotic product was added after grinding the raw material and then mixed them uniformly. All piglets were housed in an environmentally controlled room, equipped with water nipples. Feed and water were available *ad libitum* throughout the duration of the experiment and was administrated in the flour from, two meal per day. The feed structure is shown in Table 1. The room temperature was approximately 25 ± 2°C. At the end of the trial (46 ± 3 d-old), 6 piglets were selected randomly and slaghtered (2 piglets per group). Intestinal content (ileum and cecum) were collected for evaluating the microbiota and intestinal pH values.

Sample collection and microbial analyses: intestinal and faecal content

Two piglets per group were selected and euthanized for assessing the gastrointestinal microbial populations from ileum and cecum content. Intestinal content was removed immediately after killing and aseptically collected in sterile plastic bags on ice. From those content, 1 g of sample (ileum and cecum) per capita from two piglets per group were homogenized with 7 ml BHI (Brain Heart Infusion, Oxoid) broth with 2 ml glycerol, and immediately stored at - 20°C until testing (Sorescu et al., 2019). Similar, fresh faeces samples were collected randomly from each group (on 1st d, 8 d, and 16 d) and stored in the same conditions until bacterial analysis was done (no more three months). After defrost, one gram of the composite intestinal content, respectively faecal samples were supposed to decimal dilutions in 9 mL PBS (Phosphate Buffered Saline, Oxoid) solution and then very well homogenized. Microbial flora was assessed for *Lactobacillus* spp. [LABs on MRS agar (Man, Rogosa and Sharpe)], *Escherichia coli* biotype β-haemolytic [Trypticase soy agar (TSA, Sanimed) + 5% sheep blood (w/v), Dumitru et al., 2018], *Salmonella* spp. (Salmonella-Shigella agar, Oxoid), *Clostridium* spp. (Reinforced Clostridial agar, Oxoid), Coliforms (MacConkey agar, Oxoid), *Bacillus* spp. (nutrient agar) and *Enterococcus* spp. (Slanetz-Bartley agar, Oxoid). The LABs, *Clostridium* and *Enterococcus* were cultured in

anaerobic conditions (Oxoid jar with Anaerogen 2.5 L). Bacterial counts from all samples were determined by plate counting method and were $\log_{10}$ CFU transformed before statistical analysis (Vamanu et al., 2013)

Intestinal pH values

The same slaughtered piglets were used for measurement the intestinal pH (ileum and cecum). 1 g intestinal content of each piglet was collected aseptically in 9 mL distilled water (1:10 dilution) and pH values were determined by using a digital Portable meter (Waterproof, pH 7+DHS, Italy).

Statistical analysis

The analytical data were compared using variance analysis "ANOVA" with STATVIEW for Windows (SAS, version 6.0). The results were expressed as mean values and standard error of the mean (SEM), the differences between means considered statistically.

RESULTS AND DISCUSSIONS

During the experiment, the concentration of *Bacillus subtilis* ATCC 6051a used in piglets diet as source of DFM, was prepared in liquid form and contained in average 1.6 x 10^9 CFU/mL.

Table 1. Compositions of basal diet of piglets during weaning crisis

Items %	Control
Maize	33.48
Sorghum	25
Peas	17
Soybean meal	13
Maize gluten	3
Milk replacer	5
DL methionine	0.1
L- Lysine	0.21
Calcium carbonate	1.6
Phytase	0.01
Monocalcium phosphate	0.4
Salt	0.1
Premix choline	0.1
Vitamin-mineral premix*	1
Chemical composition % (g⁻ feed)	
Metabolizable energy (EM, Kcal/ kg)	3237.31
Crude protein (CP)	18.23
Lysine	1.2
Methionine + Cystine	0.59

*ME was calculated based on feed composition and theoretical coefficients.
*The vitamin-mineral premix contained (/kg feed): 10 000 IU vitamin A; 2 000 IU vitamin D3; 30 IU vitamin E; 3 mg vitamin K3; 2 mg vitamin B1; 6 mg vitamin B2; 20 mg vitamin B3; 13.5 mg vitamin B5; 3 mg vitamin B6; 0.06 mg vitamin B7; 0.8 mg vitamin B9; 0.05 mg vitamin B12; 10 mg vitamin C; 30 mg Mn; 110 mg Fe; 25 mg Cu; 100 mg Zn; 0.38 mg I; 0.36 mg Se; 0.3 mg Co; 60 mg antioxidant.

