Effect of hydrolyzed yeast from *Kluyveromyces fragilis* replacing high-dose zinc oxide on growth performance, diarrhea incidence and intestinal health of weaned piglets

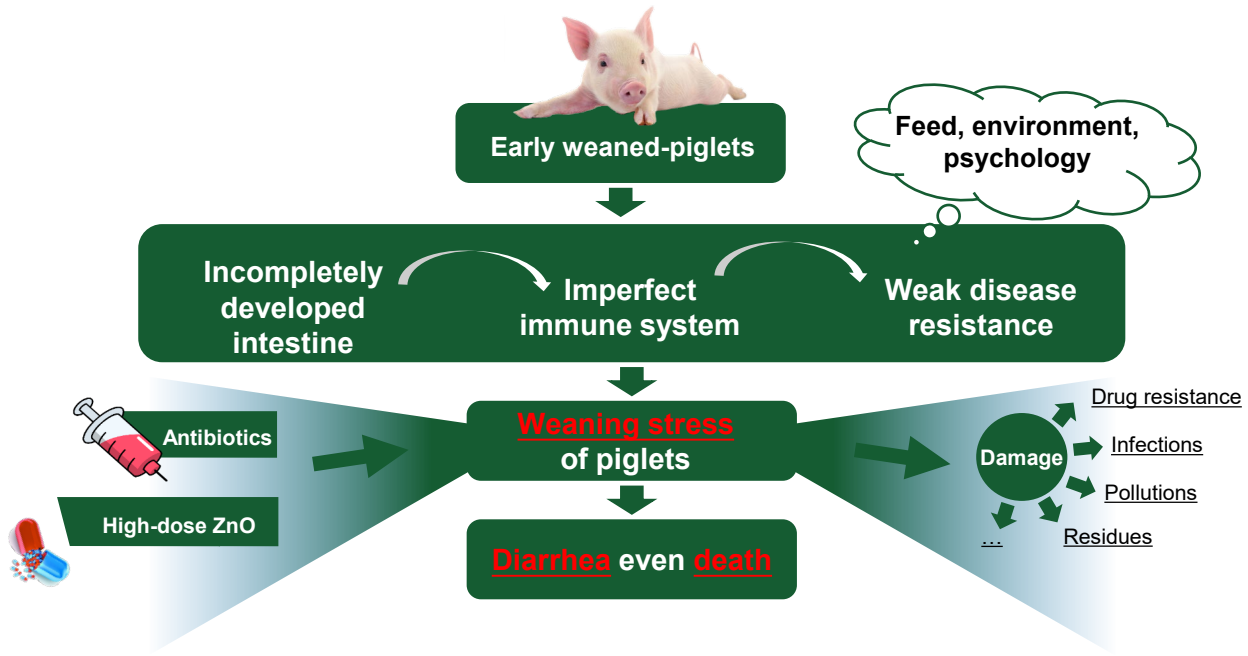
C. Yin, M. Comi, W. Chen, V. Perricone, W. Sun, Y. Pi, Y. Li, A. Agazzi, X. Li, ***X. Jiang***

Florence, Italy

2024.09.02

Outlines

01 Background



EU regulations

Antibiotics

From January 1, 2006, using antibiotic growth promoters in livestock feed is prohibited.

ZnO

In June 2022, using high-dose zinc oxide in livestock production was completely banned, and the amount of zinc added to the feed shall not exceed **150 mg/kg**.

Chinese regulations

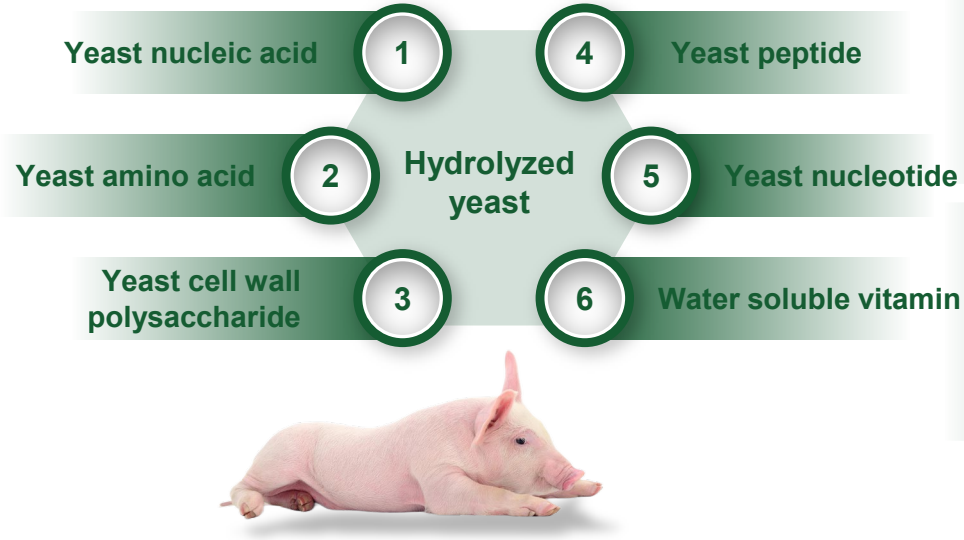
Antibiotics

From July 1, 2020, producing commercial feed containing growth-promoting drug feed additives (except traditional Chinese medicine) are not allowed.

ZnO

In 2017, Chinese government stipulated that the amount of zinc added to the feed in the form of zinc oxide in the first two weeks after weaning should not exceed **1600 mg/kg**.

- **The hydrolysate of yeast cells, obtained by autolysis or hydrolysis with external enzymes.**
- **Characteristics: non-toxic, non-polluting, no side effects, not producing drug resistance etc.**



Promoting growth of livestock

Positive effect in inducing feed intake;
Improving ADFI of animals.

Enhancing immune function

Stimulating the development of immune organs;
Promoting nonspecific immunity.

Improving antioxidant capacity

Eliminating free radicals;
Increasing antioxidant enzymes' activity.

