

# Effects of Pre and Probiotic on Growth Performance and Haematological Parameters in Pigs

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## Abstract

The present study investigates the effects of dietary supplementation with feed supplements (prebiotics and probiotics) on pig performance, the feed conversion ratio and some haematological parameters.

The research was conducted on hybrid pigs [(Yorkshire × Landrace) × Duroc] in a farm from Sălaj County, Romania. Pigs of 11 weeks age (n = 200) were divided into 4 equal groups: a group fed with the probiotic, a prebiotic-fed group, a control group and a synbiotic (pre- and probiotic) treated group. The control group was fed without the specified additives. Probiotic treated group was fed with the same feedstuff but containing the additive BetaPlus® Ultra (Biochem) (5.12x10<sup>12</sup> CFU/kg -*Bacillus subtilis* DSM 5750, 5.12x10<sup>12</sup>UFC/kg -*Bacillus licheniformis* (DSM 5749) and 921 g of betaine), in an amount of 1 kg/tons of feed. Prebiotic group had added to the feed an extract derived from the cell wall of *Saccharomyces cerevisiae* (TechnoMos® - Biochem) in a dose of 250 g/ton of feed. The weight of 25 pigs in each compartment was recorded 9 times (over 85 days). Animal health was evaluated via clinical, hematological and parasitological examinations. In day 11 and day 77 and faeces samples were taken from each investigated group. Growth rate, feed consumption and some haematological parameters were measured.

The body weight, average total weight gain and feed conversion rate increase by the dietary inclusion of the both pre- and probiotic. In the compartment treated with prebiotic, the weight of pigs has increased with 1030 g/day compared to 982 g/day value recorded in the control group. The average feed consumption was 3.21 kg/day in the control group while the group treated with prebiotic was only 2.99 kg/day. Feed conversion rate for all treated groups were lower than the control one. Haematological parameters varied in physiological limits of species.

These products show promising effects as an alternative for antibiotics in order to eliminate the use of these drugs as growth-promoting additives.

**Keywords:** *growth, performance, pigs, prebiotics, probiotics.*

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## INTRODUCTION

The efficacy of antibiotics as growth promoters in pigs is well documented in numerous studies (Awad *et al.*, 2008; Chen *et al.*, 2005; Chu *et al.*, 2011). Because of the increasing concern regarding implication of antibiotics in development of resistant bacteria (Philips, 1999), alternative methods to improve health and efficiency of growth in pigs are widely investigated

(Awad *et al.*, 2009). Researches showed mostly a limited efficiency on growth improvement of pre- and probiotics (Chen, 2005; Chu *et al.*, 2011; Cromwell, 1991). The lack in alternatives to natural growth stimulants makes further studies of probiotics and prebiotics importance. Health of animals is correlated with a specific intestinal microflora, which in turn is affected in many situations like diseases, feeds, treatments

or stress (Gibson and Fuller, 2000). In order to maintain a healthy microflora one can use specific additives (pro- and prebiotics). Probiotics were described as “live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host” (Donohue, 2006; FAO, 2002; Guarner and Schaafsma, 1998). The definition of prebiotics presents them as “food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health” (De Vrese and Schrezenmeir, 2001; Gibson and Roberfroid, 1995). A combination of these two additives is called synbiotic. The complex implications of these feed additives on intestinal flora and on immune system were documented by Gourbeyre (2011).

The aim of the study was to measure the effect of feed supplements (pre- and probiotics) on pig growth and on some haematological parameters.

## MATERIALS AND METHODS

### Treatments

The probiotic used was a commercial product (BetaPlus® Ultra – Biochem), which contained live bacterial cultures:  $5.12 \times 10^{12}$  CFU/kg *Bacillus subtilis* (DSM 5750),  $5.12 \times 10^{12}$  UFC/kg *Bacillus licheniformis* (DSM 5749) and 921 g of betaine. The prebiotic used was TechnoMos® (Biochem) which is an extract of yeast wall (*Saccharomyces cerevisiae*) containing large quantities of  $\beta$ -glucans and oligosaccharides.

