JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Influence of yeast hydrolysate supplement on growth performance, nutrient digestibility, microflora, gas emission, blood profile, and meat quality in broilers
Running Title (within 10 words)	Influence of yeast hydrolysate supplement on broiler's performance.
Author	Vetriselvi sampath[a], Kyudong Han[b,c], In Ho Kim[a]*
Affiliation	[a]Department of Animal Resource and Science, Dankook University, Cheonan, 31116, Republic of Korea [b]Department of Microbiology, College of Science & Technology, Dankook University, Cheonan 31116, Republic of Korea [c]Center for Bio Medical Engineering Core Facility, Dankook University, Cheonan 31116, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Vetriselvi Sampath (https://orcid.org/0000-0002-6726-8568) Kyudong Han (https://orcid.org/0000-0001-6791-2408) In Ho Kim (http://orcid.org/0000-0001-6652-2504)
Competing interests	Authors declared no potential conflict of interest relevant to this article.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
Acknowledgements	All authors have appreciatively acknowledged the Center for Bio-Medical Engineering core-facility at

	Dankook University for providing valuable reagents and equipment.
Availability of data and material	Based on reasonable request, data sets of this study will be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization of this study and writing: VS, KH, and IHK; Data curation: VS and KH, Formal analysis: VS; Review & editing: KH, and IHK; Supervision- IHK.
Ethics approval and consent to participate	Prior to the trail, the experimental protocols were revised well and approved by Animal Research Ethics Committee of Dankook University (DK-1-2018).

3 **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	In Ho Kim
Email address – this is where your proofs will be sent	inhokim@dankook.ac.kr
Secondary Email address	
Address	Department of Animal Resource & Science, Dankook University No.29 Anseodong, Cheonan, Choongnam, 330-714, South Korea
Cell phone number	
Office phone number	+82-41-550-3652
Fax number	+82-41-565-2949

4

5

ABSTRACT: A total of 1512 Ross 308 broilers (one - day - old) were assigned (random blocks) to 1of 3 dietary treatments with 28 replicates of 18 chicks/cage. The dietary treatments were Corn-soybean-meal based basal diet supplemented with 0, 0.1%, and 0.2% of commercial yeast hydrolysate (YH (*Saccharomyces cerevisiae*)). The graded level of YH supplementation has linearly increased broilers body weight gain on d 21, 35, and overall (p=0.044, 0.029, and 0.036, respectively) experimental period. In addition, the increased level of YH supplementation has linearly reduced feed conversation ratio of broilers on d 21, 35, and overall trial period (p=0.041, 0.052, and 0.032, respectively). However, the feed intake and mortality of broilers were not affected by the graded level of YH supplementation. Though nutrient digestibility of dry matter (p=0.012) and nitrogen (p=0.021) was linearly increased in broilers fed YH supplementation, at the end of the trial it fails to affect the total track digestible energy. Dietary inclusion of YH supplementation showed a beneficial effect on the microbial population as linearly improved *lactobacillus* (p=0.011) and reduced *E. coli* counts (p=0.042). An increasing level of YH supplementation has tended to decrease NH_3 (p=0.069) and linearly decrease H_2S (p=0.027) of noxious gas emission in broilers. Moreover, dietary YH supplements trend to increase the glucose (p=0.066) and reduced cholesterol (p=0.069) level. At the end of the test, YH supplementation elicited a linear reduction in drip loss on days 5 and 7, respectively (p=0.045, and 0.021). Furthermore, dietary inclusion of YH supplementation had linearly increased villus height (p=0.051) but fails to affect crypt depth. Therefore, in terms of positive effects on the broiler's overall performance, we suggest that dietary supplements containing graded YH levels in the broilers diet could serve as a potential alternative for growth promoters.

Key words: yeast hydrolysate, brewer yeast, broilers, growth performance.

INTRODUCTION

Poultry production is one of the largest meat producers globally [1] and broilers are raised especially for meat production. Besides, it becomes the best and cheapest source of animal protein (nutritious egg and meat) for human consumption, and plays a significant role in improving the nutritional status of human beings. Compared to other meat-producing animals, modern broilers grow rapidly and meet the protein needs of consumers' in a very short period of time. Such broiler meat demand has been increased due to the growing population. In order to produce high-quality meat, farmers have preferred to use cost-effective antibiotics since 1951 [2]. Previously, Ogle Maureen [3] stated that broilers fed with antibiotics grew 50% faster than the basal diet. However, in the past few decades, antibiotic growth promoters (AGP) uses in poultry feedstuff has become the hottest debate among many researchers due to its bacterial resistance. Consequently, the European Union (EU) has restricted the use of antibiotics in the livestock industry since 2003 [4]. In addition, several publications pointed out a close connection between antibiotic usage and poultry production, thereby increasing the resistance of bacteria that cause health issues to consumers [5]. These anxieties prompted scientists to discover a suitable alternative that could boost the quality of meat and poultry production. As a result, organic acids, probiotics, yeast, and phytogenic additives have been used as an excellent source of substitutes for AGP in monogastric animal nutrition [4]. Subsequently, these additives have been adequately addressed in previous studies.