Improving intestinal flora

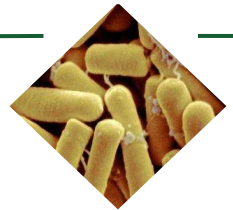
Inhibiting the growth and reproduction of harmful intestinal bacteria;
Maintaining intestinal health.

(FU et al., J ANIM SCI BIOTECHNO , 2023; LIN et al., POULTRY SCI , 2022; FU et al., ANIM NUTR , 2019.)

01

Main source of hydrolyzed yeast

- *Saccharomyces cerevisiae*
- Beer yeast
- Molasses yeast
- Candida albicans
- Bread yeast
- *Kluyveromyces fragilis*



Accepted Manuscript

Title: Effect of time and dietary supplementation with processed yeasts (*Kluyveromyces fragilis*) on immunological parameters in weaned piglets

Authors: Birgit Keimer, Robert Pieper, Annette Simon, Jürgen Zentek

PII: S0377-8401(18)30225-6
DOI: https://doi.org/10.1016/j.anifeedsci.2018.09.008
Reference: ANIFEE 14066

To appear in: *Animal Feed Science and Technology*

Received date: 19-2-2018
Revised date: 8-7-2018
Accepted date: 17-9-2018

Please cite this article as: Keimer B, Pieper R, Simon A, Zentek J, Effect of time and dietary supplementation with processed yeasts (*Kluyveromyces fragilis*) on immunological parameters in weaned piglets, *Animal Feed Science and Technology* (2018), https://doi.org/10.1016/j.anifeedsci.2018.09.008

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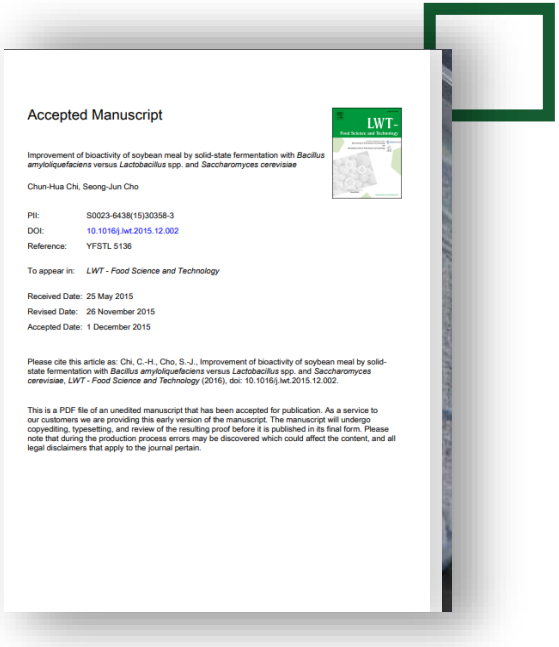
Saccharomyces cerevisiae & its hydrolysate



Accepted Manuscript

Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*

Chun-Hua Chi, Seong-Jun Cho

PII: S0023-6438(15)30358-3
DOI: 10.1016/j.lwt.2015.12.002
Reference: YFSTL 5136

To appear in: *LWT - Food Science and Technology*

Received Date: 25 May 2015
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Accepted Date: 1 December 2015

Please cite this article as: Chi, C.-H., Cho, S.-J., Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*, *LWT - Food Science and Technology* (2016), doi: 10.1016/j.lwt.2015.12.002.

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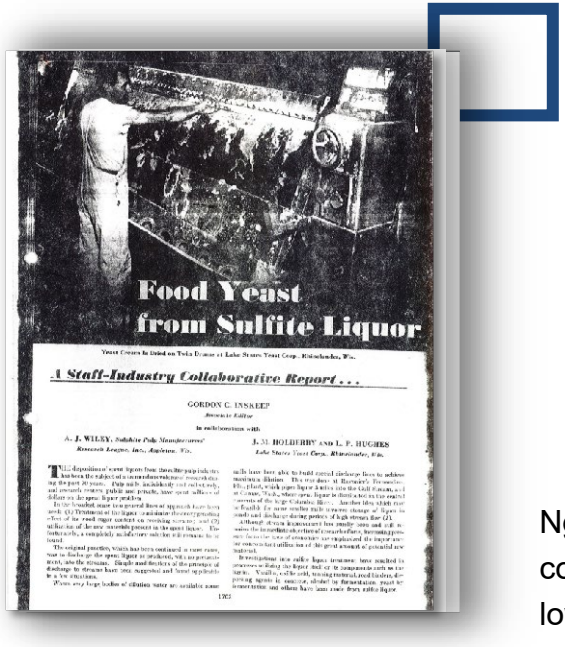
Ascomycete yeast, unicellular eukaryotic microorganism

1. Strong acid resistance
2. Short growth cycle
3. Strong fermentation capacity
4. Easy to cultivate on a large scale

Fu et al. (2021) reported that adding brewer's yeast hydrolysate to the feed of weaned piglets improved the growth performance by promoting intestinal barrier function, indicating that brewer's yeast hydrolysate can be used as a potential alternative to antibiotic growth promoters in production.

Kluyveromyces fragilis & its hydrolysate


Food Yeast from Sulfite Liquor

A Staff-Industry Collaborative Report . . .