E1+*BS* 1% and E2+*BS* 3% experimental groups received the same diet feed, the difference consisting in the percentage of DFM-probiotic product (*BS*), respectively 1% and 3% (v/w g^{-1} feed).

Intestinal and faecal microbiota of piglets

Ileum *Lactobacillus* spp. increased in piglets fed E1+*BS* 1% with 3.35% compared to C group, whereas *E. coli* concentration decreased with 26.22% at the administration of *BS* 1% *vs.* C, respectively with 2.70% in piglets diet E2+*BS* 3% (Figure 1). A critical role in animal nutrition, performance, health and the quality of the product produced is occurred by the intestinal microbiota.

It was reported that the utilization of *Bacillus* spp. reduce the intestinal count of *Escherichia coli* which is an enterotoxigenic bacteria, responsible of diarrhoea incidence, and in the last form determining the piglet's mortality (Poulsen et al., 2018). Moreover, the *Clostridium* counts along the ileum of 46 ± 3 d-old piglets was reduced by *BS* supplementation, which decreases the pathogens around 10% in E1, and proximately with 1.5% in E2 *vs.* C group. Similar, the addition of *BS* 1% influence *Enteroccocus* spp. which are present in low counts in E1 *vs.* C group.

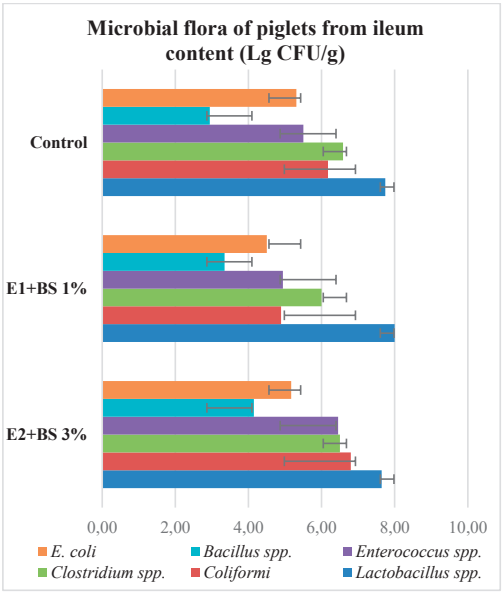

Figure 1. Microbiota from ileum piglets content with *BS*

Compared with C group, E1 and E2 shown an abundance in *Bacillus* counts (more than 13.60% to 41.15%); the coliforms grown was influenced by the concentration of *BS,* a decrease with 26.4% in low dose, and 10.0% in E2. Alternatively, the piglets diet supplemented with *BS* influenced the colonization of lactic acid bacteria between 18% to 25% in the cecum content, with a slow decrease of *E. coli* in the experimental groups (Figure 2).

