#### 

# JAST (Journal of Animal Science and Technology) TITLE PAGE

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Effect of Saccharomyces cerevisiae boulardii on sows farrowing duration and reproductive performance, and weanling piglets performance and IgG concentration
Running Title (within 10 words)	Saccharomyces cerevisiae boulardii in sows and weanling piglets
Author	Haiqing Sun 1, Fernando Bravo de Laguna 2, Shuai Wang 2, Fengju Liu 3, Liang Shi 1, Haidi Jiang 1, Peng Qin 3, Xiaoxia Hu 1, Jiajian Tan 1,4
Affiliation	<ol> <li>1 Guangxi Yangxiang Co., Ltd., Guigang, Guangxi, 537100, PR China</li> <li>2 Lallemand SAS, Blagnac Cedex, France</li> <li>3 Beijing Hilink International Biotechnology Co., Ltd., Beijing, 100102, PR China</li> <li>4 Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, PR China</li> </ol>
ORCID (for more information, please visit https://orcid.org)	Haiqing Sun (https://orcid.org/0000-0002-5390-2612) sunhaiqing1985110@163.com Fernando Bravo de Laguna (https://orcid.org/000-0003-3166-9527) fbravodelaguna@Iallemand.com Shuai Wang (https://orcid.org/0000-0002-0963-5526) swang@Iallemand.com Fengju Liu (https://orcid.org/0000-0001-6078-1425) liu19861205@163.com Liang Shi (https://orcid.org/0000-0002-4085-6730) gxyxsl@163.com Haidi Jiang (https://orcid.org/0000-0002-1468-6459) 15994563071@163.com Xiaoxia Hu (https://orcid.org/0000-0002-0258-6485) 15977584352@163.com Jiajian Tan (https://orcid.org/0000-0002-2080-8627) tjjggyx@sina.com Peng Qin (https://orcid.org/0000-0002-0849-280X) acrobatcn@sina.com No potential conflict of interest relevant to this article was reported.
<b>Funding sources</b> State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
Acknowledgements	We acknowledge Guangxi Yangxiang and Beijing Hilink International Biotechnology for their logistics and technical support during the experiment, and Mr. Zhao Shanzhan for the execution of the trial and caring of the animals.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
<b>Authors' contributions</b> Please specify the authors' role using this form.	Conceptualization, Bravo de Laguna F, Sun H, Wang S, Liu F and Qin P. Data curation, Shi L, Jiang H, Hu X and Tan J. Formal analysis, Bravo de Laguna F. Methodology, Bravo de Laguna F, Sun H, Wang S, Liu F and Qin P. Software, Shi L, Jiang H, Hu X and Tan J.

	Validation, Bravo de Laguna F, Sun H and Wang S. Investigation, Bravo de Laguna F, Sun H, Wang S, Liu F and Qin P. Writing – original draft, Bravo de Laguna F and Wang S. Writing - review & editing: All
Ethics approval and consent to participate	The experimental protocol was approved by the Ethical Committee of Guangxi Yangxiang Co., Ltd. (Ethics approval number: JN.No201805 10c1001030)

3
4

# CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Fernando, Bravo de Laguna
Email address – this is where your proofs will be sent	fbravodelaguna@lallemand.com
Secondary Email address	fbravodelaguna@gmail.com
Address	19, Rue des Briquetiers. 31702, Blagnac, France
Cell phone number	+34 606 43 42 76
Office phone number	
Fax number	

#### 6 Abstract

7 We studied the effects of Saccharomyces cerevisiae boulardii CNCM I-1079 (LSB) supplemented to lactating sows 8 on reproductive traits and farrowing duration and to piglets from day 7 of life on post-weaning performance and IgG 9 concentration. Ninety-six Landrace x Yorkshire sows started the trial 5 days before the expected farrowing date. Sows 10 were distributed into 2 groups according to parity number and backfat thickness: control (CON: regular lactation diet) 11 and LSB (CON + LSB at  $2 \times 10^9$  colony forming units (CFU)/kg of feed). Seven days after birth, litters were randomly 12 selected from each group and supplemented creep feed with or without LSB at  $2 \times 10^9$  CFU/kg. At weaning, piglets 13 from CON sows were shifted to a commercial farm and allocated to 14 pens in groups of 25 piglets/pen according to 14 the creep feed supplemented during lactation. Piglets followed a 3-phase feeding program: creep, prestarter and starter, 15 with or without LSB at  $2 \times 10^9$  CFU/kg LSB in creep and prestarter, and  $1 \times 10^9$  CFU/kg LSB in starter. The piglets 16 were vaccinated against classical swine fever on days 41 and 72 of life. One day before each vaccination and at the 17 end of the trial, blood samples were collected from 15 randomly selected piglets per treatment and assessed for total 18 IgG. Supplemented sows with non-supplemented litters displayed the lowest backfat thickness loss during lactation 19 (p<0.05). The LSB supplementation shortened farrowing duration (p<0.05) and increased feed intake (p<0.05) during 20 the first week of lactation. The LSB-fed piglets were heavier at the end of creep (p < 0.05), prestarter (p < 0.05), and the 21 trial (p < 0.05); grew faster during creep (p < 0.05), starter (p < 0.05), and overall (p < 0.05); and displayed an improved 22 feed conversion ratio during creep (p<0.05). Total IgG content was higher at days 40 (p<0.05) and 71 (p<0.05) in 23 LSB-fed piglets. We conclude that supplementing sows with Saccharomyces cerevisiae boulardii CNCM I-1079 from 24 late gestation until weaning shortens farrowing duration, increases feed intake, and minimizes backfat losses during 25 lactation. When supplemented to piglet diet, post-weaning performance is improved. This improvement observed 26 could be linked to a better immune status, as suggested by the higher IgG.

27

Keywords: sows; weanling piglets; *Saccharomyces cerevisiae boulardii*; live yeast; farrowing duration; IgG
 29

30

#### Introduction

From the end of gestation [1] and during lactation, sows live in a catabolic state as they are not able to meet the energy requirements of their metabolic processes (i.e., maintenance, milk production, and growth) and hence need to mobilize body reserves [2]. Therefore, any help in optimizing the utilization of nutrients is of immense importance, for example for enhanced performance of the progeny since most of the energy would be used for milk production, and during parturition, which is a process with great energy expenditures [3]. A successful farrowing implies more

36 piglets weaned and sold [4]. Difficulties during parturition can lead to decreased milk production, which results in 37 reduced litter performance and increased mortality during lactation [5]. A slow farrowing process leads to an increase 38 in the proportion of stillborn piglets, and has been associated with a higher percentage of sows with high body 39 temperature [6], which also represents an energy cost at a time when energy-saving is imperative. Colostrum 40 production, and its intake by piglets after birth is of critical importance for their survival rate and later performance, 41 even after weaning [7], since it is high in essential nutrients and immunoglobulins. When the piglets ingest colostrum, 42 they uptake these compounds and, as a result, improve their immunity. Live yeast and probiotics supplementation 43 around the time of farrowing and during lactation have proven to show positive effects on colostrum quality [8], litter 44 performance [9], or maintenance of body reserves. More specifically, Saccharomyces cerevisiae var. boulardii 45 CNCM-1079 (LSB) supplemented to lactating sows is reported to increase the IgG and IgA content in colostrum [10] 46 and the average daily feed intake, thereby increasing milk production, which translates into an increased litter growth 47 [11].