GORDON C. INSKEEP

J. J. WILEY, Sulfite Pulp Manufacturer

J. M. HOLDERBY AND L. F. HUGHES

Unicellular microorganism, facultative anaerobe

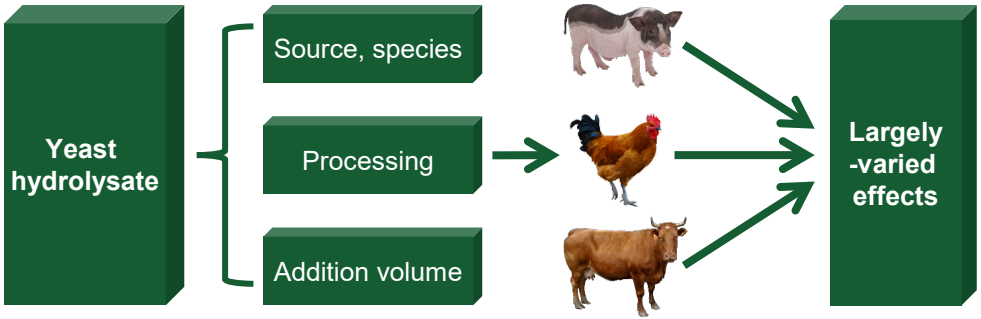
1. Simple nutritional requirements
2. Vigorous growth
3. Wide temperature adaptability range
4. High ability to absorb carbohydrates

Nguyen et al. (1998) reported that the cell wall components of *Kluyveromyces fragilis* contain a high concentration of glucan, which has the function of immunomodulation and lowering serum cholesterol levels;

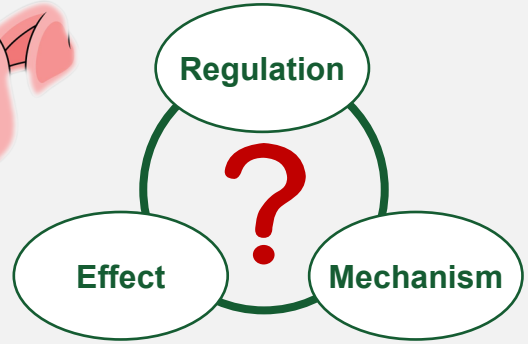
Keimer et al. (2018) reported that *Kluyveromyces fragilis* has a higher concentration of amino acids (lysine etc.) than *Saccharomyces cerevisiae*.

Nutritional value of *Kluyveromyces fragilis* and *Saccharomyces cerevisiae* hydrolysates

Items	*Kluyveromyces fragilis* hydrolysate	*Saccharomyces cerevisiae* hydrolysate
Nutrient levels (%)		
Mannan	5.48	5.00
β-glucan	9.66	8.00
Lysine	1.23	0.50
Methionine	0.20	0.10
Arginine	0.88	0.40
Histidine	0.17	0.20
Leucine	0.59	0.60
Cysteine	0.20	0.10



Hydrolyzed yeast from *Kluyveromyces fragilis* (HK)



Gut health

Alternatives to 'Antibiotics' and 'Zn'

Research aim and significance

Aim

- To investigate the regulatory mechanism of dietary *Kluyveromyces fragilis* hydrolysate (HK) supplementation on growth performance and intestinal health of weaned piglets.

Significance

- Providing scientific basis for the application of HK in weaned piglets;
- Providing theoretical basis for the development of new green antibiotics and high-dose ZnO substitutes.

02 Experiments

Study 1

Effects of different doses of dietary HK supplementation on growth performance, diarrhea incidence and blood indicators of weaned piglets

Experimental animals

A total of 100 weaned piglets (Duroc x Landrace x Yorkshire; body weight 7.03 ± 0.14 kg).

Experimental design

According to initial body weight, the animals were randomly assigned to 4 treatments, with 5 replicates (pens) per treatment and 5 piglet per replicate (pen).

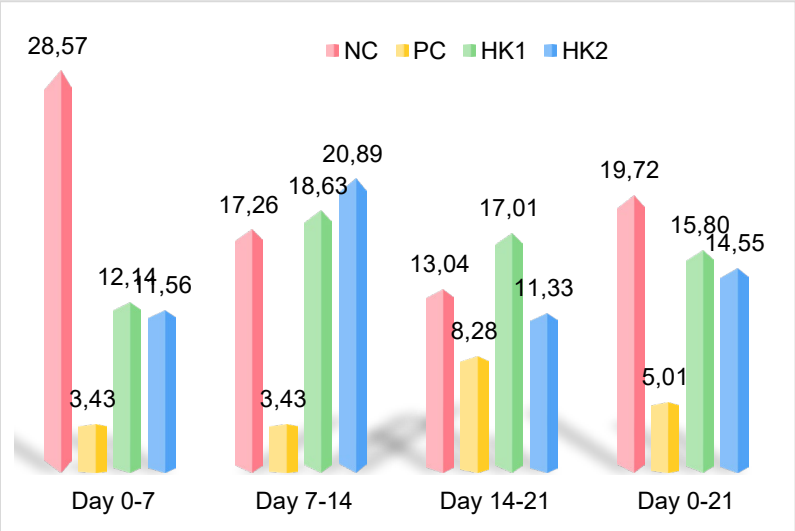
Treatments / Growing stage	NC	PC	HK1	HK2
0-21 d	-	2 g/kg	7.5 g/kg	10 g/kg

NOTE: NC represents negative control group; PC represents positive control group supplemented with ZnO; HK represents treatment group supplemented with Kluyveromyces fragilis hydrolysate.