The prebiotic has important effects on the gastrointestinal system: it controls the gastric pH, it regulates the gut motility and stimulates the non-specific immunity. It also regulates the intestinal microorganism flora by stimulating the growth of normally present bacteria. Synbiotic is obtained by combining pro- and prebiotics and has a stimulating effect on the live bacteria from probiotics together with the local ones.

### Animals and protocol

Pigs (hybrid [(Yorkshire  $\times$  Landrace)  $\times$  Duroc]) of 11 weeks ( $n = 200$ ) were divided into 4 equal groups: a group fed with the probiotic (PROB), a prebiotic-fed group (PREB), a control group (C) and a symbiotic (pre- and probiotic) treated group (SYNB). The control group was fed with a feed without the specified additives. Probiotic treated

group was fed the same feedstuff but containing the additive Beta Plus® Ultra (Biochem) in an amount of 1 kg/tonne of feed. Prebiotic group had added to the feed TechnoMos® (Biochem) in a dose of 250 g/tonne of feed. SYN group received in feed a mixture of both additives with the same dose. Animals were separated in groups of 50 pigs in controlled environment with *ad libitum* access to water and food. Administration of products, body weight and food intake (quantity of food) were monitored for 85 days. Pigs were fed with a starter fodder from day 0 to day 21; growing fodder was given from day 22 to day 56 and from day 57 to day 85 was considered a finishing period. In days 11 and 77 samples were collected for haematological and parasitological examinations. During the study, animals were observed for health changes and the absence of parasites was certified by parasitological examination of faeces (Willis method).

### Haematological analysis

For haematological investigation, blood was collected (with EDTA) from the auricular vein. Haematological analyses were the following: haematocrit, haemoglobin, determination of total number of erythrocytes and leucocytes (tab.1). Determinations were performed by automated methods with Abacus Junior Vet haematology analyser.

### Growth performance

25 animals (randomly selected) from each group were weighted 9 times during the study. Weight gain for each dietary treatment was calculated and for each period (total body weight gain TBWG = weight at day 85 – weight at day 0). Feed consumption was recorded during the whole study for each treatment and the feed conversion rate (FCR) was calculated subsequently.

### Statistical analysis

Statistical analyses were conducted with the GraphPad InStat to determine if variables differed between groups. The Kolmogorov-Smirnov test was used to test the normal distribution of the data before statistical analysis was performed. Results are expressed as means  $\pm$  SD. All the investigated parameters were compared between groups by one-way ANOVA and subsequent Welch test. Probability values of less than 0.05 ( $P < 0.05$ ) were

considered significant and over 0.05 insignificant ( $P>0.05$ ).

## RESULTS AND DISCUSSION

### Haematological analysis

We have not observed significant changes of haematological parameters and the variations were between the physiological limits depending on species and age. No significant differences were observed compared to control group.

The haematocrit presented values between  $36.74\pm 1.73\%$  (PROB-group) -  $40.25\pm 1.69\%$  (SYNB-group) for day 11 and  $37.86\pm 1.09\%$  (PROB-group) -  $40.12\pm 0.98\%$  (PREB-group) for day 77. According to these values is the evolution of RBC levels, the values being relatively constant in all groups for both determinations ( $5.99\pm 0.60$  -  $7.04\pm 0.52$  T/L). (Tab. 2).

We monitored the level of total number of leucocytes. The most important difference was observed in case of administration of probiotic (PROB-group), which produced an important increase ( $P<0.05$ ) of total leucocytes; the level of this parameter was  $14.37\pm 1.27$  G/L for first determination, and  $20.24\pm 1.08$  G/L for the end of the study. Also, the increasing of total

leukocytes was important for PREB-group ( $13.25\pm 1.97$  G/L -  $17.92\pm 1.11$  G/L) (tab. 2). Generally, the administration of probiotics induce the immunostimulation by different mechanisms, one of the most important is the production of cytokines, which are involved in the control of leucopoiesis.

