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Dietary supplementation with hydrolyzed yeast and its effect on the performance, intestinal microbiota, and immune response of weaned piglets

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Abstract: The objective of this study was to evaluate the effects of autolyzed yeast on performance, cecal microbiota, and leukogram of weaned piglets. A total of 96 piglets of commercial line weaned at 21-day-old were used. The experimental design was a randomized block design with four treatments (diets containing 0.0%, 0.3%, 0.6%, and 0.9% autolyzed yeast), eight replicates, and three animals per pen in order to evaluate daily weight gain, daily feed intake, and feed conversion in periods of 0 to 15, 0 to 26, and 0 to 36 days. Quadratic effects of autolyzed yeast inclusion were observed on the feed conversion from 0 to 15 days, on daily weight gain from 0 to 15 days, 0 to 26 days and, 0 to 36 days, indicating an autolyzed yeast optimal inclusion level between 0.4% and 0.5%. No effect from autolyzed yeast addition was observed on piglet daily feed intake, cecal microbiota, and leukogram; however, *i.m.* application of *E. coli* lipopolysaccharide reduced the values of total leukocytes and their fractions (neutrophils, eosinophils, lymphocytes, monocytes, and rods). Therefore, autolyzed yeast when provided at levels between 0.4% and 0.5% improved weaned piglets' performance.

Key words: immune system, leukogram, nutrition, swine, weaning.

INTRODUCTION

In commercial swine production system, early-weaning is a common management practice. It causes great stress to the piglets and results in low feed intake (Leibbrandt et al. 1975, Le Dividich & Sève 2000), impairment of intestinal mucosal integrity (Hampson et al. 1986, Pluske et al. 1997, Spreeuwenberg et al. 2001), and predisposition for disease occurrence (Morés & Amaral 2001) in the post-weaning period.

Thus, in order to minimize the impact of weaning and other environmental challenges, a nutritional strategy is utilized in production system by using ingredients with high digestibility and additives in pre-starter and starter diets. However, in face of increasing restrictions on the use of some of these additives (e.g. antibiotics), interest has increased in studies on functional ingredients, such as autolyzed yeast (AY), which is rich in beta-glucans, favoring immune response mechanisms (Goodridge et al. 2009, Saleh et al. 2015), mannan-oligosaccharides, which have prebiotic action (Spring et al. 2000) and, nucleotides, which are important for the repair and development of fast-growing tissues such as, the intestinal mucosa (Yamamoto et al. 1997).

The yeast cell wall consists of three major groups of polysaccharides: mannose polymers (mannoproteins), glucose polymers (beta-glucans), and N-acetylglucosamine polymers (chitin) that make up over 90% of it, whereas only small and variable amounts of lipid have been reported (Aguilar-Uscanga & François 2003). However, monogastric animals do not have enzymes to digest polysaccharides such as those present in the yeast cell wall, which compromises the animal's access to these intracellular constituents (cytoplasmic proteins, storage carbohydrates, and membrane-bound subcellular structures, such as mitochondria, the nucleus, and the vacuole, as well as, peptides, nucleic acids, vitamins of B complex and, minerals, mainly K) (Hunter & Asenjo 1988). Therefore, ways to breakdown yeast cell walls have been developed to improve the bioavailability of yeast components for animal nutrition.

According to Liu et al. (2016), the methods used for the breakdown of the yeast cell wall to isolate the bioactive compounds may be mechanical (ball mill grinding, high-pressure homogenization, and ultrasound extraction) or non-mechanical (electrical, enzymatic, physical, and chemical). AY is a dried yeast cell product, obtained as a by-product of the sugar cane alcohol production process and submitted to the process of autolysis (Nogueira et al. 2017). Autolysis occurs when live yeasts are subjected to controlled conditions of pH, temperature and to drying, usually performed in a spray-drying process.

The objective of this research was to evaluate the effects of the dietary inclusion of autolyzed yeast (*Saccharomyces cerevisiae*) on the performance (daily feed intake, daily weight gain, and feed conversion), intestinal microbiota, and leukograms of weaned piglets.