Recently, yeast and its byproducts such as yeast cell wall, yeast extract, and yeast hydrolysate (YH) have become a popular additive in poultry diets due to its nutritional ingredients, and immunological properties [6,7]. Besides, YH has a strong fermentation capacity, high nutritional value, and can reduce potential toxicity. It is an end product of

hydrolysis extraction and naturally comprises of yeast extracts and cell walls [8]. In addition, YH contains ample of B-vitamins, amino acids, nucleotides, β -glucan, and mannan-oligosaccharides (MOS). However, β-glucan and MOS were commonly used as prebiotics in order to regulate the immune response of animals [9]. Likewise, nucleotides are considered as one of the most essential YH ingredients as it regulates the immune function to enhance the growth performance and repairs the digestive tract of animals [10,11]. Moreover, dietary nucleotides can satisfy the corporal needs in the exponential growth, malnourishment, and immune challenge. Previously, many studies showed the positive effects of yeast cells [12,13] and cell wall components [14,15] on the intestinal health of poultry under various conditions. However, the application of YH supplement in broiler feed is still scarce. Therefore, we aimed to assess the efficacy of YH supplementation into broilers diet to examine their growth performance, nutrient digestibility, fecal microflora, noxious gas emission, blood profile, villi length and crypt depth, and meat quality.

MATERIALS AND METHODS

Prior to the trail, the experimental protocols were revised well and approved by Animal Research Ethics Committee of Dankook University (DK-1-2018).

Composition of main supplement

The main supplement YH (*Saccharomyces cerevisiae*) used in this experiment was commercially prepared in the name of CALMORIN. It was obtained from Daeho Co., Ltd. (Gyeonggi-do, Republic of Korea) and added to broiler feed at a prescribed level. The active ingredients presented in YH supplement were 40% of crude protein, 4.9 % of glutamic acid, 3.5% of nucleotides, 23% of β-glucans, and 15%.MOS.

Broiler husbandry

This experiment was conducted at Dankook University "Poultry farm" located at Jeonui (South Korea). Before the trial, all equipment and rearing houses were disinfected. A total of 1512 Ross308 a day-old broiler chick (mixed sex) with an initial weight of 42.23 ± 0.05g (mean ± SD) were procured from Cherry-Buro hatchery (Cheonan, South Korea) and reared in multi-layer battery cages for 35-days in a pleasant environment of 33±1℃ room temperature for first 3 days. Later the room temperature was slowly reduced up to 24℃ (60% humidity) and maintained until the end of the trail. The nipple type water troughs and feeder were attached to each cage that allows birds to enjoy *ad libitum* water and feed throughout the experiment. To maintain a hygienic environment the rearing room was routinely cleaned until the end of the test.

Experimental design and diet

Chickens were randomly distributed (28 replicates/treatment, 18 chickens/cage) into one of 3 different treatment groups and fed with Corn-soybean-meal based basal diet supplemented with 0, 0.1%, and 0.2% of commercial YH. The experimental diets of broiler starter (1-7d), grower (7-21d), and finisher (21-35d) were formulated to the norms of NRC [16] (Table1). Basal diet and YH supplements were mixed using feed mixer DDK-801 (Daedong Tech, Gyeonggi-do, Republic of Korea).

Sampling and chemical analysis

The nutritional diet was offered to broilers for 35 days. At initial, days 7,21, and 35 broilers were weighed. The amount of diet consumed and residual (each cage) were recorded on each day to evaluate the feed intake (FI). At the end of the trail, body weight (BW), FI, feed conversion ratio (FCR) was calculated, as well mortality rate was also recorded.

From day 28-35, broilers diet was mixed with 0.2% of chromium oxide. On day 35,

fresh excreta samples (56 birds/treatment) were randomly collected (2 birds/ cage) using stainless steel collection tray. The excreta samples were pooled and transported to the laboratory, and stored at -20℃ to examine the nutrient digestibility of dry matter (DM), nitrogen (N), and energy (E). Prior to analysis, freeze-dried samples were placed in a digital hot air-drying convection oven at 105℃ for24 hours. The samples were then taken out from the oven, pulverized well and sieved using a 1mm screen sieve. DM procedures was carried out according to the method of Upadhaya et al [4]. N was determined by Tecator ™ Kjeltec8400 (Hoeganaes, Sweden) analyzer. To determine E feed and a fecal sample was taken and placed in Parr 6400 (Parr instrument Co., Moline, IL, USA) oxygen bomb calorimeter, and the heat combustion in the sample was measured. The chromium absorption was identified by UV-1201 spectrophotometry (Shimadzu, Kyoto, Japan) and the results were recorded for statistical analysis. The total digestibility (cumulative result) was calculated using the equations of Upadhaya et al [4].

At the end of the trial (day 35), 56 broilers/treatment were randomly selected and the fresh excreta samples were collected (2 birds/ cage) in micro-tubes and placed in sterile plastic bags. The samples were then placed in an insulated ice container and taken to the research laboratory for microbial study. To confirm the existence of microbes, 1gm of fresh excreta sample was taken and diluted 9 ml of 1 % peptone broth and mixed well with a vortex mixer. Then 0.02% of peptone solution was poured into (*Salmonella*-Shigella, MacConkey and *Lactobacilli* medium III) agar plates, respectively. The *Salmonella*-Shigella and MacConkey agar plates were incubated at 37 °C for 1 day. A day later the plates were taken out from the incubator and bacterial colonies were counted. At once, *Lactobacilli* medium III agar plates which incubated at 39 °C, for 2 days were taken out and counted immediately.