YIN et al., ITAL J ANIM SCI, 2023

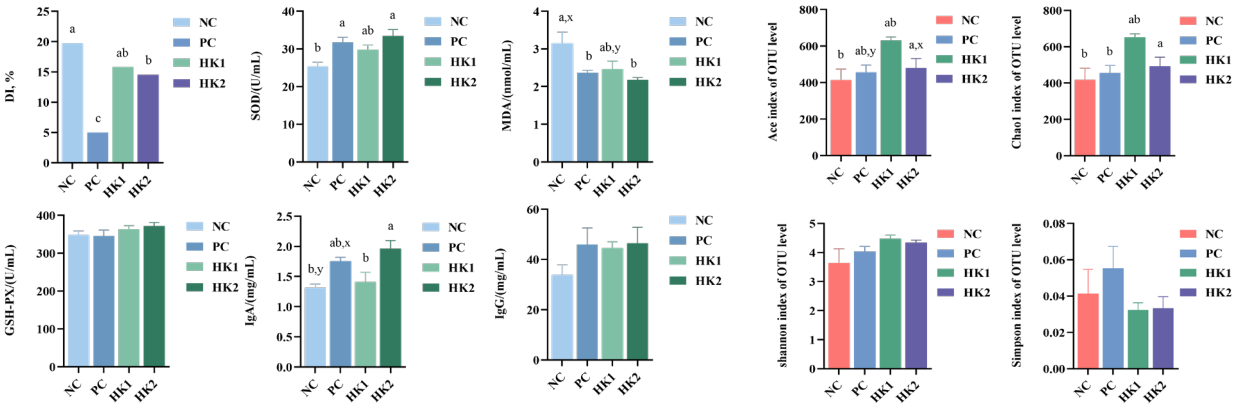
Study 1 - Results

Items	NC	PC	HK, g/kg		SEM	*P*-value
			7.5	10		
BW, kg						
Day 0	7.03	7.03	7.03	7.03	0.67	0.999
Day 7	7.13^{y}	7.82^{x}	7.39^{xy}	7.37^{xy}	0.69	0.058
Day 14	8.50	8.80	8.66	8.74	0.83	0.856
Day 21	10.84	10.77	10.79	10.77	1.13	0.999
ADG, g						
Day 0-7	14.30^{b}	113.20^{a}	51.47^{ab}	48.53^{ab}	20	0.034
Day 7-14	195	140	178	186	41	0.815
Day 14-21	334	281	290	271	51	0.597
Day 0-21	181	178	179	178	26	0.999
ADFI, g						
Day 0-7	123^{y}	176^{x}	158^{xy}	159^{xy}	14	0.081
Day 7-14	330^{xy}	352^{x}	351^{xy}	309^{y}	22	0.082
Day 14-21	563	513	557	534	65	0.909
Day 0-21	339	347	355	334	29	0.915
G:F ratio						
Day 0-7	0.210	0.625	0.356	0.301	0.144	0.233
Day 7-14	0.593	0.398	0.497	0.592	0.110	0.641
Day 14-21	0.588	0.516	0.511	0.532	0.061	0.640
Day 0-21	0.536	0.498	0.501	0.529	0.041	0.886



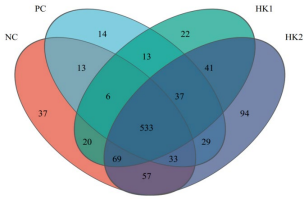
Dietary HK with 10 g/kg reduced the diarrhea incidence of weaned piglets

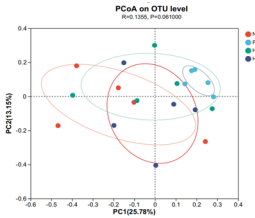
Study 1 - Results

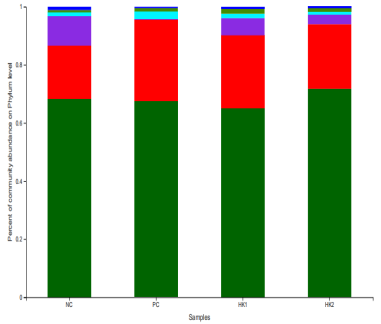


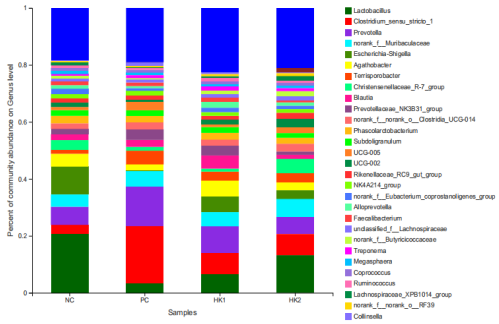
- Dietary supplementing HK improved growth of piglets by increasing plasma **SOD activity**, **IgA levels**, reducing **MDA concentration** and **diarrhea incidence**, and improving **microbial community composition;**
- **10 g/kg HK** has the best effect and can replace high dose ZnO

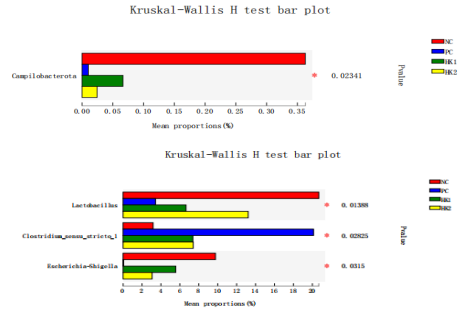
(YIN et al., Ital J Anim Sci , 2023)











Study 1 - Summary

1. Dietary supplementing 10 g/kg HK reduced the diarrhea rate of weaned piglets from 0 to 21 days, increased plasma SOD activity and IgA levels, and reduced plasma MDA content;

2. High dose of dietary ZnO reduced the diarrhea incidence of weaned piglets from 0 to 21 days, increased plasma SOD activity, and reduced plasma MDA content;

3. The effect of adding 10 g/kg HK to the diet was better than that of 7.5 g/kg HK.

Under the conditions of this study, the optimal dietary HK addition is 10 g/kg.

Study 2

Effects of different doses of dietary HK as antibiotics and ZnO substitute on growth performance, diarrhea incidence and intestinal health of weaned piglets

Experimental animals

A total of 160 weaned piglets (Duroc x Landrace x Yorkshire; body weight 8.87 ± 0.58 kg).

Experimental design

According to initial body weight and '2×2' factorial design, the animals were randomly assigned to 4 treatments, with 8 replicates (pens) per treatment and 5 piglet per replicate (pen).