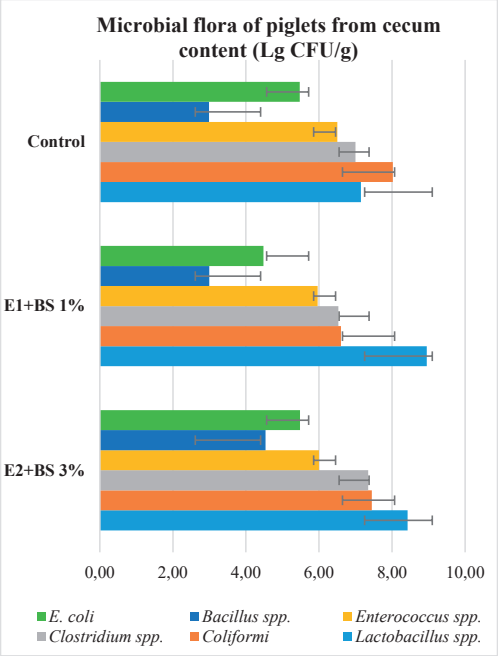

Figure 2. Microbiota from cecum piglets content with *BS*

The counts of Coliforms in the piglets cecum content were diminished between 22% (E1) and around 8% (E2) ad *BS* addition. The data present in the current study, indicate an improvement of the intestinal microbial flora of piglets in E1+*BS* 1% *vs.* E2+*BS* 3%.

According to the results of Alexopoulos et al. (2004), an important aspect of *Bacillus* is their efficacity on health status during piglets weaning crisis. Furthermore, DFM improved the number of lactic acid bacteria, decreasing the number of Gram-negative bacteria from the *Enterobacteriaceae* family, results that can be observed and in our study. The piglets age is an important factor that influences the gastrointestinal microbiota (Slifierz et al., 2015); the microbial flora on 46 ± 3 days was furthermore clearly different from that on 30 ± 3.

The modifications in diet, environment conditions, stress complicates can influence the interpretation of microbial flora in the early piglets weaning (Poulsen et al., 2018).

DFMs are implied in enhancing the gastrointestinal health by increasing the growth of helpful bacteria such as lactobacilli (Giang et al., 2010) and Bifidobacteria, through reducing the growth of harmful bacteria from the general family of Gram-negative *Enterobacteriaceae* (Liu et al., 2018; Bajagai et al., 2016). The decrease of pathogenic bacteria and equilibrium of intestinal microbiota may correspond to the animal ability to digest and ferment nutrients (Kenny et al., 2011), in our case young animals knowing that the enzymatic system is not very well developed (Habeanu et al., 2015). The presence of spores, as *Bacillus subtilis* can produce extracellular enzymes (cellulase, protease, amylase etc.) which can increase the gastrointestinal activity of piglets (Bajagai et al., 2016).

According to He et al. (2017), the addition of *Bacillus subtilis* in piglet diets determines a decrease of bacterial diversity which is associated with gastrointestinal disorders responsible for the development of diarrhoea incidences; the administration of probiotic in piglets diet could protect and ameliorate the intestinal disturbances characteristic periods of weaning (Huang et al., 2004; Prieto et al., 2014). Probiotics are supposed to improve the health of animals by preventing gut microbiota imbalance and improving gut health by adjusting the intestinal bacteria (Veizaj-Delia and Pirushi, 2012; Lescheid, 2014). Mackie et al. (1999) affirmed that the gut of piglet in utero is sterile, and after birth, the bacteria will colonize it, received from the sow and sow feces. Additionally, Baker et al. (2014) reported that utilization of DFM in sow, can be a potential source to reduce the environmental pathogens as *Clostridium* populations and their decrease from piglets gastrointestinal tract.

Table 2 shows the relative abundance of bacteria in piglets faces samples collected on day 1, 8 and 16 following the experimental protocol. On the first day of the experiment, the *Lactobacillus* spp. did not register significant differences (P = 0.2103), comparatively with the second period

were the bacteria counts tended to decrease in E1+*BS* 1%, respectively E2+*BS* 3% (P = 0.0107). Furthermore, on 16-d of the experimental trial, the lactic acid bacteria were not affected when piglets were fed with the diet that contained different levels of *BS* (P = 0.5257).