On day 35, fresh excreta samples (approximately 300 g) were randomly collected from

56 birds/treatment (2 birds/ cage), pooled well and stored in an airtight plastic box of 2.6 L with a slight hole on one side, fasten tightly with adhesive tape and fermented at 25℃ for 7 days. On 8^{th} day, a 100 ml sample was taken away from the headspace (2cm) for the air circulation, and the box was re-sealed. To know the crust formation on the surface the sample container was manually shaken for about 30 seconds. Finally, NH_3, H_2S, Methyl mercaptans, CO_2, and acetic acid concentrations were measured using the methods of Nguyen and Kim. [17].

On day 35, 0.5ml blood samples was collected from brachial vein of 56 birds (randomly selected) using a sterilized syringe and stored in (K_3EDTA) (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) heparinized and non-heparinized tubes for blood urea nitrogen (BUN) analysis and serum creatinine analysis, respectively. Within one hour of collection, all samples centrifuged (3,000 rpm × g) at 4°C for 15 min to separate the serum. Abbott Spectrum urea Series II -nitrogen test kit (Abbot Laboratories, Dallas, TX, USA) was used to analyze BUN. In addition, Astra-8 (CA 92621) Analyzer kit (Beckman Instruments, Inc., Brea, USA) was used to determine creatinine concentrations. The total cholesterol and triglyceride concentration in the serum samples were determined using an automatic biochemical analyzer RA-1000 (Bayer Corp., Tarrytown, NY, USA). The immunoglobulin (IgG) was determined using HITACHI 747 automatic biochemistry analyzer (Tokyo, Japan). After blood collection same broilers were weighed individually and taken to slaughtering room and killed by cervical dislocation. The abdominal fat, liver, gizzard, spleen, bursa of fabricius, and breast muscle were carefully removed by the experts. The relative organ weight was weighed individually and estimated as the mass BW. The respective meat samples were taken to laboratory, and breast meat was separated for meat quality analysis. The color parameters such as redness, lightness, and yellowness standards of each sample (surface) were measured at 3 locations with a portable

Konica Minolta CR-400 chroma meter (Osaka, Japan). The pH, **water holding capacity (WHC)**, and cooking loss were calculated following the detailed methods of Sampath et al. [18]. Drip loss was carried out according to the procedure of Honikel et al [19].

On day 35, 2cm intestinal tract tissue samples were collected from the ileocolic junction (ileum), mid-gut (jejunum), and duodenum region and placed into neutral buffered formalin for fixation and intestinal segments were fixed for morphometric analysis and histochemical staining in 10% buffered formalin solutions, respectively. The classification criteria for villus height was based on the appearance of lamina propria intact. Histological experiment samples were performed on 5 μm sections, stained by haematoxylin and eosin, and examined by Olympus AX70 microscope (Olympus Cooperation, Tokyo, Japan). The intestinal villus height (VH) and crypt depth (CD) were measured according to the methods of Hampson [20].

Statistical analysis

The cage was defined as the experimental unit during the trial period. The test data was analyzed in a randomized complete block design using the General Linear Model procedure (SAS Institute, Cary, NC). The polynomial contrasts of increased dietary YH supplementation was examined by linear and quadratic effects. Mean values of less than 0.05 were considered as significant, and 0.10 were considered as trends.

RESULTS

The growth performance of broilers fed YH supplement is presented in Table 2. The dietary inclusion of YH supplementation has linearly increased broilers BWG on d 21, 35, and overall (p=0.044, 0.029, and 0.036 respectively) experimental period. In addition, the increased level of YH supplementation has linearly reduced broilers FCR on d 21, 35, and overall trial period (p= 0.041, 0.052, and 0.032). However, FI and mortality of broilers were not affected

by the graded level of YH supplementation. Though apparent total digestibility of DM (p=0.012), and N (p=0.021) showed linear improvement in broilers fed YH supplementation at the end of the trial, it failed to affect the energy (Table 3). Dietary inclusion of YH supplementation showed a beneficial effect on the microbial population as linearly improved *lactobacillus* (p=0.011), reduced *E. coli* (p=0.042), and no effect on *salmonella* counts (Table 4). Throughout the experiment, dietary YH supplementation has failed to affect the gas emission of Methyl mercaptans, Acetic acid, and CO_2. However, the increased level of YH supplement has tended to linearly reduce NH_3 (p= 0.069) and H_2S (p=0.027), respectively (Table 5). Though, dietary supplement with YH has trend to increase glucose (p=0.066) and decreased cholesterol (p=0.069) they did not affect BUN, creatinine, calcium, growth hormone, IGF, and triglyceride throughout the trail (Table 6). YH supplementation elicited a linear reduction in drip loss (p= 0.045, and 0.021) on day 5 and 7, respectively (Table 7). Furthermore, dietary inclusion of YH supplement had linearly increased villus height (p=0.051), but had no effect on crypt depth (Table 8).