MATERIALS AND METHODS

All experimental procedures were previously approved by the Animal Ethics Commitee of the Veterinary Medicine and Animal Science College from São Paulo University (USP), under protocol number 3184160317 and, in accordance with directive 2010/63/EU.

Ninety-six commercial hybrids piglets weaned at average age of 21-day-old and with an average initial body weight of 6.00 ± 0.3 kg were distributed in a randomized block design (eight replicates) for evaluating the growth performance of animals receiving diets containing 0.0%, 0.3%, 0.6%, and 0.9% AY for 36 days. The criteria for the formation of the blocks were sex and initial body weight.

The piglets were housed in groups of three animals per 1.70-m^2 metal nursery pens, equipped with a feeder, a nipple-type drinker, and a heater with electric resistance. During the experimental period, the animals received feed and water *ad libitum*, and the air temperature inside the experimental barn was controlled and recorded, with mean minimum and maximum temperatures of 18°C ± 2.17°C and 25°C ± 1.54°C, respectively.

According to manufacturer, the AY product had 3.5% of Nu, 22% of B-Gl; 12% of MOS. Thus, its inclusion at 0.3% to 0.9% in piglet diets provided 105 to 315 ppm of Nu; 660 to 1980 ppm of B-GL and, 360 to 1080 ppm of MOS. The following average AY composition was established in the formulation of the diets: 95.15% dry matter, 41.10% crude protein, 2.51% ether extract, 7.04% mineral matter, 1.40% crude fiber, 0.17% calcium, 0.56% total P, 0.19% available P, 2.14% digestible Ile, 3.14% digestible Lys, 0.76% digestible Met, 2.10% digestible Thr, 0.40% digestible Trp, 2.22% digestible Val, and 1.03% digestible S-containing amino acids. The available P and digestible amino acid values were estimated considering

the P availability and amino acid digestibility coefficients of yeast from alcohol distillery recommended by Rostagno et al. (2011). The other nutritional values established in formulation were obtained by laboratorial analyses.

The established inclusion levels of AY were based on results of previous studies (Jiang et al. 2015, Shen et al. 2009) and, its inclusion in diets was performed by replacing the corresponding amount of soybean meal 45%.

The piglets received three types of diets: pre-starter 1, pre-starter 2, and starter diets from 21 to 36 days, from 36 to 47 days, and from 47 to 63 days of age, respectively. In the formulation of the experimental diets (Tables I and II), we sought to meet the nutritional requirements proposed by Rostagno et al. (2011).

The growth performance (mean daily feed intake, mean daily gain, and feed conversion) was assessed from the weighing of the animals at the initial, 15^{th}, 26^{th}, and 36^{th} days of the experiment, based on the feed provided and the feed leftovers.

The slaughter was performed on the 40^{th} day of experiment, for isolation and removal of the cecum, 4 days after the last weighing of the growth performance evaluation. In six of the eight replicates per treatment, one piglet was slaughtered that was closest to the average pen weight, comprising a total of 24 piglets, for determination of total coliform, *Escherichia coli*, and lactic-acid bacteria counts in the cecum contents.

After slaughter, the animals' ceca were sent to the laboratory in refrigerated isothermal boxes. Each cecum was opened with sterile tweezers and scalpel, 25 g of the cecal contents was weighed into sterile bags, and 225 mL of 0.1% buffered peptone water (0.1% BPW) was added, obtaining a dilution of 10^{-1}. From the first dilution, serial dilutions were performed in test tubes containing 9 mL of 0.1% BPW until a 10^{-9} dilution was reached.

The coliforms and *E. coli* counts were counted using Petrifilm plating (Plate 3M™ Petrifilm™ for counting the *E. coli* coliforms), with 1 mL of the 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9} dilutions being placed in each Petrifilm plate, followed by incubation at 35°C ± 1°C for approximately 48-h. During reading, plates that had between 15 and 150 colonies were considered countable, with the colonies being classified as coliforms and *E. coli* according to the manufacturer's specification.