Treatments / Growing stage	- ZnO		+ ZnO (2 g/kg)	
	NC-	HK-	NC+	HK+
Early nursery stage (0-14 d)	-	10 g/kg HK	-	10 g/kg HK
Late nursery stage (14-42 d)	-	5 g/kg HK	-	5 g/kg HK

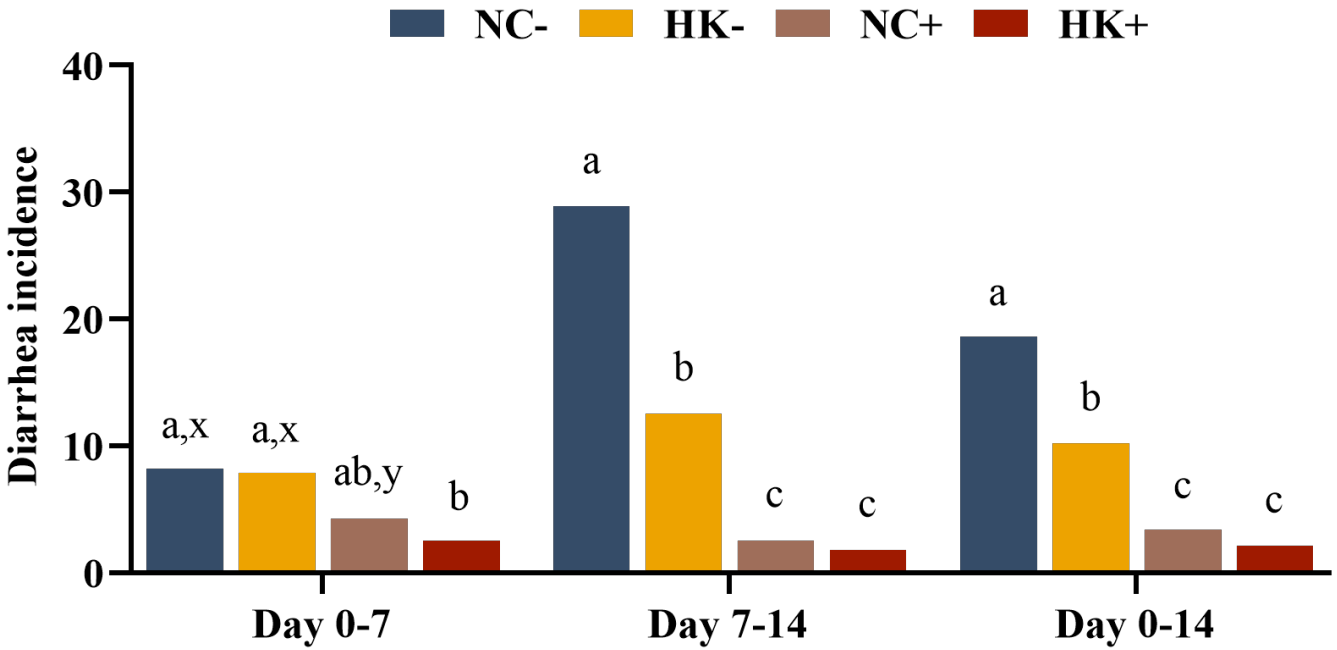
NOTE: NC represents control group; HK represents treatment group which supplemented with Kluyveromyces fragilis hydrolysate; '+' and '-' represents whether or not supplement ZnO; no ZnO was added in post nursery stage.

YIN et al., ANIM NUTR, Revise

Study 2 - Results – Growth performance

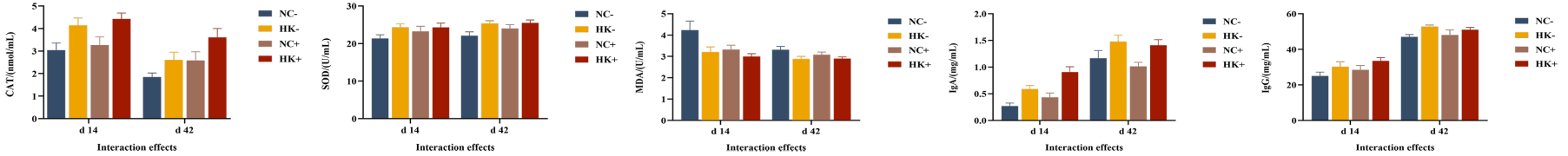
	BW, kg				ADG, g				ADFI, g				G:F			
	d 0	d 14	d 28	d 42	d 0-14	d 14-28	d 28-42	d 0-42	d 0-14	d 14-28	d 28-42	d 0-42	d 0-14	d 14-28	d 28-42	d 0-42
Interaction effects																
NC-	8.87	9.88	14.43	20.64	72	325	444	280	251	592	860	568	0.24	0.55	0.52	0.49
HK-	8.87	10.34	15.31	23.19	105	355	563	341	269	626	941	612	0.37	0.58	0.61	0.57
NC+	8.87	10.89	15.00	22.46	145	293	533	324	278	596	910	595	0.53	0.50	0.59	0.55
HK+	8.87	11.02	16.07	24.24	154	360	583	366	292	710	1009	658	0.53	0.53	0.58	0.57
SEM	0.575	0.589	0.875	1.151	15.0	27.2	36.2	18.0	22.6	55.6	61.9	38.0	0.047	0.037	0.029	0.025
Main effects																
ZnO																
-	8.87	10.11^{b}	14.87	21.91^{y}	89^{b}	340	503^{y}	311^{y}	260	609	900	590	0.31^{b}	0.56	0.56	0.53
+	8.87	10.96^{a}	15.53	23.35^{x}	149^{a}	327	558^{x}	345^{x}	285	653	960	626	0.53^{a}	0.51	0.59	0.56
SEM	0.393	0.405	0.615	0.843	11.7	20.4	27.8	14.3	15.6	39.6	44.6	26.9	0.036	0.026	0.024	0.020
Diet																
NC	8.87	10.39	14.71^{b}	21.55^{b}	109	309^{y}	488^{b}	302^{b}	264	594	885^{y}	581	0.39	0.52	0.55	0.52^{y}
HK	8.87	10.68	15.69^{a}	23.71^{a}	130	358^{x}	573^{a}	353^{a}	281	668	975^{x}	635	0.45	0.55	0.60	0.57^{x}
SEM	0.393	0.418	0.607	0.811	13.5	19.5	26.5	13.2	15.6	39.6	44.6	26.9	0.046	0.026	0.023	0.018
P-value																
ZnO	0.934	<0.001	0.162	0.064	<0.001	0.604	0.067	0.063	0.152	0.410	0.226	0.270	<0.001	0.161	0.370	0.278
Diet	0.934	0.165	0.046	0.008	0.168	0.064	0.007	0.007	0.332	0.165	0.073	0.107	0.212	0.427	0.150	0.075
ZnO × diet	1.000	0.434	0.842	0.608	0.436	0.469	0.242	0.605	0.903	0.451	0.848	0.773	0.229	0.920	0.085	0.366