Table 2. Effect of *Bacillus subtilis* ATCC 6051a on fecal microbiota in weaning piglets (16-d)

Item		C	E1+*BS* 1%	E2+*BS* 3%	*SEM*	*P*
***Lactobacillus* spp., log CFU/g**	I	8.20	8.47	9.04	0.19	0.2103
	II	9.01^{ab}	7.78^{ab}	7.58^{b}	0.22	0.0107
	III	8.44	8.00	8.76	0.26	0.5257
Coliforms, log CFU/g	I	6.23^{a}	7.00^{a}	6.63	0.11	0.0184
	II	6.12	5.79	5.92	0.14	0.6663
	III	6.01	6.82	6.29	0.17	0.1675
***Clostridium* spp., log CFU/g**	I	6.33	6.61	6.66	0.11	0.4443
	II	5.93^{a}	6.36^{b}	7.60^{ab}	0.26	0.0184
	III	6.05	6.37	6.20	0.11	0.5576
***Enterococcus* spp., log CFU/g**	I	5.71^{ab}	7.93^{a}	7.71^{b}	0.26	0.0001
	II	5.33	4.71	5.41	0.23	0.4407
	III	5.41	5.84	5.81	0.14	0.4072
***Bacillus* spp., log CFU/g**	I	5.69	5.57	5.28	0.10	0.2843
	II	4.45	4.79	4.40	0.16	0.5958
	III	4.18	4.18	3.94	0.09	0.5024
***Salmonella* spp., log CFU/g**	I	abs	Abs	abs	nd	nd
	II	abs	Abs	abs	nd	nd
	III	abs	Abs	abs	nd	nd
***E. coli*, log CFU/g**	I	nd	Nd	nd	nd	nd
	II	5.36	4.94	5.02	0.13	0.4576
	III	>4.42	3.78	3.91	0.18	0.3288

*Where: I: 1-d of the experiment, without BS (30±3 days); II: 8-d of the experiment, with BS (38±3 days); III: 16-d of the experiment, with BS (47±3 days); Abs - absent; BS: *BS - Bacillus subtilis* ATCC 6051a (1% and 3%) in a dose of 1.6 x 10^9 UFC/mL/ g^{-1} feed; nd: not applied (*E. coli* < 1 x 10^3 UFC/g, absent); Experimental groups: C (Control), E1+*BS* 1%; E2+*BS* 3%; SEM: standard error of the means; ^{a,b}Means in the same row with the same common superscript are significantly different ($P \leq 0.05$).

The genus *Lactobacillus* as a Gram-positive bacteria is not involved a significantly growing in the presence of *BS* (P = 0.5257).

Lactobacillus is considered a beneficial bacterium for the equilibrium of intestinal microbiota, due to its healthfulness effects such as the prevention of diarrhoea and intestinal infections (Hu et al., 2014). Previous studies have found that the fecal *Lactobacillus* abundances' where not affected at *Bacillus subtillis* addition in piglets diet, and their abundance are in opposition with the *E. coli* concentrations (Konstantinov et al., 2006).

Coliforms were insignificantly ($P > 0.05$) on 8-d and 16-d of the experiment, whereas the *Clostridium* spp. counts significantly increased (P = 0.0184, on 8-d) at the addition of *BS* 1% and 3% *vs.* C group. Furthermore, DFM-product used as a source of probiotic in piglets' diets, did not influence the concentration of *Enterococcus* spp. and *Bacillus* spp. among dietary treatments. Vanhoutte et al. (2006) have been reported that an increase of *Lactobacillus* bacteria is in a relative decrease with *Clostridium* and Coliforms.

The administration of *BS* involves a decrease of *E. coli* on 16-d, but no significant differences were observed in the relative densities of total colonies bacteria ($P > 0.05$). Vanhoutte et al., 2006). *E. coli* is one of the major sources of intestinal pathogens, and some strains can produce serious illnesses, including diarrhoea. In the post-weaning, piglets fed supplementation with probiotics is essential for the prevention of diarrhoea, which is usually caused by β-hemolytic enterotoxigenic *E. coli* strains (García-Meniño et al., 2018).

Salmonella spp. was absent in all experimental groups, respectively analysis of microbial intestinal content and faces of piglets (30 ± 3 days) with and without *BS* supplementation.