For the counts of lactic-acid bacteria, 1 mL of the 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7},and 10^{-9}dilutions was inoculated into individual plates using the "pour plate" method. A volume between 18 mL and 20 mL of agar medium MRS (OXOID) was added to the inoculated plates; then, homogenization was carried out, and after solidification, 10 mL of the same medium was added for the overlay insert. Plates were incubated at 30°C ± 1°C for 48-h. During reading, plates with 25 to 250 colonies were considered countable, and from the plates read in this interval, five colonies were removed to proceed the catalase test and gram staining, according to APHA (1992). Colonies with gram-positive and catalase-negative cocci/bacilli were classified as lactic-acid bacteria. The calculation of the number of colony-forming unit/g (CFU/g) was based on the proportion of colonies confirmed as lactic-acid and the dilution used in the count.

On the day following slaughter, one of the two remaining piglets per pen (for a total of 32 animals) was randomly selected and given intramuscular injections of 80 µg/kg body weight lipopolysaccharide (LPS) of the cell wall of *Escherichia coli* serotype 055:B5 (Sigma Aldrich L2880, St. Louis, MO) dissolved in 1.0 mL of 0.01 M saline (PBS) at pH 7.4. Immediately prior to application and within 2 to 3 hours after application of the LPS, blood was collected by

vacuum system (4 mL tubes) using 40 x 9-mm needles to determine the total and differential leukocyte count using an automatic veterinary cell counter (MC-6200 vet cell, Shenzhen Maxcom 59 Electronic, China).

For all variables studied, a regression analysis was performed considering four treatments (inclusion of 0.0%, 0.30%, 0.60%, and 0.90% AY in diets) through the PROC GLM command of SAS (2010). For the growth performance study, a randomized block

Table I. Percentage of pre-starter1 (PS1), pre-starter 2 (PS2), and starter (S) diets.

Ingredients (%)	PS1	PS2	S
Corn	51.745	55.675	63.565
Soybean meal 45%	20.000	24.000	29.000
SPC 60%[1]	3.000	2.500	-
Whey	3.300	2.000	-
Milk concentrate[2]	3.000	1.000	-
Sugar	4.000	3.000	-
Maltodextrin	4.000	2.000	-
Soy oil	2.500	2.800	2.310
Corn gluten 60%	2.500	2.000	1.000
Limestone	0.820	0.880	0.860
Dicalcium phosphate	1.680	1.560	1.520
Sodium chloride	0.500	0.500	0.500
L-Lysine HCl 78%	0.870	0.630	0.400
DL-Methionine 99%	0.300	0.210	0.110
L-Threonine 98%	0.340	0.220	0.110
L-Tryptophan 98%	0.080	0.050	0.010
L-Isoleucine 99%	0.140	0.020	-
L-Valine 99%	0.270	0.140	-
Zinc oxide 76%	0.340	0.200	-
Tiamulin 10%	0.010	0.010	0.010
Chloride choline 60%	0.090	0.090	0.090
Adsorbent[3]	0.250	0.250	0.250
B.H.T.[4]	0.015	0.015	0.015
Mineral premix[5]	0.100	0.100	0.100
Vitamin premix[6]	0.150	0.150	0.150
Autolyzed yeast[7]	0.000	0.000	0.000
Total	100.00	100.00	100.00

[1]Soy protein concentrate; [2]Nutrient information (%): crude protein 4.0; ether extract 1.5; lactose 73.00; crude fiber 0.01; calcium 0.88; phosphorus 1.05; amino acids, digestible: lysine 0.22; threonine 0.12; tryptophan 0.04; cystine 0.21; methionine 0.07; isoleucine 0.12; valine 0.11; metabolizable energy 3,202 kcal/kg; [3]Commercial product: Notox, calcium-sodium aluminosilicate; [4]B.H.T.: butylated hydroxytoluene antioxidant; [5]Mineral premix providing per kg of feed: Fe 100 mg, Cu 10 mg, Se 30 mg, Mn 40 mg, Zn 100 mg, Co 1 mg, and I 1.5 mg; [6]Vitamin premix providing per kg of feed: vitamin A 9,000 I.U., vitamin D_3 2,250 I.U., vitamin E 22.5 mg, vitamin K_3 22.5 mg, vitamin B_1 2.03 mg, vitamin B_2 6 mg, vitamin B_6 3 mg, vitamin B_{12} 30 µg, folic acid 0.9 mg, pantothenic acid 14.03 mg, niacin 30 mg, biotin 0.12 mg and, choline 400 mg; [7]The inclusion of 0.30%, 0.60% and 0.90% AY in the diets was performed by replacing the corresponding amount of soybean meal 45%.