Weaning is a critical moment in the piglets' life cycle, when they are exposed to environmental, social, and nutritional changes [12]. Through nutritional means we can alleviate the weaning stress. Live yeast supplementation helps postweaning piglets to deal with the nutritional changes. *Saccharomyces cerevisiae* var. *boulardii* has beneficial effects on immunomodulation and microbiota balance [13], with positive consequences in piglet performance. However, the effects of its supplementation in both lactation and creep feed on litter performance, as well as the impact of supplementation in the creep feed in non-supplemented sows' litters, on litter and post-weaning performance have never been investigated.

This study investigated the following effects: 1) supplementation of the live yeast LSB to sows during late gestation and lactation on farrowing duration and reproductive performance and 2) supplementation of LSB to piglets from week 1 of life until the end of weaning on post-weaning performance and IgG concentration, without the influence of the maternal dietary regime.

59

# **Materials and Methods**

- The experimental protocol was approved by the Ethical Committee of Guangxi Yangxiang Co., Ltd. (Ethics
  approval number: JN.No201805 10c1001030).
- 62 Experimental design, animals, housing, and diets

63 Ninety-six Landrace x Yorkshire (LY) sows of parities 3-6 ( $3.98 \pm 1.24$ ; mean  $\pm$  SD) started the trial when they

64 were moved to the farrowing room 5 days prior to the expected farrowing date. In total, 5 slatted-floor farrowing

65 rooms with 28 cages each were used in the experiment. Not all the cages in each room were used as they were reserved 66 for the foster sows. At the beginning of the trial, the sows were equally distributed into 2 groups according to the 67 parity number and backfat thickness (BFT) (Figure 1): control (CON: regular lactation diet) and LSB (CON + LSB at 68  $2 \times 10^9$  colony forming units (CFU)/kg of feed). The test product was Levucell SB<sup>®</sup> (Lallemand SAS, France). All 69 the diets utilized in the trial (Table 1) were formulated according to the National Research Council recommendations 70 [14]. There was a total of 48 replicates per treatment, as the experimental unit was the sow. Sows were fed twice a 71 day a total of 2.8 kg/sow/day of the lactation diet in two equal meals from the beginning of the experiment until 72 farrowing. One hour after each meal, the sows were monitored to confirm if they had consumed all the allowance. 73 Twenty-four hours after farrowing, the litters were homogenized to 11-13 piglets. This fostering was always made 74 between litters in the same treatment. The sows were fed ad libitum and had free access to water during the entire 75 period of lactation. From day 7 of life, all the litters were offered a creep feed (Table 1). There were 2 different creep 76 feeds: with or without LSB at  $2 \times 10^9$  CFU/kg. The litters from each sow group were randomly selected and equally 77 allotted to one of the creep feeds so that half of the litters were offered the supplemented creep feed, and the other half 78 the non-supplemented one. At wearing  $(22.7 \pm 0.68 \text{ days})$ , piglets from CON sows were moved to a commercial post-79 weaning farm (Figure 1) and allocated in 14 concrete-floor pens in groups of 25 piglets/pen according to the creep 80 feed received during lactation (Figure 1; LSB-supplemented (LSB) or non-supplemented (CON)), so that the average 81 initial BW was as similar as possible between pens. The building was equipped with wind blowers and water curtain 82 cooling systems to maintain the environmental temperature. The piglets followed a 3-phase feeding program (Table 83 1): creep, Prestarter, and starter, for 14, 16, and 25 days, respectively, with or without the LSB at  $2 \times 10^9$  CFU/kg LSB 84 in creep and prestarter feeds, and  $1 \times 10^9$  CFU/kg LSB in starter feed. Creep feed supplemented in post-weaning was 85 the same as the one supplemented during lactation (Figure 1). The piglets had free access to feed and water throughout 86 the experimental period. The post-weaning experimental diets (Table 1) were medicated with ZnO at 3 kg/ton, 2 kg/ton, 87 and 1.5 kg/ton in creep, prestarter, and starter, respectively. In addition, diets included 7.5 ppm of Nosiheptide and 50 88 ppm of Quinocetone, as well as 300 ppm of Oxytetracycline for the creep feed only.

89 Sampling and measurements

Body weight (BW) and BFT (Renco Lean-Meater, Renco Corporation, Minneapolis, USA) were measured at the
beginning of the trial and reassessed at day 21 after farrowing. Additionally, BW was recorded 1 day after farrowing
(Figure 1). Average daily feed intake (ADFI) during the period from birth to day 21 was recorded (Figure 1), as well
as the number of total piglets born, born alive, stillborn, and present at day 21 were noted. The farrowing duration in

94 minutes was measured for each sow as the difference between the time of birth of the first piglet and the expulsion of 95 the placenta. The suckling piglets were weighed at birth, and at days 7 and 21 after farrowing (Figure 1). After weaning, 96 piglets were weighed at the time of the distribution in the pens, during their changes in diet and at the end of the trial 97 (Figure 1). Total feed intake per phase was measured as the difference between feed supplied and the remaining feed 98 at the end of each feeding phase, and the ADFI was calculated accordingly (Figure 1). Average daily gain (ADG) and 99 ADFI were utilized to determine the feed conversion ratio (FCR) per phase and overall. All the piglets were vaccinated 100 against porcine circovirus type 2 (Wuhan Keqian Biology Co., Ltd., China) at day 25 of life (3 days after weaning), 101 and against the classic swine fever (CSF; Wuhan Keqian Biology Co., Ltd., China) on days 41 and 72 of life (18 and 102 49 days after weaning, respectively), according to the suppliers' recommendations. One day before each vaccination 103 against CSF, and at the end of the trial (day 77 of life), blood samples of 15 randomly selected piglets per treatment 104 were collected. The piglets were bound, and blood was collected from their anterior vena cava. To obtain the serum, 105 the blood was left for 15 minutes at environmental temperature for natural coagulation and centrifuged for 20 minutes 106 at 3000 rpm. The serum supernatant was collected carefully and kept at -20°C until analysis. The samples were 107 assessed for their total IgG content by ELISA (NJJCBIO, China).