DISCUSSION

Recently, YH has become a trending supplement in broiler feed and showed a potential approach in enhanced feed intake and reduced environmental pollution [21,22]. In this trial, we observed that dietary supplement with YH had linearly increased BWG of broilers which was consistent with Emmanuel et al. [23] who noted that broilers fed yeast products as yeast cell wall (1.5g/kg) and autolyzed whole yeast (2.0 g/kg) had increased the BWG during starter, grower, and finisher phases. Furthermore, Onifade, [24] reported that dried yeast containing *S. cerevisiae* had improved the BWG of broilers. Only a few documents were found in the addition of YH in broilers, so we made comparisons with other yeast products and other

monogastric animal such as pig. In 2012, Zhao et al. [25] observed that the nursery pigs' diet contains yeast extract had improved ADG and ADFI. Likewise, the findings of Li et al. [26] showed that live yeast (*S. cerevisiae*) supplement had significantly enhanced BW, ADG, and feed intake of weaner pigs during the overall experiment. In contrast, this study revealed that dietary YH supplementation has failed to affect the FI of broilers, during the entire experiment. The reason for increased feed intake in Li et al. [26] study may be due to the enhanced palatability of dietary yeast supplements. The enhanced BWG with the increased YH level in this study may have been linked to improving intestinal health leading to better FCR, which is associated with increased enzyme activities and led to increase the protein absorption and nutritional utilization. In 2018, Araujo et al. [27] demonstrate that YH supplement had significantly improved the BWG and FCR of breeder hens (35wk old). In contrast, our study reveals that broiler fed dietary supplement with YH had linearly decreased FCR compare to those fed with control diet. The inconsistent results may be due to the difference in YH dosage, quantity, or animal's age. Thus, it should be further explored in the future using different dosages, methods, and techniques.

As described by Zhang et al. [28] the yeast products often enhance feed intake and nutrient digestibility of broilers. Furthermore, soybean-meal diets contain oligosaccharides and non-starch polysaccharides (NSP) as β-glucans, cellulose, and arabinoxylans could positively affect their digestibility and utilize all nutrients [29]. In 2014, studies of Li and Kim. [30] showed that dietary supplementation with yeast cell wall extract (0.10%) had improved the digestibility of pigs, similarly our research also showed an increased digestibility of DM, and N with the graded level of (0, 0.1%, and 0.2%) YH which contributes to the growth performance of broilers. Additionally, Keimer et al. [31] noted that 1% of hydrolyzed yeast supplement had increased the nutrient digestibility of energy in weaning pigs. However,

219 in this study a dietary supplement with 0.1%, 0.2% of YH has failed to affect broilers nutrient
220 digestibility of energy. The inconsistent results regarding nutrient digestibility of energy may
221 be due to the difference in yeast types, dosage levels or animals.

222 The intestinal tract of the chicken is not only highly complex but also contains dynamic
223 microbial populations [32] which plays a significant role in improving intestinal health. In the
224 current study, excreta *Lactobacillus* and *E. coli* counts were positively affected by YH
225 supplementation. Besides, Boontiam et al. [8] found an increased *lactobacillus* count of fecal
226 microbial shedding in weaner pigs fed hydrolyzed yeast supplementation. The increased
227 *lactobacillus* counts and decreased *E. coli* counts in this study with the inclusion of YH
228 supplement was agreed with the results of Sweeney et al. [33] who noted a significant reduction
229 in *E. coli* counts with yeast supplement. Throughout the experiment *Salmonella* counts remain
230 unaffected with a dietary YH supplement. The discrepancies between our study and earlier
231 studies may be due to the dose of YH supplementation, feeding type, as well as intestinal
232 morphology and functions of broilers. Therefore, further study is needed to know the exact
233 mechanisms of dietary YH supplement on excreta microflora of broilers. Ammonia (NH_3),
234 Methane (CH_4), Hydrogen sulfide (H_2S), and Carbon dioxide (CO_2) were considered as the
235 most hazardous gases in poultry [34]. In addition, odor emission from livestock industries
236 contributes to civil complains thereby affecting the environment, animals' health and
237 production [35]. Such hazardous gas of H_2S, and NH_3 was linearly reduced in broilers fed
238 dietary YH supplement. Yan et al. [36] stated that harmful gas emission has close relation with
239 nutrient digestibility. We assume that increased digestibility may lower the bacterial
240 fermentation in the large intestine, thereby it reduces the NH_3, and H_2S gas emissions.

241 The humoral immune response of animals could be identified by serum IGF (Insulin-
242 like growth factor). Boontiam et al. [8] stated that serum protein level variations could affect

243 animal productivity and immunity. In addition, Zhang **et al. [28]** reported that the yeast extract
244 had decreased the blood cholesterol content in pigs and this statement was agreed with our
245 findings that dietary supplement with YH has linearly reduced cholesterol content in broilers.
246 A Study by Zhang et al. [28] noted that dietary supplement with yeast had enhanced the growth
247 hormones (GH) and the immune response of pigs. Also, Kim et al. [37] stated that the dietary
248 inclusion of 1.0% of the yeast in rats' diet had enhanced its GH level. However, in this study
249 except for a linear increase in glucose and linear reduction in cholesterol level, no significant
250 effects were observed on other measured blood indices with the graded level of YH supplement.
251 To date, the influence of supplementing YH to the broiler, obtain a high effect on the blood
252 profile has not been elucidated. Thus, comparisons could not be made with other studies.