Piglets intestinal pH

Along the gastrointestinal tract, administration of *BS* in piglets feed as probiotic treatment affected the pH level (Table 3. Merchant et al. (2011) affirmed that the pH in the small intestine of piglets is around 6 to 7, which is optimal interval for spores of *Bacillus* to germinate, grow and to act efficiently. *Bacillus* spp. due to

their capacity to survive to the gastrointestinal conditions, they are able to resist feeding processing and digestion of the stomach.

Table 3. The intestinal pH values from piglets in the weaning crisis fed control diet or supplemented with DFM for 16-day experimental period

Segment	C	E1+*BS* 1%	E2+*BS* 3%
Ileum	6.75 ± 0.91	6.62 ± 0.50	7.78 ± 0.59
Cecum	6.46 ± 0.33	5.26 ± 0.30	7.10 ± 1.01

Experimental groups: C (Control); E1+*BS* 1%; E2+*BS* 3%.

Interestingly, the ileum pH in the C group was around 6.8 while in E1+*BS* 1% registered 6.63, respectively 7.8 in E2+*BS* 3%. In cecum content, the pH of C group was 6.5 *vs*. 5.3 of E1+*BS* 1%, where E2+*BS* 3% registered an average of 7.1.
Weaning as a stressful period of piglets is influenced by numerous factors that contribute to physiological and microbial diversity in the gut (Lalles et al., 2007). The pH of intestinal digests can represent an indicator of the population of pathogens that colonize the gastrointestinal tract of piglets and in the end the intentness of diarrhea process to develop. An acidic environment encourages the proliferation of beneficial bacteria while inhibiting the growth of pathogenic bacteria (Fuller, 1977 cited by Heo et al., 2012).
Our pH values are in concordance with the results reported by Heo et al. (2012), which confirms that the pH of different areas of the gastrointestinal tract of piglets in the weaning crisis is, for example, in range of 6.0 to 7.4 in ileum segment, respectively 5.4 to 6.7 in caecum content. Dumitru et al. (2018; 2019) presents some results of the probiotic properties of *Bacillus* spp. including pH and bile salts resistance, these being significant criteria for selecting a probiotic product for use in animal nutrition.

CONCLUSIONS

Feed supplementation with *Bacillus subtilis* ATCC 6051a probiotic prepared at 1% was shown to have a positive effect during the post-weaning period on endogenous microbiota, fecal microbial count and intestinal pH evolution of piglets. Supplementation of the compound feed with *BS* 1% reduced the multiplication of Coliforms, *Clostridium* spp. and *E. coli* β-hemolytic in the intestinal and faecal contents of piglets.

ACKNOWLEDGEMENTS

This research was funded by the Romanian Ministry of Research and Innovation through Sub-program 1.2 - Institutional Performance, Program 1 - Developing national R & D, National Research and Development and Innovation Contract no.17 PFE/17.10.2018 and Project 8PCCDI/2018 pc2.

REFERENCES

Alexopoulos, C., Georgoulakis, I. E., Tzivara, A., Kyriakis, C. S., Govaris, A., Kyriakis, S. C. (2004). Field Evaluation of the Effect of a probiotic-containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the health status, performance, and carcass quality of grower and finisher pigs. *Journal of Veterinary Medicine*, Series A, 51(6), 306–312.

Bajagai, Y. S., Klieve, A. V., Dart, P. J., Bryden, W. L. (2016). Probiotics in animal nutrition e production, impact and regulation. In: Makkar HPS, editor. *FAO animal production and health paper*, 179. Rome, Italy: Food and Agriculture Organization of the United Nation.

Baker, A. A., Davis, E., Spencer, J. D., Moser, R., Rehberger, T. (2013). The effect of a *Bacillus*-based direct-fed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. *Journal of Animal Science*, 91(7), 3390–3399.

Casula, G., Cutting, S. M. (2002). *Bacillus* probiotics: Spore germination in the gastrointestinal tract. *Applied and Environmental Microbiology*, 68, 2344–2352.