#### 108 Statistical analysis

109 The data were analyzed using SPSS Statistics 26.0 (IBM). Prior to the analysis, all the variables were assessed for 110 normality according to the Kolmogorov-Smirnov test. When they were normally distributed, the reproductive 111 performance variables, BFT and BW at farrowing, as well as ADFI of sows during the first week of lactation were 112 submitted to an analysis of variance with sow dietary treatment, room, parity and their interactions as main effects. 113 The litter performance variables between days 7 and 21, BW and BFT at weaning, BW and BFT loss during lactation, 114 and the ADFI of sows in weeks 2, 3, and overall were analyzed submitted to an analysis of variance and analyzed 115 according to a  $2 \times 2$  factorial approach with sow dietary treatment, litter diet, room, parity and their interactions as 116 main effects. For litter size variables, the number of piglets at the beginning of the analyzed period was used as a 117 covariate. For litter performance variables, the body weight of the litter at the beginning of the analyzed period was 118 used as a covariate. The post-weaning piglets' performances and IgG concentration were submitted to an analysis of 119 variance with treatment as the main effect. Alternatively, if the variables were not normally distributed, data were 120 processed using Kruskal-Wallis non-parametric test, with treatment as the main effect. The experimental unit was the 121 sow for lactation variables, the pen for post-weaning variables, and the piglet for IgG concentration in the blood. The

122 variability of data is expressed as the standard error of means (SEM). For all the statistical procedures a probability

123 value lower than 0.05 was considered significant, and a probability value between 0.05 and 0.1 was considered a trend.

124

### **Results**

125 No dietary treatment effect was depicted on the reproductive performances (Table 2). After fostering, average litter 126 size resulted in 12.09 and 11.96 piglets/litter for CON and LSB sows, respectively, and in 12.25, 11.91, 11.68, and 127 12.22 piglets/litter for CON sows with non-supplemented and supplemented litters, and LSB sows with non-128 supplemented and supplemented litters, respectively. At day 7, the average litter size was 12.21, 11.68, 11.32, and 129 12.00 piglets/litter, for CON sows with non-supplemented and supplemented litters, and LSB sows with non-130 supplemented and supplemented litters, respectively. Moreover, average litter weight after cross fostering was 17.86 131 and 17.88 kg for CON and LSB sows, respectively, and 17.90, 17.82, 17.62, and 18.13 kg for CON sows with non-132 supplemented and supplemented litters, and LSB sows with non-supplemented and supplemented litters, respectively. 133 At day 7, average litter weight was 27.61, 26.63, 25.37, and 25.57 kg for CON sows with non-supplemented and 134 supplemented litters, and LSB sows with non-supplemented and supplemented litters, respectively. We found a 135 significant interaction (p < 0.05) between sow diet and litter diet in litter weight gain during lactation, suggesting that 136 LSB supplementation to the litters improved litter gain compared to the non-supplemented litters in the LSB sows. 137 The LSB supplementation to sows increased the ADFI during the first week of lactation (p<0.05).

Sow diet	CON		L	SB	SEM		<i>p</i> -value	
Litter diet	CON	LSB	CON LSB		SEM	SD	LD	$SD \times LD$
Litter size (n)								
Total born	14	.32	13	.74	0.433	0.313 <sup>1</sup>	-	-
Born alive	13	.30	13	.01	0.393	$0.574^{1}$	-	-
Stillborn	1.04		0.	0.76		0.303 <sup>2</sup>	-	-
Stillborn (%)	6.73		5.	5.25		$0.395^{2}$	-	-
At day 7	11	.94	11	.76	0.096	$0.173^{3}$	-	-
At day 21	10.84	10.83	10.86	11.00	0.146	$0.631^4$	0.717	0.705
Litter weight (kg)								
At day 7	26	.93	26	.00	0.461	$0.165^{5}$	-	-
At day 21	73.87	74.19	73.28	74.82	1.176	$0.989^{6}$	0.576	0.713
Litter gain (kg)								
Days 0-7	9.	05	8.	12	0.461	$0.165^{1}$	-	-
Days 8-21	47.33	47.65	46.75	48.24	1.176	$0.989^{6}$	0.576	0.713
Days 0-21	56.59	56.23	54.36	58.36	1.373	$0.500^{6}$	0.498	0.038
ADFI (kg/d)								
Week 1	4.	.37	4.	78	0.094	$0.002^{1}$	-	-
Week 2	6.68	6.99	6.99	6.87	0.133	$0.579^{7}$	0.573	0.216
Week 3	7.82	7.76	7.52	7.74	0.128	0.3567	0.625	0.402
Overall	6.32	6.38	6.37	6.47	0.114	$0.626^{7}$	0.591	0.880

138 Table 2. Effect of LSB supplementation on reproductive performance and lactation feed intake of sows

- 139 Abbreviations: CON: Control lactation/creep feed diets; LSB: Control diet +  $2 \times 10^9$  CFU/kg of Saccharomyces
- 140 cerevisiae var. boulardii CNCM I-1079; SEM: Standard Error of the Mean; SD: sow diet; LD; litter diet; ADFI: 141 average daily feed intake
- 142 <sup>1</sup>Analysis of variance (with room, parity, sow diet, and their interactions as effects; the interactions were non-143 significant, therefore, they were removed from the model
- 144 <sup>2</sup>Non-parametric test (Kruskal-Wallis) with sow diet as effect
- 145 <sup>3</sup>Analysis of variance (with room, parity, sow diet, and their interactions as effects; number of piglets at the beginning 146 of the period was used as covariate); the interactions were non-significant, therefore, they were removed from the
- 147 model
- 148 <sup>4</sup>Analysis of variance (with room, parity, sow diet, litter diet, and their interactions as effects; number of piglets at the
- 149 beginning of the period was used as covariate); the interactions with room and parity were non-significant, therefore, 150 they were removed from the model
- 151 <sup>5</sup>Analysis of variance (with room, parity, sow diet, and their interactions as effects; litter weight at the beginning of
- 152 the period was used as covariate); the interactions were non-significant, therefore, they were removed from the model
- 153 <sup>6</sup>Analysis of variance (with room, parity, sow diet, litter diet, and their interactions as effects; litter weight at the
- 154 beginning of the period was used as covariate); the interactions with room and parity were non-significant, therefore, 155 they were removed from the model
- 156 157 <sup>7</sup>Analysis of variance (with room, parity, sow diet, litter diet, and their interactions as effects;); the interactions with
- room and parity were non-significant, therefore, they were removed from the model
- 158
- 159 At day 109, the average sow body weight was 265.10, 259.49, 257.80, and 265.43 kg for CON sows with non-
- 160 supplemented and supplemented litters, and LSB sows with non-supplemented and supplemented litters, respectively.
- 161 And after farrowing, it was 242.64, 238.91, 237.41, and 244.62 CON sows with non-supplemented and supplemented
- 162 litters, and LSB sows with non-supplemented and supplemented litters, respectively. Average BFT at day 109 was
- 163 15.92, 15.91, 15.91, and 15.87 for CON sows with non-supplemented and supplemented litters, and LSB sows with
- 164 non-supplemented and supplemented litters, respectively. There was a significant difference in BFT loss during
- 165 lactation, where the supplemented sows displayed a lower loss compared to the CON sows (p < 0.05); furthermore, the
- 166 sows with non-supplemented litters tended to lose less BFT than the sows with supplemented litters (p < 0.1). Overall,
- 167 the non-supplemented litters from LSB sows displayed the lowest loss, however, there was no interaction between
- 168 sow diet and litter diet (Table 3).