253 It is a complex concept to determine poultry meat quality because it always depends
254 on consumer preferences [38]. In 2007, Duclos et al. [39] stated that the carcass must have
255 maximum meat yield with low-fat content to produce good meat quality. However, this
256 statement was agreed with our study that broilers fed YH supplement has reduced cholesterol
257 content. According to Lee et al. [40] chicks fed yeast supplement has enhanced the water
258 holding capacity and tenderness. However, in this study, the YH supplementation failed to
259 affect WHC. The quality of meat can be assessed by drip loss. Such drip loss had linearly
260 reduced in this study at days 5 and 7 with YH supplementation, and this finding was correlated
261 with the study of Li et al. [41] who noted a significant reduction in drip loss (day 7) in pigs fed
262 increased level of yeast supplement. Intestinal villi height and crypt depth indicate the
263 proliferation and absorptive capacity [42] of developed intestinal components [43]. Such,
264 intestinal villus to crypt ratios is commonly used to assess the effects of dietary regimens on
265 gut microanatomy. Keimer et al. [31] demonstrate that weaner pigs had significantly increased
266 villus height, and reduced crypt depth in the colon when fed with 1% hydrolyzed yeast, and

267 this result agreed with our findings graded level of YH supplement linearly increased villus
268 height. Our study showed that crypt depth was not affected by YH supplement, but a
269 controversial result was observed in the study of Carlson [44] who observed shorter crypt
270 depths in nursery pigs fed a yeast diet. The dissimilarity between our study and previous studies,
271 may be due to the complex effects of YH supplement or intestinal villus height and crypt depth
272 of different animals.

273 **CONCLUSION**

274 Our findings showed that incorporating YH supplement in the diet of broilers could
275 significantly enhance the growth performance of BWG and nutrient digestibility of DM and N,
276 shifted microbiota by raising excreta *Lactobacillus* counts and decreased *E. coli* counts. In
277 addition, YH supplement had linearly decreased drip loss, noxious gas (NH_3 and H_2S), and
278 cholesterol level. Based on the positive results, we recommend that YH supplement could be
279 used as an excellent alternative solution to boost the production performance of broilers.

280

REFERENCES

1. Rout SK, Pradhan CR, Rajashree Rath, Panda N, Panigrahi B, Pati PK. Influence of probiotics and acidifier supplementation on growth, carcass characteristics and economics of feeding in broilers. Indian J. Anim. Nutr. 2016;33: 97-101. https://doi.org/10.5958/2231-6744.2016.00017.7
2. Castonon JR. History of the Use of Antibiotics. Poult. Sci. 2011. https://doi.org/10.3382/ps.2007-00249
3. Ogle Maureen. "Riots, Rage, and Resistance: A Brief History of How Antibiotics Arrived on the Farm". Sci. Am. 2013.
4. Upadhaya SD, Lee KY, Kim IH. Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. J. Appl. Anim. 2016; 44 :232–242. https://doi.org/10.1080/09712119.2015.1031775
5. Laube H, Friese A, Von Salviati C, Guerra B, Käsbohrer A, Kreienbrock L, Roesler U. Longitudinal monitoring of extended-spectrum-beta-lactamase/AmpC-producing Escherichia coli at German broiler chicken fattening farms. Appl. Environ. Microbiol.2013; 79 (16):4815-4820. https://doi.org/10.1128/AEM.00856-13
6. Jung EY, Hong YH, Kim JH, Park Y, Bae SH, Chang UJ, et al. Effects of yeast hydrolysate on hepatic lipid metabolism in high-fat-diet-induced obese mice: yeast hydrolysate suppresses body fat accumulation by attenuating fatty acid synthesis. Ann Nutr Metab 2012; 61:89-94. https://doi.org/10.1159/000338441
7. Molist F, Eerden EV, Parmentier HK, Vuorenmaa J. Effects of inclusion of hydrolyzed yeast on the immune response and performance of piglets after weaning. Anim Feed Sci Technol. 2014; 195:136-41. https://doi.org/10.1016/j.anifeedsci.2014.04.020