experimental design with eight replicates and three animals per experimental unit was used. In the study of the total coliform, *Escherichia coli*, and lactic-acid bacteria counts in the cecum contents, a randomized block design with six replicates and one animal per experimental unit was used, and the microbial count values CFU/g were $\log_{10}$ transformed. For the total and differential counts of leukocytes, we used the experimental design of a randomized block design with repeated measures over time (two blood draws: one before and one at 2 h to 3 h after LPS solution application), eight replicates, and one animal per experimental unit.

RESULTS AND DISCUSSION

No effects of the inclusion of AY (P> 0.05) on daily feed intake were observed in any of the periods studied, nor were any effects on the feed conversion in the periods from 0 to 26 and from 0 to 36 days (Table III). Regression analysis showed a quadratic effect of AY levels on the DWG ($y = 0.2652 + 0.0958x - 0.0941x^2$) and FCR ($y = 1.3480 - 0.4247x + 0.4948x^2$) at 0 to 15 days and, showed a quadratic effect on the DWG at 0 to 26 days ($y = 0.3841 + 0.1465x - 0.1688x^2$) and, at 0 to 36 days ($y = 0.4489 + 0.1460x - 0.1726x^2$) (Table III).

The recommended levels of AY inclusion, calculated on the basis of the regression equations in the present study (between 0.40% and 0.50%), are higher than those suggested by Bael & Roxas (2013) and Molist et al. (2014), who recommended 0.30% and 0.20% brewery hydrolyzed yeast in the diets of weaned piglets, respectively. Although the authors have studied another source of yeast, the species *Saccharomyces cerevisiae* is the most used

Table II. Calculated (*CNV*) and analyzed (*ANV*) nutritional values of pre-starter (PS1 and PS2) and starter (S) diets.

	PS1				PS2				S			
	0.0% AY	**0.3% AY**	**0.6% AY**	**0.9% AY**	**0.0% AY**	**0.3% AY**	**0.6% AY**	**0.9% AY**	**0.0% AY**	**0.3% AY**	**0.6% AY**	**0.9% AY**
CNV												
ME[1]	3,378	3,378	3,378	3,378	3,365	3,365	3,365	3,365	3,298	3,298	3,298	3,298
CP[2]	18.52	18.51	18.50	18.48	19.24	19.23	19.21	19.20	19.21	19.20	19.19	19.18
Ca[3]	0.82	0.82	0.82	0.82	0.80	0.80	0.80	0.80	0.77	0.77	0.77	0.77
Total P[4]	0.65	0.65	0.65	0.65	0.62	0.62	0.62	0.62	0.61	0.61	0.61	0.61
Dig. P[5]	0.45	0.45	0.45	0.45	0.41	0.41	0.41	0.41	0.38	0.38	0.38	0.38
Dig. Lys[6]	1.45	1.45	1.45	1.45	1.33	1.33	1.33	1.34	1.17	1.17	1.17	1.18
Dig. Met[7]	0.55	0.55	0.55	0.55	0.47	0.47	0.47	0.47	0.37	0.37	0.37	0.37
Dig. Thr[8]	0.91	0.91	0.91	0.91	0.84	0.84	0.84	0.84	0.74	0.74	0.74	0.74
Dig. Trp[9]	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24	0.21	0.21	0.21	0.21
Dig. Met+Cys[10]	0.81	0.81	0.81	0.81	0.75	0.74	0.74	0.74	0.65	0.65	0.65	0.65
Dig. Ile[11]	0.80	0.80	0.80	0.80	0.73	0.73	0.73	0.73	0.72	0.72	0.72	0.72
Dig. Val[12]	1.00	1.00	1.00	1.00	0.92	0.92	0.92	0.93	0.80	0.80	0.80	0.80
ANV												
CP[2]	18.60	18.36	18.40	18.28	19.10	19.00	19.20	19.00	19.00	19.15	18.90	19.04
Ca[3]	0.84	0.83	0.82	0.83	0.81	0.79	0.81	0.80	0.78	0.76	0.75	0.76
Total P[4]	0.63	0.61	0.64	0.63	0.62	0.61	0.64	0.63	0.62	0.61	0.61	0.60