### Growth performance

The weight gain of studied animals in the starter period was 25.5 kg for control group (tab.3), with a fodder consumption of 2074.5 kg (tab. 4). In the growing period, the highest weight gain (38.3 kg) was measured for the PROB group with a feed consumption of 5318.5 kg. The finishing period shows different evolution of the weight gain, PREB group having the highest value (31.8 kg) but the lowest feed consumption being recorded in PROB group (4333 kg).

The PROB group had a limited weight gain for the finishing period to only 26.1 kg. The results obtained for the whole study shows that best weight evolution have been registered in the prebiotic treated group (87.6 kg) and the lowest feed consumption had the PROB group (tab. 3, tab. 4). These aspects are underlined by the evolution

**Tab. 1.** The methods used for haematological investigation

No.	INVESTIGATED PARAMETERS	METHOD	UNIT OF MEASUREMENT	REFERENCES*
1.	Haematocrit (PVC)	Calculation - the number of red blood cells	%	36-43
2.	Haemoglobin (Hgb)	Cyanmethaemoglobin method	g/dl	9-13
3.	Total number of erythrocytes (RBC)	Impedance method	T/L	5-7
4.	Total number of leucocytes (WBC)	Impedance method	G/L	11-22

\* Merck Veterinary Manual (2014)

**Tab. 2.** Haematological investigated parameters (average  $\pm$  SD) of the experimental group

GROUPS	PARAMETERS							
	PVC (%)		Hgb (g/dl)		RBC (T/L)		WBC (G/L)	
	Day 11	Day 77	Day 11	Day 77	Day 11	Day 77	Day 11	Day 77
PROB	36.74 $\pm$ 1.73	37.86 $\pm$ 1.09	11.32 $\pm$ 0.92	11.98 $\pm$ 1.11	6.83 $\pm$ 0.41	5.99 $\pm$ 0.60	14.37 $\pm$ 1.27	20.24 $\pm$ 1.08*
PREB	37.05 $\pm$ 2.83	40.12 $\pm$ 0.98	11.10 $\pm$ 0.88	11.23 $\pm$ 0.10	6.46 $\pm$ 0.50	6.77 $\pm$ 0.42	13.25 $\pm$ 1.97	17.92 $\pm$ 1.11*
SYNB	40.25 $\pm$ 1.69	39.76 $\pm$ 2.32	10.72 $\pm$ 0.35	12.04 $\pm$ 0.60	7.01 $\pm$ 0.66	6.06 $\pm$ 0.23	19.62 $\pm$ 2.82	19.96 $\pm$ 1.45
C	37.10 $\pm$ 1.02	38.82 $\pm$ 1.43	11.00 $\pm$ 1.30	10.84 $\pm$ 0.87	7.04 $\pm$ 0.52	6.66 $\pm$ 0.34	17.74 $\pm$ 3.01	18.54 $\pm$ 1.22

PROB=probiotic; PREB=prebiotic; SYNB=synbiotic; C=control; \*=significant difference

of feed conversion ratio (FCR) (tab. 5), which indicate a good value for the probiotic treated group while control had a FCR of 3.172.

The group treated with synbiotic presented an overall good weight gain (86.3 kg) but with a relative high feed consumption (11,762.0 kg) and a feed conversion ratio of 2.896.

The comparative analyses of the data indicates that highest weight gain was measured in the prebiotic treated group but the lowest feed consumption was of the probiotic treated group, and this is why the best FCR was calculated for the PROB group. The recorded differences between the four investigated groups for all growth parameters are not statistically assured.

The impact of prebiotic, probiotic and synbiotic products on the health and growth performance of animals is based on their capacity to alter the bacterial structure on the intestine stimulating the normally present bacteria and not the pathogenic microorganisms. This additives impact directly on the immune system present in the digestive tract (De Vrese and Schrezenmeir, 2008).

Similar results were obtained in a study performed on mice by Steřanút (2015) where the lowest feed conversion rate (FCR) was measured for probiotic treated group and all other groups presenting higher values. In an investigation of the performance impact of similar additives on broiler chickens it was concluded that the growth parameters were improved by the dietary inclusion of the both pre- and probiotic compared with the control group (Szakacs *et al.*, 2015).