8. Boontiam W, Wachirapakorn C, Phaengphairee P. Effects of hydrolyzed yeast supplementation on growth performance, immunity, antioxidant capacity, and microbial shedding in weaning pigs. Vet World 2020;13(9): 1902–1909
9. Sauerwein H, Schmitz S, Hiss S. Effects of a dietary application of a yeast cell wall extract on innate and acquired immunity, on oxidative status and growth performance in weanling piglets and on the ileal epithelium in fattened pigs. J Anim Physiol Anim Nutr. 2007; 91:369. https://doi.org/10.1111/j.1439-0396.2006.00663.x
10. Sauer N. Nucleotides modify growth of selected intestinal bacteria in vitro. Livest Sci. 2010; 133:161-3. https://doi.org/10.1016/j.livsci.2010.06.053
11. Superchi P, Saleri R, Borghetti P, Angelis ED, Ferrari L, Cavalli V, et al. Effects of dietary nucleotide supplementation on growth performance and hormonal and immune responses of piglets. Animal. 2012; 6: 902-8. https://doi:10.1017/S1751731111002473
12. Bradley GL, Savage TF, Timm KI. The effects of supplementing diets with *Saccharomyces cerevisiae* var. boulardii on male poult performance and ileal morphology. Poult Sci. 1994; 73:1766-70. https://doi.org/10.3382/ps.0731766
13. Sandikci M, Erin U, Onol AG, Kum S. The effect of heat stress and the use of Saccharomyces cerevisiae or (and) bacitracin zinc against heat stress on the intestinal mucosa in quails. Rev. Méd. Vét. 2004;1: 552-556.
14. Brümmer M, Jansen van Rensburg C, Moran CA. **Saccharomyces cerevisiae cell wall products: the effects on gut morphology and performance in broiler chickens.** S. Afr. J. Anim. Sci. 2010; 40: 14-21. https://doi.org/10.4314/sajas.v40i1.54125
15. Solis de los Santos F, Donoghue AM, Farnell MB, Huff GR, Huff WE, Donoghue DJ. Gastrointestinal maturation is accelerated in turkey poults supplemented with a

mannooligosaccharide yeast extract (Alphamune). Poult. Sci..2007; 86:921-930. https://doi.org/10.1093/ps/86.5.921

16. NRC [National research council]. Nutrient Requirements of Poultry. 9th rev. ed. Washington, DC: Natl. Acad. Press; 1994.

17. Nguyen DH, Kim IH. Protected Organic Acids Improved Growth Performance, Nutrient Digestibility, and Decreased Gas Emission in Broilers. *Animals*. 2020; 10:416. https://doi:10.3390/ani10030416.

18. Sampath V, Shanmugam S, Park JH, Kim IH. The Effect of black pepper (*Piperine*) extract supplementation on growth performance, nutrient digestibility, fecal microbial, fecal gas emission, and meat quality of finishing pigs. *Animals.* 2020; 10 (11):1965. https:// doi: 10.3390/ani10111965.

19. Honikel KO. Reference methods for the assessment of physical characteristic of meat. *Meat. Sci.* 1998; 49: 447–457.

20. Hampson DJ. Alteration in piglet small intestine structure at weaning. Res. Vet. Sci.1986; 40: 32-40.

21. Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase J. Appl. Anim. Res.2016; 45:538-542. https://doi.org/10.1080/09712119.2016.1219665

22. Attia Y, Abd-El Rahman S. Impact of multienzymes or Yeast Sacc supplementation on growth performance and some carcass parameters of broiler chicks fed triticale containing diets. Archiv Fur Geflugelkunde 2001; 65: 168–177.

350 23. Emmanuel UA, Medani EA, Edwin PC, Apeh AO, Mohammed AQ, Harriet G, Hadden
351 G, Paul AI . Influence of dietary supplementation of autolyzed whole yeast and yeast cell
352 wall products on broiler chickens. Asian-australas. J. Anim. Sci. 2020; 33(4): 579-587.
353 https://doi.org/10.5713/ajas.19.0220

354 24. Onifade AA. Growth performance, carcass characteristics, organs measurement and
355 hematology of broiler chickens fed a high fiber diet supplemented with antibiotics or dried
356 yeast. Food Nahr. 1997; 41:370–374. https://doi.org/10.1002/food.19970410612

357 25. Zhao PY, Jung JH, Kim IH. Effect of mannan oligosaccharides and fructan on growth
358 performance, nutrient digestibility, blood profile, and diarrhea Score in Weanling Pigs. J
359 Anim Sci. 2012; 90:833-9. https://doi.org/10.2527/jas.2011-3921

360 26. Li J, Li D, Gong L, Ma Y, He Y, Zhai H. Effects of live yeast on the performance, nutrient
361 digestibility, gastrointestinal microbiota and concentration of volatile fatty acids in
362 weanling pigs. Arch Anim Nutr. 2006; 60:277-88.
363 https://doi.org/10.1080/17450390600785343

364 27. Araujo LF, Bonato M, Barbalho R, Araujo CSS, Zorzetto PS, Granghelli CA, et al.
365 Evaluating hydrolyzed yeast in the diet of broiler breeder hens J. Appl. Poult. 2018; 27:65–
366 70. https://doi.org/10.3382/japr/pfx041

367 28. Zhang JY, Park JW, Kim IH. Effect of supplementation with brewer's yeast
368 hydrolysate on growth performance, nutrients digestibility, blood profiles and meat quality
369 in growing to finishing pigs. Asian-Australas J Anim Sci. 2019; 32(10): 1565–1572.
370 https://doi.org/10.5713/ajas.18.0837

371 29. Knudsen KEB. Fiber and nonstarch polysaccharide content and variation in common crops

372 used in broiler diets. Poult Sci. 2014; 93:2380-93. https://doi.org/10.3382/ps.2014-03902

373 30. Li J, Kim IH. Effects of Saccharomyces cerevisiae cell wall extract and poplar propolis
374 ethanol extract supplementation on growth performance, digestibility, blood profile, fecal
375 microbiota and fecal noxious gas emissions in growing pigs. *Anim. Sci. J.* 2014;85: 698-
376 705. https://doi.org/10.1111/asj.12195

377 31. Keimer B, Kröger S, Röhe I, Pieper R, Simon A, Zentek J. Influence of differently
378 processed yeast (Kluyveromyces fragilis) on feed intake and gut physiology in weaned
379 pigs. J Anim Sci 2018; 96:194-205. https://doi.org/10.1093/jas/skx031 .