[1] Metabolizable energy (kcal/kg); [2] Crude protein (%); [3] Total Ca (%); [4] Total P (%); [5] Digestible P (%); [6] Digestible Lys (%); [7] Digestible Met (%); [8] Digestible Thr (%); [9] Digestible Trp (%); [10] Digestible Met+Cys (%); [11] Digestible Ile (%); [12] Digestible Val (%).

in the fermentative processes in sugar cane alcohol distilleries and breweries.

Yeasts are recognized as sources rich in nucleotides (Nu), beta-glucans (β-GL), and mannan-oligosaccharides (MOS) (Aguilar-Uscanga & François 2003, Kogan & Kocher 2007, Lipkeand Ovalle 1998), and the AY studied had 3.51% Nu, 22.01% β-GL, and 12.00% MOS. Therefore, its inclusion at levels of 0.3% to 0.9% in the diets of the piglets supplied 105 to 315 ppm Nu, 660 to 1,980 ppm β-GL, and 360 to 1,080 ppm MOS.

Nu have important functions in the body, being precursors of nucleic acids (DNA and RNA), biological regulators, source of energy (ATP) (Lerner & Shamir 2000, Sauer et al. 2011) and, can be synthesized by the "*de novo* pathway" using amino acids as precursors, with a high energy cost or by the "*salvage* pathway" from products of digestion of nucleic acids from the diet. Considering certain disease states and periods of limited nutrient intake or even rapidly growing tissues such as the intestinal epithelium, Nu can be considered nutritionally essential because "*de novo* pathway" synthesis may be insufficient (Hess & Greenberg 2012, Sauer et al. 2011). Thus, their presence in diets may benefit the gastrointestinal morphology and function, the immune system and, the intestinal microbiota of monogastric animals (Sauer et al. 2012).

The conditions imposed by weaning, which usually occurs in commercial farms at approximately 3 to 4 weeks of age result in low feed intake, especially in the first week after weaning (Campbell et al. 2013). This decrease in feed intake is responsible for a reduction in the villi height and alterations in the depth of the intestinal crypts, decreasing the digestive capacity and nutrient uptake (Lallès et al. 2007, Pluske et al. 1997). Therefore, in the present study, the probable re-establishment of intestinal integrity and functions due to the high concentration of Nu in AY may have been a determinant of the better performance of weaned piglets, as the inclusion of Nu in the diets at the level of 500 ppm improved performance and decreased the incidence of diarrhea (Martinez-Puig et al. 2007), and at the 200 ppm level, it increased the final weight and daily weight gain of weaned piglets (Andrade et al. 2016).

Despite the high concentration of β-GL and MOS in yeast cells and the improved growth performance of weaned piglets from the addition of these compounds to their diets (Le Mieux et al. 2003, Li et al. 2006, Mendes et al. 2010, Rozeboom et al. 2005, Tuoi et al. 2016, Valpotić et al. 2016, Wang et al. 2008), this effect can be considered indirect, as the main action attributed to β-GL is the improvement of the immune response (Li et al. 2005, Zhou et al. 2013), and the main action attributed to MOS is the selective action on the intestinal microbiota (Halas & Nochta 2012, White et al. 2002).