Feed products that are classified as probiotics, prebiotics and competitive exclusion cultures have been utilized as pathogen reduction strategies in animal food with varying degrees of success. The efficacy of these products is often due to specific microbial ecological factors, which alter the competitive pressures experienced by microbial population of the gut (Callaway *et al.*, 2008).

The probiotics and prebiotics have different properties regarding pig health and growth. Probiotics impact on pathological microorganisms growth, by producing substances like bacteriocins (antibiotics) or acids like acetic and propionic (Cotter *et al.*, 2005). Because of their

**Tab.3.** Average growth rate of the pigs in the studied groups (kg/stage)

Period\Treatment	PREB	PROB	SYNB	C
Starter	21.5	20.5	23.5	25.5
Growing	34.3	38.3	35.7	36.6
Finishing	31.8	26.1	27.1	21.4
TOTAL	87.6	84.9	86.3	83.5

**Tab.4.** Total feed consumption for each growing period and for the total time period (kg)

Period\Treatment	PREB	PROB	SYNB	C
Starter	1733.5	1635	1843.5	2074.5
Growing	5127.5	5318.5	5435	5473
Finishing	4466	4333	4486	4649.5
TOTAL	11,327.00	11,286.50	11,762.50	12,197.00

**Tab.5.** Feed conversion rate calculated for each growing stage and for the total period

Period\Treatment	PREB	PROB	SYNB	C
<b>Starter</b>	1.790	1.692	1.659	1.764
<b>Growing</b>	3.320	2.953	3.238	3.248
<b>Finishing</b>	3.119	3.528	3.520	4.719
<b>TOTAL</b>	2.872	2.825	2.896	3.172

capacity to adhere to the epithelial cells of the gastrointestinal tract probiotic bacteria reduce the pathogens and toxins by enhancing the intestinal barrier (Gourbeyre *et al.*, 2011). A number of 12 strains of *Bifidobacteria* were analysed in order to determine which has a higher impact on growth performance of weaning piglets. The results highlighted that *B. animalis* subsp. *lactis Ra 18*, at  $10^{11}$  CFU per pig per day, appeared to be the best probiotic choice for improved growth performance (Modesto *et al.*, 2009).

Prebiotics ferment ingredients in a selective way, which impact on the composition of intestinal flora. The exact mechanism is not clear yet, but is considered to be related to the production of small chain fatty acid (Scaldaferri *et al.*, 2013).

A combination of probiotic(s) and prebiotic(s) also called synbiotics (Gibson and Roberfroid, 1995; Gourbeyre *et al.*, 2011), it is considered to help the passage of probiotics through digestive tract, and potentiates their effect (Zimmerman *et al.*, 2001).

The results of Chu *et al.* (2011) study suggest that synbiotics have similar effects with antibiotics on the nutrient digestibility and faecal microflora composition in growing pigs. In this study the growth performance of pigs was not affected by supplementation with synbiotics but digestibility of crude protein and dry matter were higher.

Gourbeyre *et al.* (2011) observed that prebiotics, probiotics or synbiotics can influence the intestinal microflora and modulate the immune response. These substances influence the innate and adaptive immune responses. This action is caused by stimulation of the cytotoxic activity of NK cells and macrophage phagocytosis.

The general impact of prebiotics and probiotics on pig health is due to the maintenance of a normal ecology on the gastrointestinal tract and the improved digestion and increased feed intake (Awad *et al.*, 2009).

## CONCLUSIONS

The analysis of the results reveals that the administration of pro-, pre- and synbiotic did not have negative effect on the health of animals. The investigated parameters have shown different aspects depending on the experimental group:

1. The treated group with probiotic presented the lowest feed conversion rate because of the reduced total consumed fodder.

2. The treated group with prebiotics had the best evolution regarding weight gain, but with a superior FCR than the group treated with probiotics.

3. All the treated groups presented better results compared with the control group regarding the investigated growth parameters.

4. The probiotic and prebiotic determined stimulation of the immune system by increasing of the total number of leukocytes.

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