169 Table 3. Effect of LSB supplementation on body condition of sows

Sow diet	CO	DN	LS	B	SEM	<i>p</i> -value		
Litter diet	CON	LSB	CON	LSB	SEN	SD	LD	$SD \times LD$
Body weight (kg)								
At day 109	264	1.75	263	.71	2.655	$0.771^{1}$	-	-
After farrowing	243	3.72	243	.42	2.755	$0.936^{1}$	-	-
At day 21	237.88	236.13	236.49	239.53	3.225	$0.817^{2}$	0.882	0.583
Loss	6.97	4.85	4.71	6.36	1.717	$0.870^{2}$	0.918	0.414
Backfat thickness (mm)								
At day 109	16	.42	16.	34	0.269	$0.827^{1}$	-	-
At day 21	15.27	15.46	16.24	15.43	0.345	$0.308^{2}$	0.500	0.287
Loss	0.97	1.21	-0.04	0.95	0.226	$0.048^{2}$	0.057	0.239

170 Abbreviations: CON: Control lactation/creep feed diets; LSB: Control diet +  $2 \times 10^9$  CFU/kg of

171 Saccharomyces cerevisiae var. boulardii CNCM I-1079; SEM: Standard Error of the Mean; SD: sow diet;

172 LD; litter diet

- <sup>1</sup>Analysis of variance (with room, parity, sow diet, and their interactions as effects); the interactions were
- 174 non-significant, therefore, they were removed from the model
- <sup>2</sup>Analysis of variance (with room, parity, sow diet, litter diet, and their interactions as effects); the interactions with
   room and parity were non-significant, therefore, they were removed from the model
- 177
- 178 The LSB supplementation to sows shortened the farrowing duration (p < 0.05) by nearly 100 minutes (-27%; Table
- 4). The piglets began the post-weaning period with  $7.60 \pm 0.34$  kg on average. The LSB-fed piglets displayed a heavier
- 180 BW at the end of creep (Table 4; p < 0.05), prestarter (p < 0.05), and the trial (p < 0.05). The ADG during creep (p < 0.05),
- 181 starter (p < 0.05), and overall (p < 0.05) was greater in the LSB-fed piglets; these differences were mainly due to a higher
- ADFI of the LSB-fed piglets: in the first 3 days (p < 0.05), between days 4 and 7 post-weaning (p < 0.05), during the
- 183 first week post-weaning (p<0.05) and overall (p<0.05), and to a better immune status suggested by the higher total
- 184 IgG concentration (Table 5) at days 40 (p < 0.05) and 71 of life (p < 0.05). Additionally, the LSB-fed piglets tended to
- 185 a higher ADFI in starter (*p*<0.1). Growth and intake results translated into a better FCR of the LSB-fed piglets during
- 186 creep (p < 0.05), and a trend to a better overall FCR (p < 0.1).

		U	-	01
Items	CON	LSB	SEM	<i>p</i> -value <sup>1</sup>
Farrowing duration (min)	317.73	221.11	31.60	$0.027^{1}$
BW (kg)				
Day 22	7.65	7.56	0.090	-
Day 36	10.82	11.31	0.042	$< 0.001^{2}$
Day 52	18.07	18.60	0.146	$0.039^{2}$
Day 77	35.41	36.94	0.181	$< 0.001^{2}$
ADG (g/d)				
Days 22-36	230	264	2.97	$< 0.001^{2}$
Days 37-52	453	456	10.60	$0.900^{2}$
Days 53-77	693	734	3.62	$0.012^{2}$
Days 22-77	618	652	3.12	$< 0.001^{2}$
ADFI (g/d)				
Days 22-36	313	340	2.43	$< 0.001^{2}$
Days 37-52	740	759	14.31	$0.524^{2}$
Days 53-77	1206	1248	10.10	$0.066^{2}$
Days 22-77	843	874	5.43	$0.016^{2}$
Days 22-25	136	155	4.06	$0.039^{2}$
Days 25 29	189	234	6.33	$0.005^{2}$
Days 22-29	166	200	2.52	$< 0.001^{2}$
FCR				
Days 22-36	1.364	1.286	0.013	$0.014^{2}$
Days 37-52	1.637	1.668	0.017	0.396 <sup>2</sup>
Days 53-77	1.741	1.701	0.017	$0.269^{2}$
Days 22-77	1.615	1.586	0.007	$0.054^{2}$

#### 187 Table 4. Effect of LSB supplementation on farrowing duration and post-weaning performance

188 Abbreviations: CON: Control lactation/post-weaning diets; LSB: Control diet +  $2 \times 10^9$  CFU/kg in

189 lactation, creep and prestarter feeds, and  $1 \times 10^9$  CFU/kg in starter feed of *Saccharomyces cerevisiae* 

var. *boulardii* CNCM I-1079; SEM: Standard Error of the Mean; BW: body weight; ADG: average
 daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio

<sup>1</sup>Analysis of variance (with room, parity, sow diet, and their interactions as effects); the interactions
 were non-significant, therefore, they were removed from the model

<sup>194</sup> <sup>2</sup>Analysis of variance (with post-weaning treatment as effect; initial body weight was used as

195 covariate)

Item	CON	LSB	SEM	<i>p</i> -value <sup>1</sup>
IgG (mg/ml)				
Day 40	9.51	11.32	0.267	0.002
Day 71	9.57	10.88	0.277	0.027
Day 77	10.20	9.69	0.413	0.548