Study 2 - Results - Dirrhea incidence

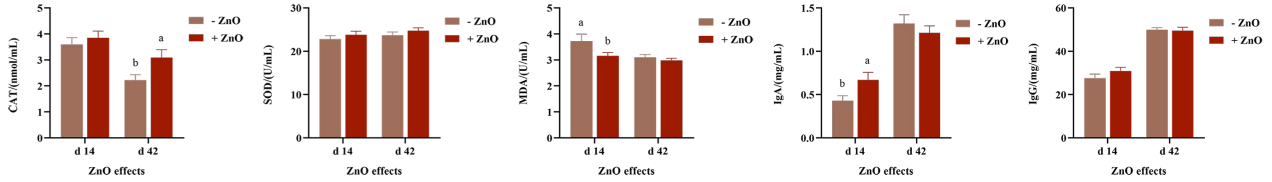


Study 2 - Results - Plasma indices

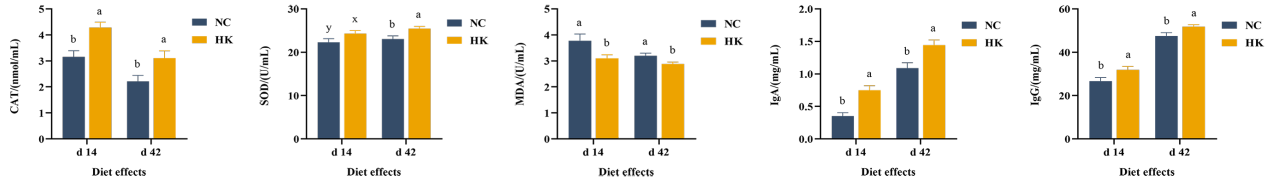
Interaction effects



ZnO effects



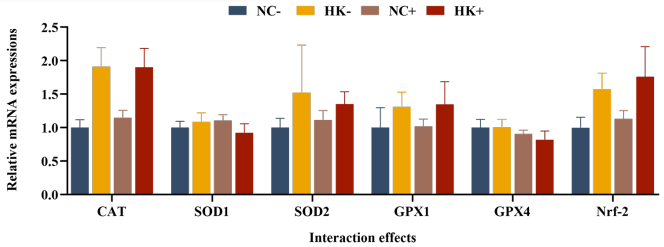
Diet effects

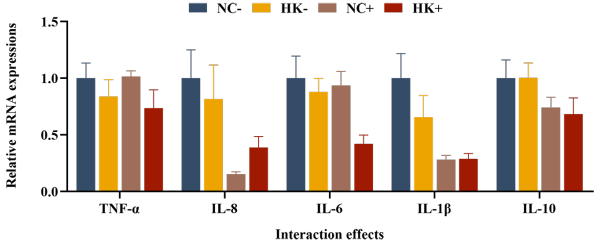


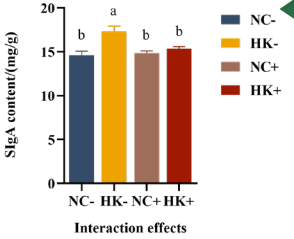
- Dietary supplementing HK enhanced antioxidant and immune levels by increasing the plasma **CAT and SOD activities**, **IgA and IgG concentrations** of weaned piglets, reducing the **MDA concentration**;
- ZnO enhanced the antioxidant and immune levels by increasing the **plasma CAT activity and IgA concentration** of weaned piglets.

Study 2 - Results - Gene expression

Interaction effects

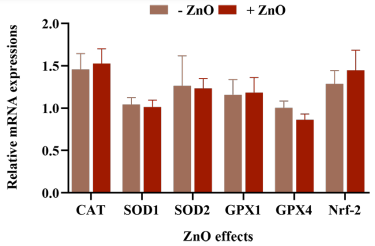


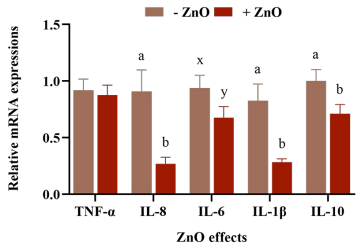


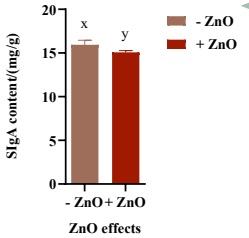


The expression of **sIgA mRNA** in jejunal mucosa had a significant interaction effect.

ZnO effects

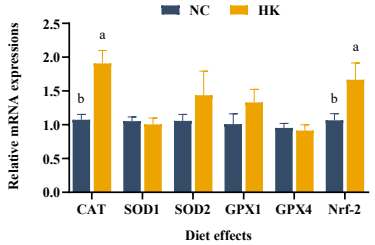


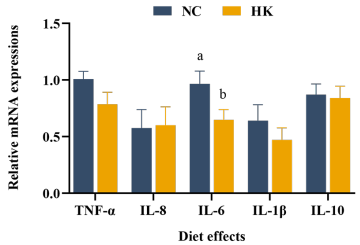


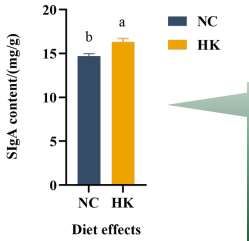


- HK improved the intestinal antioxidant and immune levels by increasing the expression of ***CAT* and *Nrf2 mRNA*** and **sIgA content** in the jejunal mucosa of weaned piglets, reducing the expression of ***IL-6 mRNA***;
- ZnO improved the intestinal immune level by reducing the expression of ***IL-8, IL-1β and IL-10 mRNA*** in the jejunal mucosa of weaned piglets.