The total coliform, *Escherichia coli,* and lactic-acid bacteria counts in the piglets' cecum content were not influenced (P> 0.05) by the AY levels in the diets (Table IV). This result partially agrees with those obtained by White et al. (2002), where the addition of 3% dry brewer's yeast as a source of MOS in the diets of weaned piglets showed no effect on the number of total coliforms and *Escherichia coli*, despite the increase in the lactobacilli in the feces of animals receiving diets containing yeast. Van der Peet-Schwering et al. (2007) also did not observe alterations in the intestinal microbiota of piglets after weaning when they were provided with diets that included *Saccharomyces cerevisiae*.

Beneficial microorganisms exist in the gut, and these can aid in digestion and, by competitive action, reduce the proliferation of pathogenic species that can cause inflammation

in the intestinal mucosa and produce toxic metabolites and diseases. Among the beneficial bacteria are those of the genera *Lactobacillus* and *Bifidobacterium*, and among the noxious ones are *Escherichia coli* and those of the genera *Clostridium* and *Salmonella* (Fouhse et al. 2016).

The prebiotic action of purified MOS, which has been proven in piglets (Miguel et al. 2004), mainly has been attributed to its nondigestion by the swine, allowing the MOS to serve as a substrate for the growth of beneficial microorganisms and to bind to the fimbriae of pathogenic bacteria, inhibiting the adherence of these bacteria to the cells of the intestinal mucosa and eliminating them in the feces (Halas & Nochta 2012). Although the AY studied showed high concentration of MOS, this component was linked to the proteins of the cell wall, which is also composed of β-glucans and, to a lesser extent, chitins (Magnani & Castro-Gómez 2008, Orlean 2012). For the MOS to exert prebiotic action, the yeast cell must be subjected to mechanical and/or chemical processes that break the cell wall and then treated with proteases, breaking the mannoproteins of the outer cell wall and releasing soluble mannan, which, subsequently, is separated from the insoluble and purified glucans (Yamabhai et al. 2016).

Thus, if yeast cell wall processing is not sufficient to ensure the adequate release of MOS, the prebiotic action of the MOS will be compromised, which may have occurred with the AY assessed in the present study, explaining the absence of effects on the microbiota of the cecum. Another probable explanation for the lack of treatment effects on the bacterial populations in the cecum is related to the low microbiological challenge imposed on the animals during the research; that is, the isolated experimental facilities were cleaned and disinfected, undergoing a sanitary wash before housing the piglets. In addition, the pre-starter diets contained tiamulin as a performance enhancer and included pharmacological doses of zinc (zinc oxide) as an antimicrobial agent.

Interactions ($P > 0.05$) among the collection, diet time, and diet were not identified on the leukograms of the piglets (Table V). The bacterial LPS used had an effect on the immune system, altering the leukograms of the piglets ($P < 0.001$), as indicated by the values of leukocytes, neutrophils, eosinophils, lymphocytes and monocytes, which were reduced 2 to 3 hours after application (Table V). These results agree with those found by Collier et al. (2011), who found that the leukocyte population was reduced 1 hour after piglets were challenged with LPS and returned to the normal physiological level after 24-hour. This response probably resulted from inflammatory processes induced by the application of the LPS solution, as a rapid migration of leukocytes from the blood to the site of application, resulting in changes in the leukogram (Dhabhar 1998).

As an inducer of the immune response, LPS has been used in research with several animal species, among them swine (Johnson 1997, Saleh et al. 2016). After inoculation, it induces symptoms of acute bacterial infection in piglets, with consequent depression, anorexia, and fever (Klasing & Leshchinsky 2000, Liu et al. 2008). LPS may also exacerbate the production of proinflammatory cytokines and produce tissue damage, with decreased muscle tone and cardiac output resulting in hypotension and poor tissue perfusion and therefore in cell death (Machado et al. 2004).

Among the yeast components, β-GL are considered efficient immunomodulatory agents (Petravić-Tominacet al. 2010, Stier et al. 2014, Volman et al. 2008); however, in this study, increasing the levels of AY inclusion did not alter the white blood cell count of animals challenged or not with 80 µg of LPS/kg of

body weight. Possibly, the LPS dose triggered physiological responses that made it difficult to identify the action of AY on the immune system. Furthermore, although it was used to evaluate the immune response of piglets (Lozano et al. 2014, Sugiharto et al. 2014), the leukogram does not seem to be the best way to identify the possible immunomodulatory action of yeast because, in other studies, the action of the hydrolyzed yeast was verified in the concentrations of the acute phase proteins, IgM, and IgG (Molist et al. 2014).