196 Table 5. Effect of LSB supplementation on total IgG concentration in post-weaning piglets ´serum

197 Abbreviations: CON: Control post-weaning diet; LSB: Control diet  $+ 2 \times 10^9$  CFU/kg in creep and 198 prestarter feeds, and  $1 \times 10^9$  CFU/kg in starter feed of *Saccharomyces cerevisiae* var. *boulardii* CNCM I-

199 1079; SEM: Standard Error of the Mean; IgG: immunoglobulin G

<sup>1</sup>Analysis of variance (with post-weaning treatment as effect)

201

202

#### Discussion

203 The first phase of the study showed the effect of the supplementation of a specific live yeast strain to sows beginning 204 from the last days of gestation until weaning on the farrowing duration and performance from a productivity standpoint. 205 We did not observe any effect of LSB supplementation on reproductive performance; however, the LSB supplementation shortened the farrowing duration by nearly 100 minutes. According to Oliviero et al. [15], farrowing 206 207 duration is positively correlated with the BFT at farrowing. In their study, they used Finnish Yorkshire x Finnish 208 Landrace sows, with an average BFT at farrowing of 14.5 mm (ranging from 7.5 to 24.5), which was lower compared 209 to our observations (15.9 mm; ranging from 12 to 23), and the farrowing duration was on average 272 minutes, which 210 was shorter but comparable to our observations (277 minutes). The differences in the relative increase of duration by 211 unit of backfat may be due to the different genetics of the sows in both trials. However, we did not observe any 212 difference in the BFT at farrowing between treatments, therefore the effect on the farrowing duration might be 213 explained through other mechanisms, for instance sow comfort and well-being, which are important to alleviate 214 maternal stress around farrowing, as stress has adverse effects on farrowing duration and offspring's development 215 [16]. One of the markers of sow comfort is the degree of constipation around farrowing. Oliviero et al. [15] indicated 216 that farrowing duration was increased in sows displaying severe constipation. Indeed, live yeast supplementation helps 217 to minimize constipation and to increase comfort, likely through the modulation of the microbiota [9]; these authors 218 indicated that the utilization of live yeast in the sows limits constipation. Tan et al. [17] reported better constipation 219 score at the end of gestation when the sows were fed Saccharomyces boulardii alone or in combination with konjac 220 flour for two subsequent cycles. In that study, sows supplemented with Saccharomyces boulardii displayed the highest 221 percentage of non-constipated sows, and the lowest percentage of sows with extremely severe constipation. Moreover, 222 the feed intake during lactation of the second supplementation cycle was higher in the supplemented sows than in the 223 control sows. The mechanism behind reduced constipation may be linked to higher intestinal motility [18], which 224 could be a consequence of a better use of the dietary fiber when live yeast is supplemented. Additionally, we

225 hypothesize that constipation, and therefore the cumulated fecal material in the hind gut, may partially block the birth 226 canal, hence impairing and prolonging the farrowing process. The advantageous effect of the LSB supplementation 227 on early lactation feed intake observed in our study could be connected to potentially minor constipation, since 228 constipation reduces feed intake in lactating sows [19]. Hence, we can further hypothesize that the benefits of the LSB 229 supplementation in reducing constipation around farrowing resulted in a better feed consumption immediately after 230 farrowing, which keeps stimulating the sows' feeding behavior along with lactation, as illustrated by an overall greater 231 feed intake for the LSB-fed sows. However, in our study constipation was not measured and deserves further 232 investigation.

233 It might be surprising that the lowest BFT loss was observed in the non-supplemented litters from the LSB-fed 234 sows. This could be explained keeping in view 2 possible reasons: first, the growth of these litters was numerically 235 the lowest during lactation; and second, higher milk production can be linked to a higher backfat mobilization and a 236 higher feed intake [20]. In our study, we could assume that the milk production of the supplemented sows with 237 supplemented piglets was higher, as alluded by the upsurge in litter weight gain during lactation, as well as an 238 increased backfat loss compared to supplemented sows with non-supplemented litters. In both supplemented and non-239 supplemented sows, the supplemented litters provoked a bigger backfat loss, although only significant in the 240 supplemented sows. In the supplemented sows, the increased backfat loss could be explained by the numerically 241 heavier litter weight, implying an increased milk production and body reserves mobilization. However, in the non-242 supplemented sows, it is possible that milk production was not enough, and they needed to mobilize body reserves. 243 The faster farrowing process in the study may have also contributed to the conservation of body reserves of the LSB-244 fed sows, both during the farrowing per se and after the process. Indeed, Tummaruk and Sang-Gassanee [6] reported 245 that when farrowing was prolonged, the percentage of sows with fever increased as well, and a rise in body temperature 246 had a detrimental effect on energy expenses. Owing to a quicker farrowing, the LSB-fed sows likely spared some 247 energy, minimizing body reserves mobilization. Thongkhuy et al. [21] found a positive correlation between BFT at 248 the end of gestation and milk yield, and a negative correlation with backfat loss during lactation. This could imply that 249 the more backfat at farrowing is preserved, the more the sow prioritizes the use of the body reserves for milk 250 production, and therefore the piglets' performances during lactation are improved. Since all sows in our study showed 251 the same BFT at farrowing, the analogous litter performance found during lactation would follow [21]'s hypothesis. 252 However, the energy-saving operated by the LSB-fed sows could be precluding a longer-term effect on the next 253 reproductive cycle but requires a repeated reproductive cycles study to confirm it.

254 Supplementing the sows with LSB implies a more efficient use of the feed through the modulation of the microbial 255 ecosystem since it is proven to increase the relative abundance of *Fibrobacter* family in the piglets' feces [22], using 256 fiber for their own metabolism, releasing short chain fatty acids (SCFA) into the intestinal lumen, and leaving more 257 energy available for the metabolism of the sow [23]. More efficient use of the energy from the feed together with the 258 higher feed intake of the LSB-fed sows during the first week of lactation are probably the two main reasons why 259 supplementing sows with LSB helped them to diminish backfat loss during lactation. Such observation strongly 260 suggests improved management of the body reserves and a higher efficiency in the utilization of nutrients. In summary, 261 the lower backfat loss observed in our study may be explained by the greater overall feed intake, the higher feed 262 efficiency caused by LSB, and the quicker farrowing process.