380 32. Zhu XY, Zhong T, Pandya Y, Joerger RD. 16S rRNA-based analysis of microbiota from
381 the cecum of broiler chickens. Appl Environ Microbiol. 2002;68(1):124–37.
382 https://doi.org/10.1128/AEM.68.1.124-137.2002

383 33. Sweeney RA. Generic combustion method for determination of crude protein in feeds:
384 collaborative study. J. Assoc. Off. Anal. Chem. 1989; 72:770–774.
385 https://doi.org/10.1093/jaoac/72.5.770

386 34. Gates RS, Casey KD, Wheeler EF, Xin H, Pescatore AJ. U.S. broiler ammonia emissions
387 inventory model. Atmos. Environ. (2008); 42: 3342–3350.
388 https://doi.org/10.1016/j.atmosenv.2007.06.057

389 35. Park S, Cho S, Hwang O. Effects of Italian Ryegrass (IRG) Supplementation on Animal
390 Performance, Gut Microbial Compositions and Odor Emission from Manure in Growing
391 Pigs. Agronomy, 2020; 10(5): 647. https://doi.org/10.3390/agronomy10050647

36. Yan L, Meng QW, Kim IH. The effect of an herb extract mixture on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas content in growing pigs. *Livest. Sci.* 2011; 141: 143–147. https://doi.org/10.1016/j.livsci.2011.05.011
37. Kim JM, Kim SY, Jung EY, Bae SH, Suh HJ. Yeast hydrolysate induces longitudinal bone growth and growth hormone release in rats. Phytother Res. 2009; 23:731-6. https://doi.org/10.1002/ptr.2720
38. Ishamri Ismail, Seon-Tea Joo1. Poultry Meat Quality in Relation to Muscle Growth and Muscle Fiber Characteristics. Korean J. Food Sci. An. 2017; 37(6): 873-883. https://doi.org/10.5851/kosfa.2017.37.6.87
39. Duclos MJ, Berri C, Le Bihan-Duval E. Muscle Growth and Meat Quality. J. Appl. Poult. Res. 2007; 16: 107-112. https://doi.org/10.1093/japr/16.1.107
40. Lee HS, Noh DO, Suh HJ. Promotion effects of yeast hydrolysates and a mixture of safflower seed and gasiogapi extract on longitudinal bone, proximal epiphysis, and growth hormone in rats. Prev Nutr Food Sci. 2011; 16:110-6.
41. Li JG, Zhou JC, Zhao H. Enhanced water-holding capacity of meat was associated with increased sepw1 gene expression in pigs fed selenium-enriched yeast. Meat Sci. 2011; 87:95-100. https://doi.org/10.1016/j.meatsci.2010.05.019
42. Lenhardt L, Mozes S. Morphological and Functional Changes of the Small Intestine in Growth-Stunted Broilers. *Acta* Vet. *Brno.* 2003; 72(3):353-358
43. Franco JRG, Murakami AE, Natali MRM, Garcia ERM, Furlan AC. Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. Rev. Bras. Cienc. Avic2006; 8 :4. https://doi.org/10.1590/S1516-635X2006000400006
44. Carlson MS. Effects of yeast extract versus animal plasma in weanling pig diets on growth

416 performance and intestinal morphology. J Swine Health Prod. 2005; 13:204

417

418

Table 1. Ingredient composition of experimental diets as-fed basis

Ingredient, %	Starter (1-7d)	Grower (7-21d)	Finisher (21-35d)
Corn	54.19	55.38	56.77
Soybean meal	33.80	26.1	18.23
Canola meal	5.00	10.0	15.0
Soybean oil	2.10	3.62	5.07
MDCP[1]	-	1.28	1.12
DCP[2]	1.70	-	-
Limestone	1.15	1.34	1.22
L-lysine	0.50	0.65	0.81
DL-Methionine	0.46	0.47	0.52
L-Threonine	0.20	0.25	0.32
L-Tryptophan	-	0.01	0.04
$NaHCO_3$	0.10	0.10	0.10
Salt	0.30	0.30	0.30
Vitamin premix[3]	0.20	0.20	0.20
Mineral premix[4]	0.20	0.20	0.20
Choline	0.10	0.10	0.10
Nutrient composition			
ME, kcal/kg	3,000	3,100	3,200
CP, %	23.0	21.5	20.0
Lys, %	1.50	1.40	1.30
Met + Cys, %	1.08	0.99	0.94
AP, %	0.48	0.44	0.41
Ca, %	0.96	0.87	0.81

[1]Monodicalcium phosphate. [2]Dicalcium phosphate.

[3]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3mg riboflavin; 50mg niacin; 4mg thiamine; 29mg d-pantothenic; 166mg choline; 33µg vitamin B_{12}.