Another consideration is the divergent responses of animals to dietary supplementation with β-GL due to the presentation forms (purified or complexed to the cell wall) of the β-GL, type of processing for production, and levels of inclusion (Rahar et al. 2011, Stier et al. 2014). Although the product used contained 22.01% β-GL, a large part could be bound to the other constituents of the yeast cell wall, impairing the expression of its immunomodulatory potential, as its addition to the diet in its purified form has been shown to be effective in stimulating the immune response (Ledur et al. 2012).

CONCLUSIONS

Autolyzed yeast levels between 0.4% and 0.5% improve the performance of weaned piglets; although its inclusion does not affect the microbiota and the values of total leukocytes and fractions which in turn are reduced by *E. coli* lipopolysaccharide.

Table III. Mean values of daily feed intake (DFI), daily weight gain (DWG), and feed conversion ratio (FCR) of piglets fed diets with different levels of inclusion of autolyzed yeast (AY).

		% of AY in diets					
Period	**Variable**	**0.0**	**0.3**	**0.6**	**0.9**	**Effect**	**CV (%)**
0 to 15 days	DFI (g)	359	353	375	373	NS[5]	8.87
	DWG (g)	268	279	296	273	Q[1]	7.05
	FCR	1.35	1.27	1.27	1.37	Q[2]	6.62
0 to 26 days	DFI (g)	590	616	615	582	NS[5]	7.98
	DWG(g)	384	412	412	379	Q[3]	6.15
	FCR	1.54	1.49	1.49	1.53	NS[5]	5.46
0 to 36 days	DFI (g)	736	766	764	716	NS[5]	8.30
	DWG(g)	449	476	476	440	Q[4]	6.12
	FCR	1.64	1.61	1.60	1.62	NS[5]	4.21

[1]Quadratic effect (P = 0.0237); [2]Quadratic effect (P = 0.0086); [3]Quadratic effect (P = 0.0020); [4]Quadratic effect (P = 0.0052); [5]NS = Not significant (P > 0.05).

Table IV. Total coliform (TC), *Escherichia coli* (EC), and lactic-acid bacteria (LAB) counts (log_{10} CFU/g) in the cecal contents of piglets fed diets with different inclusion levels of autolyzed yeast (AY).

		% of AY in diets					
Age	**Variable**	**0.0**	**0.3**	**0.6**	**0.9**	**Effect**	**CV (%)**
61st day	TC	8.76	8.91	8.54	7.26	NS[1]	15.47
	EC	8.75	8.91	8.54	7.22	NS	15.40
	LAB	10.34	11.25	10.00	10.48	NS	4.78

[1]NS = Not significant (P > 0.05).

Table V. Mean values of the number (10^6/L) of leukocytes (Leu.), neutrophils (Neu.), eosinophils (Eos.), lymphocytes (Lymph.), monocytes (Mon.), and rods (Rod.) from piglets fed diets with different inclusion levels of autolyzed yeast (AY).

	Leu.	Neu.	Eos.	Lymph.	Mon.	Rod.
Collection (hours after LPS application)						
0	19,893[a]	6,207[a]	375[a]	11,565[a]	915[a]	0
3	5,784[b]	845[b]	37[b]	4,788[b]	98[b]	0
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	-
Diet (% AY)						
0.0	12,056	3,695	242	7,628	442	0
0.3	12,737	3,615	162	8,495	403	0
0.6	12,793	2,926	252	8,014	589	0
0.9	13,768	3,056	167	8,570	592	0
P-value	0.4737	0.6698	0.3711	0.6489	0.2123	-
Collection x Diet						
P-value	0.8760	0.4777	0.5665	0.6792	0.2841	-

[a b] Means with different letters in the column differ (P < 0.0001). Not significant (P > 0.05).

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