263 The second step of the study aimed at assessing the effect of the live yeast supplementation to piglets from day 7 264 of life, without the influence of the maternal dietary regime on post-weaning performance. The piglets fed live yeast 265 responded better than the non-supplemented piglets as demonstrated by their greater growth, feed intake, and feed 266 efficiency. The faster ADG is in line with previous studies in weanling piglets fed Saccharomyces cerevisiae var. 267 boulardii [24]. In our study, the ADG seems to be directly related to the higher ADFI especially right after weaning, 268 as we could observe a higher ADFI in the first 3 days of study, between days 4 and 7, and as a result in the whole first 269 week post-weaning. However, the possible hypothesis to explain a faster growth and feed intake is an increased 270 apparent total tract digestibility of dry matter and gross energy [25], caused by a better integrity of the intestinal 271 epithelium [24]. On one hand, higher digestibility leaves more nutrients available for growth; on the other, feed intake 272 capacity can be earlier restored as the nutrients are absorbed leaving space in the intestinal lumen. Besides, the effects of Saccharomyces cerevisiae var. boulardii on the microbial ecosystem, leaving more energy available and 273 274 suppressing harmful bacteria, might be the cause for the positive effects on the piglets' performance. Furthermore, 275 since supplementation started from day 7 of life, piglets benefited from the live yeast for a longer period than just 276 during the post-weaning stage.

A factor that may have helped to enhance the piglets ´performance was the environmental temperature. The upper critical temperature of a piglet varies from around 31 °C at weaning until 24 °C at 30 kg [26], provided they are housed on a concrete floor as in our study. The minimum and maximum temperatures inside the facilities were 28 °C and 35 °C, respectively. We observed increased ADG and ADFI in the LSB-fed piglets compared to CON piglets, indicating that LSB could alleviate some of the heat stress' negative impact on piglets in the late post-weaning stage, which is in line with the findings of Labussière et al. [27] in finishing pigs fed LSB. 283 The potential benefits of the use of live yeast in swine production result partially from the sow (transfer of IgGs 284 from colostrum and milk [10, 28, 29], or colonization of the piglets' gastrointestinal tract from sow feces), and partially 285 from the live yeast intake of the piglets. We found differences in the IgG concentration in piglets at days 40 and 71 of 286 life. However, given that the sows did not receive live yeast during lactation and, therefore, could not be the agent of 287 the immunoglobulin transfer to the piglets, the explanations lie within the piglets. One is based on piglets capacity to 288 synthetize specific antibodies after vaccination when they are fed yeast products, and the other relies on their ability 289 to produce more IgG's [30]. These authors found that feeding recombinant yeast Pichia pastoris to post-weaning 290 piglets increased plasma IgG concentration and the specific antibodies to porcine reproductive and respiratory 291 syndrome virus. Hence, the extra synthesis would be in addition to the basal concentration. Kogan and Kocher [31] 292 have also indicated the immunomodulatory properties of yeast compounds from Saccharomyces cerevisiae. In 293 addition, the BW change of the veast-fed piglets was bigger than that of the non-supplemented ones, which is 294 consistent with our findings. There are no references in the literature about the effect of supplementing live yeast to 295 weanling piglets on plasma IgG concentration; however, White et al. [32] found a higher IgG level in serum in post-296 weaning piglets that were fed a combination of brewer's yeast and citric acid. The fact that in our study there are no 297 differences at day 77 could be due to the animals 'exposure to the farm environment, which contributed to the leveling 298 of immune status over time.

299

## Conclusion

We conclude that supplementing sows with *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 from late gestation until weaning shortens the farrowing duration, increases feed intake of sows in the first week after farrowing, and reduces backfat thickness losses during lactation. When the same is supplemented to piglets, post-weaning growth performance is improved under these trial conditions. This improvement could be due to a better immune status, as suggested by the higher IgG concentration of the LSB-fed piglets.

305

### Acknowledgments

We acknowledge Guangxi Yangxiang and Beijing Hilink International Biotechnology for their logistics and technical support during the experiment and Mr. Zhao Shanzhan for the execution of the trial and tending of the animals.

**References** 310 311 312 1. Kim SW, Weaver AC, Shen YB, Zhao Y. Improving efficiency of sow productivity: nutrition and health. J Anim 313 Sci Biotechnol. 2013;4:26. doi:10.1186/2049-1891-4-26. 314 2. Leman AD. Diseases of swine. 6th ed. US: Iowa State University; 1986. 315 Oliveira RA, Neves JS, Castro DS, Lopes SO, Santos SL, Silva SVC, et al. Supplying sows energy on the expected 3. 316 day of farrowing improves farrowing kinetics and newborn piglet performance in the first 24 h after birth. Animal. 317 2020;14:2271-6. doi:10.1017/S1751731120001317. 318 Ison SH, Jarvis S, Hall SA, Ashworth CJ, Rutherford KMD. Periparturient behavior and physiology: further 4. 319 insight into the farrowing process for primiparous and multiparous sows. Front Vet Sci. 2018;5:122. 320 doi:10.3389/fvets.2018.00122. Peltoniemi O, Oliviero C. Housing, management and environment during farrowing and early lactation. In: 321 5. 322 Farmer C, editor. The gestating and lactating sow. The Netherlands: Wageningen Academic Publishers; 2015. 323 p. 231-252. doi:10.3920/978-90-8686-803-2\_10. 324 6. Tummaruk P, Sang-Gassanee K. Effect of farrowing duration, parity number and the type of anti-inflammatory 325 drug on postparturient disorders in sows: a clinical study. Trop Anim Health Prod. 2013;45:1071-7. 326 doi:10.1007/s11250-012-0315-x. 327 7. Declerck I, Dewulf J, Sarrazin S, Maes D. Long-term effects of colostrum intake in piglet mortality and 328 performance. J Anim Sci. 2016;94:1633-43. doi:10.2527/jas.2015-9564. 329 Peng X, Yan C, Hu L, Huang Y, Fang Z, Lin Y, et al. Live yeast supplementation during late gestation and 8. 330 lactation affects reproductive performance, colostrum and milk composition, blood biochemical and

immunological parameters of sows. Anim Nutr. 2020. doi:10.1016/j.aninu.2020.03.001.