Cornea, C. P., Grebenisan, I., Mateescu, R., Vamanu, E., Campeanu, G. (2002). Isolation and characterization of new *Bacillus* spp. Strains - useful as biocontrol agents of plant pathogens. *Rom Biotechnol Lett.,* 8(1), 1115–1122.

Du, Y., Xu, Z., Yu, G., Liu, W., Zhou, Q., Yang, D., Cao, Y. (2019). A newly isolated *Bacillus subtilis* strain named ws-1 inhibited diarrhea and death caused by pathogenic *Escherichia coli* in newborn piglets. *Frontiers in Microbiology*, 10.

Dumitru, M., Habeanu, M., Tabuc, C., Jurcoane S., 2019. Preliminary characterization of the probiotic properties of a bacterial strain for used in monogastric nutrition. *Bulletin UASVM, Animal Science and Biotechnologies*, 76(2), 102–108.

Dumitru, M., Sorescu, I., Habeanu, M., Tabuc, C., Idriceanu, L., Jurcoane, S. (2018). Preliminary characterisation of *Bacillus subtilis* strain use as a dietary probiotic bio-additive in weaning piglet. *Food and Feed Research,* 45(2), 203–211.

Dumitru, M., Sorescu, I., Habeanu, M., Tabuc, C., Jurcoane, S. (2019). Preliminary characterization *in vitro* of *Bacillus licheniformis* strain for used as

dietary probiotic. *Scientific Bulletin*. *Series F. Biotechnologies*, 23, 164–172.

Fuller, R. (1977). The importance of lactobacilli in maintaining normal microbial balance in the crop. *British Poultry Science*, 18, 85–94.

García-Meniño, I., García, V., Mora, A., Díaz-Jiménez, D., Flament-Simon, S. C., Alonso, M. P., Blanco, J. (2018). Swine enteric colibacillosis in spain: pathogenic potential of mcr-1 ST10 and ST131 *E. coli* isolates. *Frontiers in Microbiology*, 9.

Giang, H. H., Viet, T. Q., Ogle, B., Lindberg, J. E. (2010). Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livest. Sci*. 129, 95–103.

Habeanu, M., Lefter, N., Gheorghe, A., Tabuc, C., Untea, A., Surdu, I., Dragomir, C. (2015). Changes in certain serum and faeces parameters in weaned piglets as a response to nutritional stress. *South African Journal of Animal Science*, 45(2), 164.

He, Y., Mao, C., Wen, H., Chen, Z., Lai, T., Li, L., Wu, H. (2017). Influence of *ad libitum* feeding of piglets with *Bacillus subtilis* fermented liquid feed on gut flora, luminal contents and health. *Scientific Reports*, 7(1).

Heo, J. M., Opapeju, F. O., Pluske, J. R., Kim, J. C., Hampson, D. J., Nyachoti, C. M. (2012). Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *Journal of Animal Physiology and Animal Nutrition*, 97(2), 207–237.

Hu, Y., Dun, Y., Li, S., Zhao, S., Peng, N., Liang, Y. (2014). *Bacillus subtilis* KN-42 on growth performance, diarrhea and faecal bacterial flora of weaned piglets. Asian-Australasian Journal of Animal Sciences, 27(8), 1131–1140.

Huang, C.H., Qiao, S.Y., Li, D.F., Piao, X.S., Ren, J.P. (2004). Effects of Lactobacilli on the performance, diarrhoea incidence, VFA concentration and gastrointestinal microbial flora of weaning pigs. *Asian Australas. J. Anim.* Sci., 17, 401–409.

Isolauri, E., Salminen, S., Ouwehand, A. C. (2004). Probiotics. *Best Practice & Research in Clinical Gastroenterology*, 18, 299–313.

Kenny, M., Smidt, H., Mengheri, E., Miller, B. (2011). Probiotics - do they have a role in the pig industry? *Animal*, 5:462-470.