Diet effects



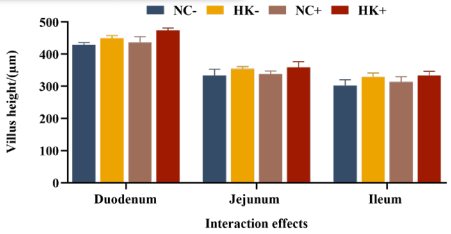


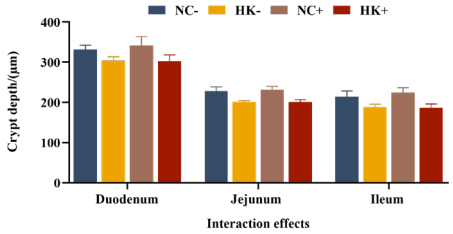


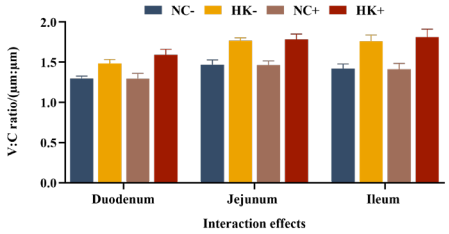
ZnO reduced the **sIgA content** in the jejunal mucosa of piglets, indicating that ZnO would cause certain damage to the intestinal immune barrier function.

Study 2 - Results - Morphology

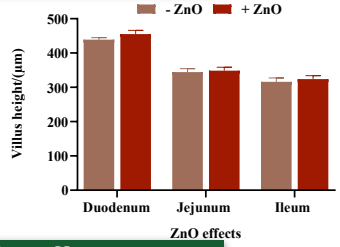
Interaction effects

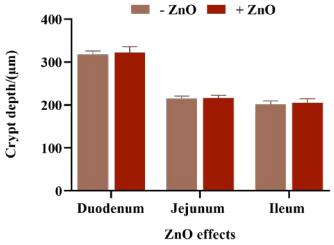


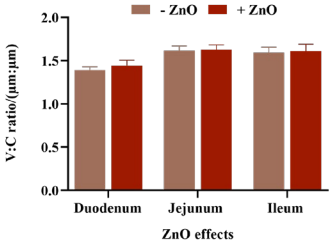




ZnO effects

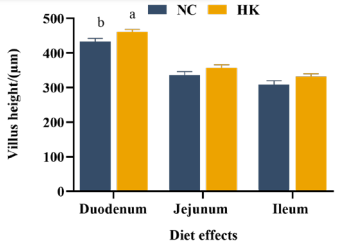


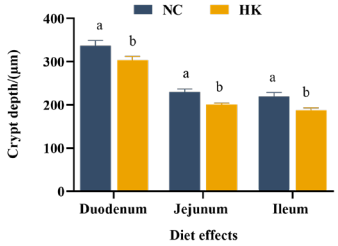


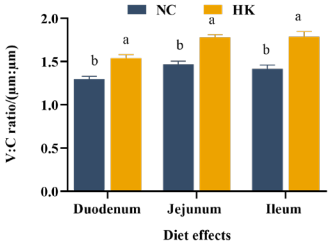


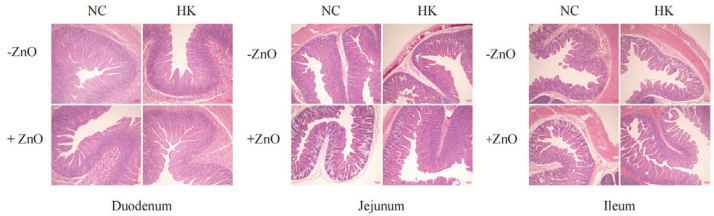
- HK improved the intestinal morphology by reducing **crypt depth** of jejunum and ileum and increasing the **ratio of villus height-to-crypt depth**;
- ZnO had no significant effect on the intestinal morphology of weaned piglets.