- 332 9. Chaucheyras-Durand F, Durand H. Probiotics in animal nutrition and health. Benef Microbes. 2010;1:3–9.
  333 doi:10.3920/BM2008.1002.
- 334 10. Guillou D, Chevaux E, Rosener D, Treut Y, Le Dividich J. Feeding live saccharomyces cerevisiae boulardii (SB)
  335 to sows increases immunoglobulin content in colostrum and milk. J Anim Sci. 2012;90.
- 336 11. Domingos RL, Silva B, Bravo de Laguna F, Araujo W, Gonçalves MF, Rebordões F, et al. Saccharomyces
- cerevisiae var. boulardii CNCM I-1079 during late gestation and lactation improves voluntary feed intake, milk
   production and litter performance of mixed-parity sows in a tropical humid climate. Anim Feed Sci Technol.
- 339 2021;272:114785. doi:10.1016/j.anifeedsci.2020.114785.
- 340 12. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. J Anim Sci Biotechnol.
  341 2013;4:19. doi:10.1186/2049-1891-4-19.
- 342 13. Stier H, Bischoff SC. Influence of Saccharomyces boulardii CNCM I-745on the gut-associated immune system.
  343 Clin Exp Gastroenterol. 2016;9:269–79. doi:10.2147/CEG.S111003.
- 344 14. National Research Council. Nutrient enquirements of swine. Washington, DC: The National Academies Press;
  345 2012.
- 346 15. Oliviero C, Heinonen M, Valros A, Peltoniemi O. Environmental and sow-related factors affecting the duration
  347 of farrowing. Anim Reprod Sci. 2010;119:85–91. doi:10.1016/j.anireprosci.2009.12.009.
- 348 16. Muns R, Nuntapaitoon M, Tummaruk P. Non-infectious causes of pre-weaning mortality in piglets. Livest Sci.
  349 2016;184:46–57. doi:10.1016/j.livsci.2015.11.025.
- Tan CQ, Wei HK, Sun HQ, Long G, Ao JT, Jiang SW, Peng J. Effects of supplementing sow diets during two
   gestations with konjac flour and Saccharomyces boulardii on constipation in peripartal period, lactation feed
   intake and piglet performance. Anim Feed Sci Technol. 2015;210:254–62. doi:10.1016/j.anifeedsci.2015.10.013.

- 353 18. Oliviero C, Kokkonen T, Heinonen M, Sankari S, Peltoniemi O. Feeding sows with high fibre diet around
  and early lactation: impact on intestinal activity, energy balance related parameters and litter
  performance. Res Vet Sci. 2009;86:314–9. doi:10.1016/j.rvsc.2008.07.007.
- Tabeling R, Schwier S, Kamphues J. Effects of different feeding and housing conditions on dry matter content
  and consistency of faeces in sows. J Anim Physiol Anim Nutr (Berl). 2003;87:116–21. doi:10.1046/j.14390396.2003.00423.x.
- 359 20. Strathe AV, Bruun TS, Hansen CF. Sows with high milk production had both a high feed intake and high body
  360 mobilization. Animal. 2017;11:1913–21. doi:10.1017/S1751731117000155.
- Thongkhuy S, Chuaychu SB, Burarnrak P, Ruangjoy P, Juthamanee P, Nuntapaitoon M, Tummaruk P. Effect of
   backfat thickness during late gestation on farrowing duration, piglet birth weight, colostrum yield, milk yield and
   reproductive performance of sows. Livest Sci. 2020;234:103983. doi:10.1016/j.livsci.2020.103983.
- Achard C, Bravo de Laguna F, Castex M, Combes S, Agazzi A, Bontempo V, et al. Effect of Saccharomyces
   cerevisiae boulardii supplementation on gut microbiota in post-weaning piglets in a context of antibiotics and
- 366 ZnO removal. Poster session presented at: ZeroZinc Summit 2019; 2019 June 17-18; Copenhagen, Denmark.
- 367 23. Jha R, Berrocoso JF. Dietary fiber and protein fermentation in the intestine of swine and their interactive effects
  368 on gut health and on the environment: a review. Anim Feed Sci Technol. 2016;212:18–26.
  369 doi:10.1016/j.anifeedsci.2015.12.002.
- 24. Di Giancamillo A, Bontempo V, Savoini G, Dell'Orto V, Vitari F, Domeneghini C. Effects of live yeast dietary
  supplementation to lactating sows and weaning piglets. Int J Probiotics Prebiotics. 2007;2:55–66.
- 25. Lu H, Wilcock P, Adeola O, Ajuwon KM. Effect of live yeast supplementation to gestating sows and nursery
  piglets on postweaning growth performance and nutrient digestibility. J Anim Sci. 2019;97:2534–40.
  doi:10.1093/jas/skz150.

- 375 26. Muirhead MR, Alexander TJL, Carr J. Managing pig health: a reference for the farm. 2nd ed. Sheffeild: 5M
  376 Enterpriese Ltd; 2013.
- 27. Labussière E, Achard CS, Dubois S, Combes S, Castex M, Renaudeau D. Saccharomyces cerevisiae boulardii
  CNCM I-1079 supplementation in finishing male pigs helps to cope with heat stress through feeding behavior
  and gut microbiota modulation. Br J Nutr. 2021:1–35. doi:10.1017/S0007114521001756.
- 380 28. Jang YD, Kang KW, Piao LG, Jeong TS, Auclair E, Jonvel S, et al. Effects of live yeast supplementation to
  381 gestation and lactation diets on reproductive performance, immunological parameters and milk composition in
  382 sows. Livest Sci. 2013;152:167–73. doi:10.1016/j.livsci.2012.12.022.
- 29. Zanello G, Meurens F, Serreau D, Chevaleyre C, Melo S, Berri M, et al. Effects of dietary yeast strains on
  immunoglobulin in colostrum and milk of sows. Vet Immunol Immunopathol. 2013;152:20–7.
  doi:10.1016/j.vetimm.2012.09.023.
- 386 30. Luo G, Yang L, Liang G, Wan X, Chen C, Wang B, et al. Construction and synergistic effect of recombinant
  387 yeast co-expressing Pig IL-2/4/6 on immunity of piglets to PRRS vaccination. Procedia Vaccinol. 2015;9:66–79.
  388 doi:10.1016/j.provac.2015.05.011.
- 389 31. Kogan G, Kocher A. Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest Sci.
  2007;109:161–5. doi:10.1016/j.livsci.2007.01.134.
- 32. White LA, Newman MC, Cromwell GL, Lindemann MD. Brewers dried yeast as a source of mannan
  oligosaccharides for weanling pigs. J Anim Sci. 2002;80:2619–28. doi:10.1093/ANSCI/80.10.2619.