Konstantinov, S. R., Awati, A. A., Williams, B. A., Miller, B. G., Jones, P., Stokes, C. R., Akkermans, A. D. L., Smidt, H., De Vos, W. M. (2006). Post-natal development of the porcine microbiota composition and activities. *Environ. Microbiol.*, 8, 1191–1199.

Lalles, J. P., Bosi, P., Smidt, H., Stokes, C. R. (2007). Weaning-A challenge to gut physiologists. *Livest Sci*., 108(1-3), 82–93.

Lescheid, D. W. (2014). Probiotics as regulators of inflammation: a review. *Funct Foods Health Dis*, 4, 299-311.

Liu, Y., Espinosa, C. D., Abelilla, J. J., Casas, G. A., Lagos, L. V., Lee, S. A., Stein, H. H. (2018). Non-antibiotic feed additives in diets for pigs: A review. *Animal Nutrition*, 4(2), 113–125.

Mackie, R. I., Sghir, A., Gaskins, H. R. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clin. Nutr*. 69, 1035S–1045S.

Merchant, H. A., McConnell, E. L., Liu, F., Ramaswamy, C., Kulkarni, R. P., Basit, A. W., Murdan, S. (2011). Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development. *European Journal of Pharmaceutical Sciences*, 42(1-2), 3–10.

Mingmongkolchai, S., Panbangred, W. (2018). *Bacillus* probiotics: an alternative to antibiotics for livestock production. *Journal of Applied Microbiology*, 124(6), 1334–1346.

Poulsen, A.-S. R., Jonge, N. de, Nielsen, J. L., Højberg, O., Lauridsen, C., Cutting, S. M., Canibe, N. (2018). Impact of *Bacillus* spp. spores and gentamicin on the gastrointestinal microbiota of suckling and newly weaned piglets. *PLOS ONE*, 13(11), e0207382.

Prieto, M. L., Laurie, O. S., Shiau, P. T., McLoughlin, P., O'Donovan, R. M. C., Kent, R. M., Cassidy, J. P., Hughes, H., Gardiner, G. E., Lawlor, P. G. (2014). Evaluation of the efficacy and safety of a marine-derived *Bacillus* strain for use as an in-feed probiotic for newly weaned pigs. *PLoS One* 9(2), e88599.

Scharek, L., Altherr, B. J., Tölke, C., Schmidt, M. F. G. (2007). Influence of the probiotic *Bacillus cereus* var. toyoi on the intestinal immunity of piglets. Veterinary Immunology and Immunopathology, 120(3-4), 136–147.

Slifierz, M. J., Friendship, R. M., Weese, J. S. (2015). Longitudinal study of the early-life fecal and nasal microbiotas of the domestic pig. *BMC Microbiology*, 15(1).

Sorescu, I., Dumitru, M., Ciurescu, G. (2019). *Lactobacillus* spp. and *Enterococcus faecium* strains isolation, identification, preservation and quantitative determinations from turkey gut content. *Rom. Biotechnol. Lett*., 24(1), 41–49.

Taras, D., Vahjen, W., Macha, M., Simon, O. (2005). Response of performance characteristics and fecal consistency to long-lasting dietary supplementation with the probiotic strain *Bacillus cereus* var. toyoi to sows and piglets. *Arch. Anim. Nutr.*, 59, 405–417.

Vamanu, E., Pelinescu, D., Avram, I., Niță, S., Vamanu, A. (2012). Study of PROBAC product influence on infant microbiota in a single-chamber colonic fermentation model GIS1. *Annals of Microbiology*, 63(3), 1029–1038.

Vanhoutte, T., De Preter, V., De Brandt, E., Verbeke, K., Swings, J., Huys, G. (2006). Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and *Saccharomyces boulardii*. *Appl. Environ. Microbiol*. 72, 5990–5997.

Veizaj-Delia, E., Pirushi, R. (2012). The utilization of probiotics as a way to improve human and animal gut health. Maced *J Anim Sci*, 2, 175–181.