[4]Provided per kg of complete diet: 12mg Cu (as $CuSO_4 \cdot 5H_2O$); 85mg Zn (as$ZnSO_4$); 8mg

Table 2. The effect of dietary yeast hydrolysate supplementation on the growth performance of broilers

Items	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
d 1 to 7						
BWG, g	159	175	187	2	0.789	0.313
FI, g	186	185	187	2	0.562	0.553
FCR	1.167	1.198	1.192	0.011	0.141	0.218
d 7 to 21						
BWG, g	583	610	684	9	0.044	0.131
FI, g	849	871	861	9	0.372	0.157
FCR	1.459	1.433	1.428	0.013	0.041	0.402
d 21 to 35						
BWG, g	814	884	928	15	0.029	0.102
FI, g	1711	1724	1717	15	0.893	0.650
FCR	2.113	2.012	1.983	0.035	0.052	0.122
Overall						
BWG, g	1557	1618	1794	15	0.036	0.164
FI, g	2745	2780	2766	17	0.582	0.299
FCR	1.766	1.731	1.719	0.017	0.032	0.142
Mortality, %	3.37	2.98	2.98	-	-	-

[1]Standard error of means.

Mn (as MnO_2); 0.28 mg I (as KI); 0.15mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

Table 3. The effect of dietary yeast hydrolysate supplementation on total tract digestibility of broilers

Items, %	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Day 35						
Dry matter	71.52	73.13	74.74	0.52	0.012	0.138
Nitrogen	68.66	69.54	71.43	1.05	0.021	0.627
Digestible energy	72.24	74.12	73.43	0.67	0.232	0.140

[1]Standard error of means.

423

424

Table 4. The effect of dietary yeast hydrolysate supplementation on microbial of broilers

Items, log_{10}cfu/g	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Day 35						
Lactobacillus	8.75	8.97	9.12	0.02	0.011	0.182
E. coli	6.39	6.20	6.16	0.04	0.042	0.589
Salmonella	4.47	4.26	4.14	0.05	0.102	0.589

[1]Standard error of means.

425

426

Table 5. The effect of dietary yeast hydrolysate supplementation on gas emission of broilers

Items, ppm	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Day 35						
NH_3	14.5	13.8	11.5	1.6	0.069	0.941
H_2S	2.8	2.1	1.5	0.4	0.027	0.708
Methyl mercaptans	9.0	7.3	6.5	1.8	0.371	0.831
CO_2	1725	1525	1425	248	0.426	0.875
Acetic acid	4.5	3.3	2.5	0.9	0.164	0.827

[1]Standard error of means.

427

428

Table 6. The effect of dietary yeast hydrolysate supplementation on blood profile of broilers

Items	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Day 35						
BUN, mg/dL	1.3	1.2	1.2	0.1	0.249	0.496
Creatinine, mg/dL	0.3	0.3	0.4	0.1	0.525	0.741
Calcium, mg/dL	9.9	10.3	10.1	0.2	0.439	0.374
Glucose, mg/dL	145.3	215.2	211.5	17.0	0.066	0.202
Growth hormone, ng/mL	0.06	0.07	0.07	0.01	0.247	0.367
IGF-1, ng/mL	14.3	14.5	13.9	0.5	0.635	0.479
Cholesterol, mg/dL	137.8	127.8	119.7	6.3	0.069	0.908
Triglyceride, mg/dL	51.0	39.3	35.0	7.1	0.142	0.681

[1]Standard error of means.

Table 7. The effect of dietary yeast hydrolysate supplementation on organ weight and meat quality of broilers

Items	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Relative organ weight, %						
Breast muscle	19.66	20.27	20.17	0.88	0.694	0.754
Liver	3.00	3.28	2.87	0.10	0.404	0.134
Spleen	0.08	0.10	0.09	0.01	0.357	0.197
Abdominal fat	1.13	1.17	1.19	0.04	0.404	0.821
Bursa of Fabricius	0.16	0.17	0.20	0.03	0.495	0.774
Gizzard	1.25	1.17	1.39	0.08	0.243	0.151
Breast muscle color						
Lightness(L*)	56.22	53.23	56.61	1.40	0.852	0.112
Redness(a*)	12.78	12.60	11.66	0.99	0.453	0.764
Yellowness(b*)	12.32	12.48	12.14	0.52	0.815	0.711
pH value	5.54	5.58	5.65	0.09	0.440	0.901
Cooking loss, %	31.69	29.20	27.02	2.02	0.153	0.952
WHC, %	51.44	53.94	56.56	3.19	0.299	0.989
Drip loss, %						
d 1	2.02	1.82	1.52	0.53	0.703	0.394
d 3	5.94	5.41	5.21	0.77	0.666	0.474
d 5	12.21	11.41	10.02	0.71	0.045	0.821
d 7	15.27	14.53	13.72	0.74	0.021	0.966

[1]Standard error of means.

Table 8. The effect of dietary yeast hydrolysate supplementation on villus height and crypt depth in broilers

Items,	YH supplementation (%)			SEM[1]	*P*-value	
	0	0.1	0.2		Linear	Quadratic
Day 35						
Villus height	657.93	668.99	683.06	36.00	0.051	0.883
Crypt depth	130.44	160.36	145.12	9.96	0.309	0.077

[1]Standard error of means.

433

434

435