## **Tables and figures**

Items Lactation Creep Prestarter Starter Ingredients (%) 49.79 Corn 60.01 19.39 57.59 Extruded corn 19.90 10.00 \_ \_ 10.00 15.00 Sorghum 9.00 5.00 Fermented soybean meal 3.00 Soybean meal 43 24.10Soybean meal 46 13.90 19.30 18.96 \_ 1.50 Lecithin powder \_ 0.50 \_ Soy oil 1.66 1.60 1.80 0.72 Whey (low protein) 15.28 7.64 \_ -Fat powder 1.11 --\_ Fish meal 6.67 --White sugar 2.5 \_ -\_ Glucose 2.75 0.32 0.43 0.52 0.52 Lys Met 0.06 0.29 0.23 0.18 Thr 0.24 0.23 0.21 0.08 0.05 Trp \_ 0.07 1.58 0.61 Limestone 0.82 0.95 Monocalcium phosphate 1.11 Dicalcium phosphate 0.62 0.81 0.89 -Sodium chloride 0.5 0.23 0.39 0.43 Other<sup>1</sup> 3.9 0.66 2.9 1.5 Calculated nutrients Moisture (%) 12.50 9.20 10.40 11.20 Crude protein (%) 16.20 18.1017.90 17.10 Ash (%) 4.90 6.80 5.50 4.70 0.60 Ca (%) 0.46 0.70 0.61 Total phosphorous (%) 0.60 0.62 0.58 0.54 Av. P (%) 0.448 0.456 0.438 0.388 Salt (%) 0.49 0.78 0.63 0.50 Crude fiber (%) 2.60 2.40 2.30 2.20 2.90 Crude fat (%) 4.00 5.10 4.30 3454 DE (kcal/kg) 3352 3430 3226 3217 3283 3191 ME (kcal/kg) 3277 Lys (%) 1.05 1.35 1.30 1.20 Met (%) 0.31 0.54 0.49 0.43 Met + Cys (%)0.59 0.81 0.78 0.70 Thr (%) 0.70 0.89 0.87 0.80 Trp (%) 0.21 0.28 0.27 0.24 Val (%) 0.75 0.90 0.89 0.80 Ile (%) 0.67 0.73 0.71 0.66 Arg (%) 1.02 0.96 1.08 1.00 0.95 1.25 SID Lys (%) 1.20 1.10 0.29 0.52 0.47 SID Met (%) 0.41 SID Met + Cys (%)0.52 0.75 0.72 0.64 SID Thr (%) 0.61 0.81 0.78 0.72 0.25 SID Trp (%) 0.18 0.24 0.21

<b>395</b> Table 1. Control experimental diets composition	395	Table 1.	Control e	xperimental	diets com	positio
--	-----	----------	-----------	-------------	-----------	---------

Abbreviations: Av: available; DE: digestible energy ; ME : metabolic energy ; SID : standardized ileal digestibility <sup>1</sup>Includes minerals and vitamins: Lactation: Na (0.2%), Cl (0.16%), Mg (0.06%), K (0.2%), Cu (20 mg/kg), I (0.14

398 mg/kg), Fe (80 mg/kg), Mn (25 mg/kg), Se (0.15 mg/kg), Zn (100 mg/kg), Vit A (2000 IU/kg), Vit D3 (800 IU/kg),

399 Vit E (44 IU/kg), Vit K (0.50 mg/kg), Biotin (0.20 mg/kg), Choline (1 g/kg), Folic acid (1.30 mg/kg), Niacin (10

- 400 mg/kg), Pantothenic acid (12 mg/kg), Vit B2 (3.75 mg/kg), Vit B1 (1 mg/kg), Vit B6 (1  $\mu$ g/kg), Vit B12 (15 mg/kg); 401 Creep: Na (0.4%), Cl (0.5%), Mg (0.04%), K (0.3%), Cu (6 mg/kg), I (0.14 mg/kg), Fe (100 mg/kg), Mn (4 mg/kg), 402 Se (0.30 mg/kg), Zn (3000 mg/kg), Vit A (2200 IU/kg), Vit D3 (220 IU/kg), Vit E (16 IU/kg), Vit K (0.50 mg/kg), 403 Biotin (0.08 mg/kg), Choline (0.60 g/kg), Folic acid (0.30 mg/kg), Niacin (30 mg/kg), Pantothenic acid (12 mg/kg), 404 Vit B2 (4 mg/kg), Vit B1 (1.5 mg/kg), Vit B6 (7 µg/kg), Vit B12 (20 mg/kg); Prestarter: Na (0.35%), Cl (0.45%), 405 Mg (0.04%), K (0.28%), Cu (6 mg/kg), I (0.14 mg/kg), Fe (100 mg/kg), Mn (4 mg/kg), Se (0.30 mg/kg), Zn (2000 406 mg/kg), Vit A (2200 IU/kg), Vit D3 (220 IU/kg), Vit E (16 IU/kg), Vit K (0.50 mg/kg), Biotin (0.05 mg/kg), Choline 407 (0.50 g/kg), Folic acid (0.30 mg/kg), Niacin (30 mg/kg), Pantothenic acid (10 mg/kg), Vit B2 (3.50 mg/kg), Vit B1 408 (1 mg/kg), Vit B6 (7 µg/kg), Vit B12 (17.50 mg/kg); Starter: Na (0.28%), Cl (0.32%), Mg (0.04%), K (0.26%), Cu 409 (5 mg/kg), I (0.14 mg/kg), Fe (100 mg/kg), Mn (3 mg/kg), Se (0.25 mg/kg), Zn (1500 mg/kg), Vit A (1750 IU/kg), 410 Vit D3 (220 IU/kg), Vit E (11 IU/kg), Vit K (0.50 mg/kg), Biotin (0.05 mg/kg), Choline (0.40 g/kg), Folic acid (0.30 411 mg/kg), Niacin (30 mg/kg), Pantothenic acid (9 mg/kg), Vit B2 (3 mg/kg), Vit B1 (1 mg/kg), Vit B6 (3 µg/kg), Vit 412 B12 (15 mg/kg)
- 413
- 414

				LSB creep (lacta	tion)						
	LS	B diet	(n=4	8 sows)							
			[	CON creep (lact	ation)	]					
						-					
				LSB creep (lacta	tion)	LSB creep (post-weaning)		SB prestarter	LSB starter		
	C	ON diet	t (n=	48 sows)		]					
			[	CON creep (lact	ation)	CON creep (post-weaning)	С	ON prestarter	CON starter		
	-5	1	7	14	21 2	22 25	36	40 41	52	71 72	77
Sow											
BW	*	*			*						
BFT	*				*						
FI			*	*	*						
Piglets											
BW		*	*		* *	•	*		*		*
FI							*		*		*
PCV2						*					
CSF								*		*	
IgG								*		*	*
									-		

#### Figure 1. Schematic trial design and observations during the experimental period

Abbreviations: BW: body weight; BFT: backfat thickness; FI: feed intake; PCV2: porcine circovirus type 2; CSF:
 classical swine fever; IgG: immunoglobulin G