Diet effects





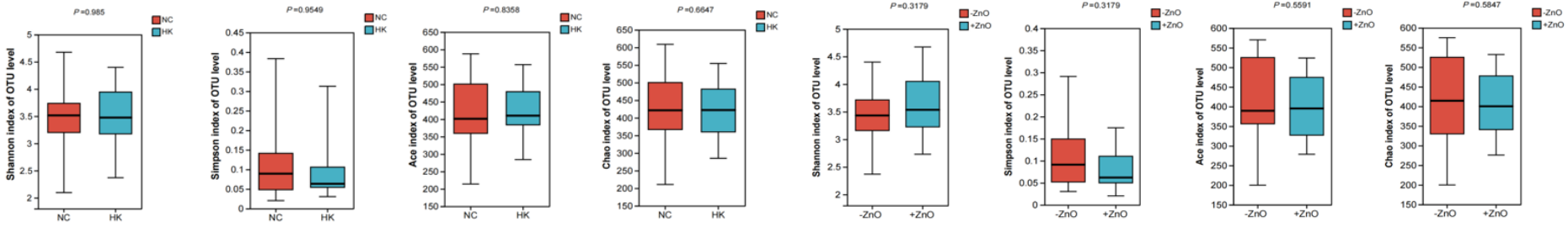




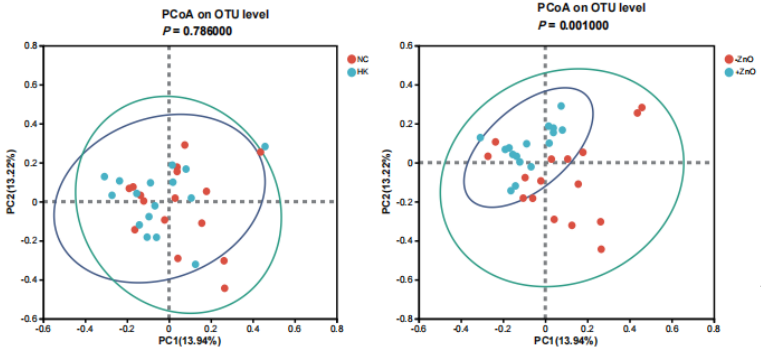
Study 2 – Results - Microbiota

Diet effects

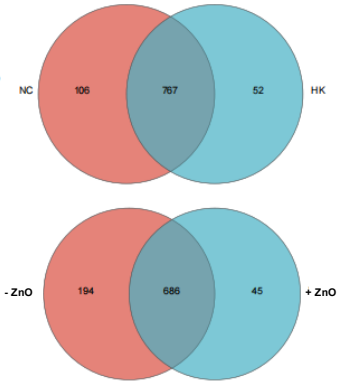
ZnO effects



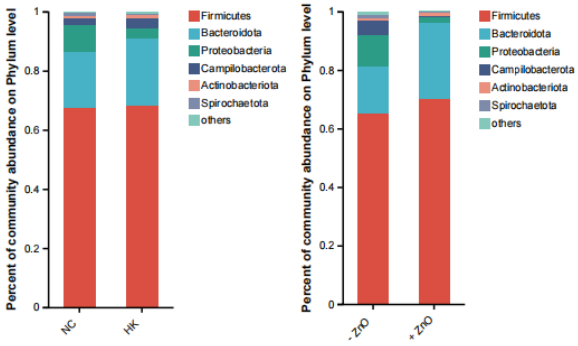
α diversity



β diversity



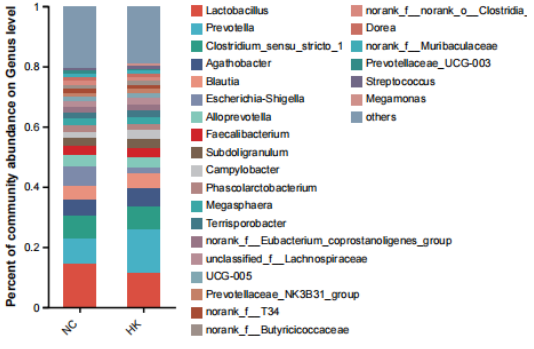
Venn diagram



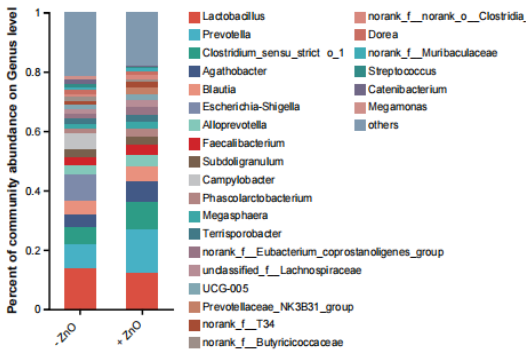
Microbial community abundance on phylum level

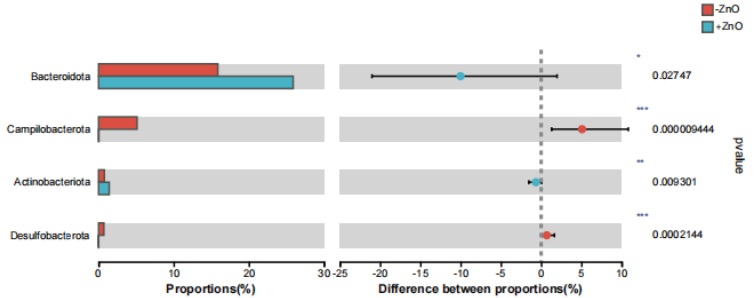
Study 2 - Results

Diet effects

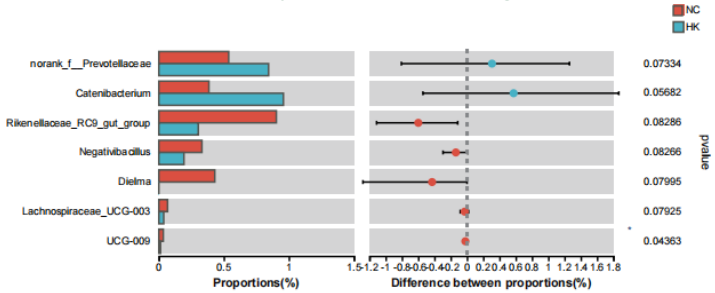


ZnO effects

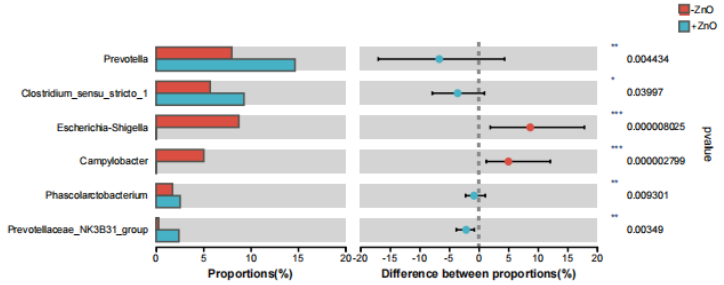




Microbial community abundance on genus level



Differences on the proportion of microbes



Differences on the proportion of microbes

Study 2 - Summary

1. Dietary HK with long-term can improve the growth performance of piglets;

2. Dietary HK reduced the diarrhea incidence of weaned piglets;

3. Dietary HK improved the antioxidant and immune functions, reduced intestinal inflammatory response, and improved intestinal tissue morphology and barrier function.

Dietary supplementing HK can replace high-dose ZnO to a certain extent.

03 Conclusions and plans

Conclusions

- Dietary HK improved growth performance and reduce diarrhea incidence of weaned piglets, which may be attributed to the enhanced antioxidant and immune functions, down-regulated intestinal inflammatory response, improved intestinal morphology and barrier function;
- HK (10 g/kg) has the potential to replace high-dose ZnO in weaned piglets.

Further plans

- Use multi-omics technology, to reveal the key metabolites of HK acting on the target organs of weaned piglets, and to clarify the key targets and intrinsic molecular mechanisms of HK regulating the antioxidant and immune functions of the intestine of weaned piglets;
- Utilize the liver of weaned piglets as the target organ, to investigate whether HK can promote animal health and growth performance by improving the antioxidant function.

中国农业科学院饲料研究所
Institute of Feed Research of CAAS

Thank you very much for your attention!

Prof. Dr. Xian-Ren Jiang
jiangxianren@caas.cn
+86 15801524430
Zhongguancun Nandajie 12, Haidian District, Beijing, 100081 China