Insect mass rearing and biorefinery technology to produce protein and by-products

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- Greenhouse gas (GHG) emissions from livestock production (including transport of livestock and feed) account for approximately 18% of global human-induced emissions.

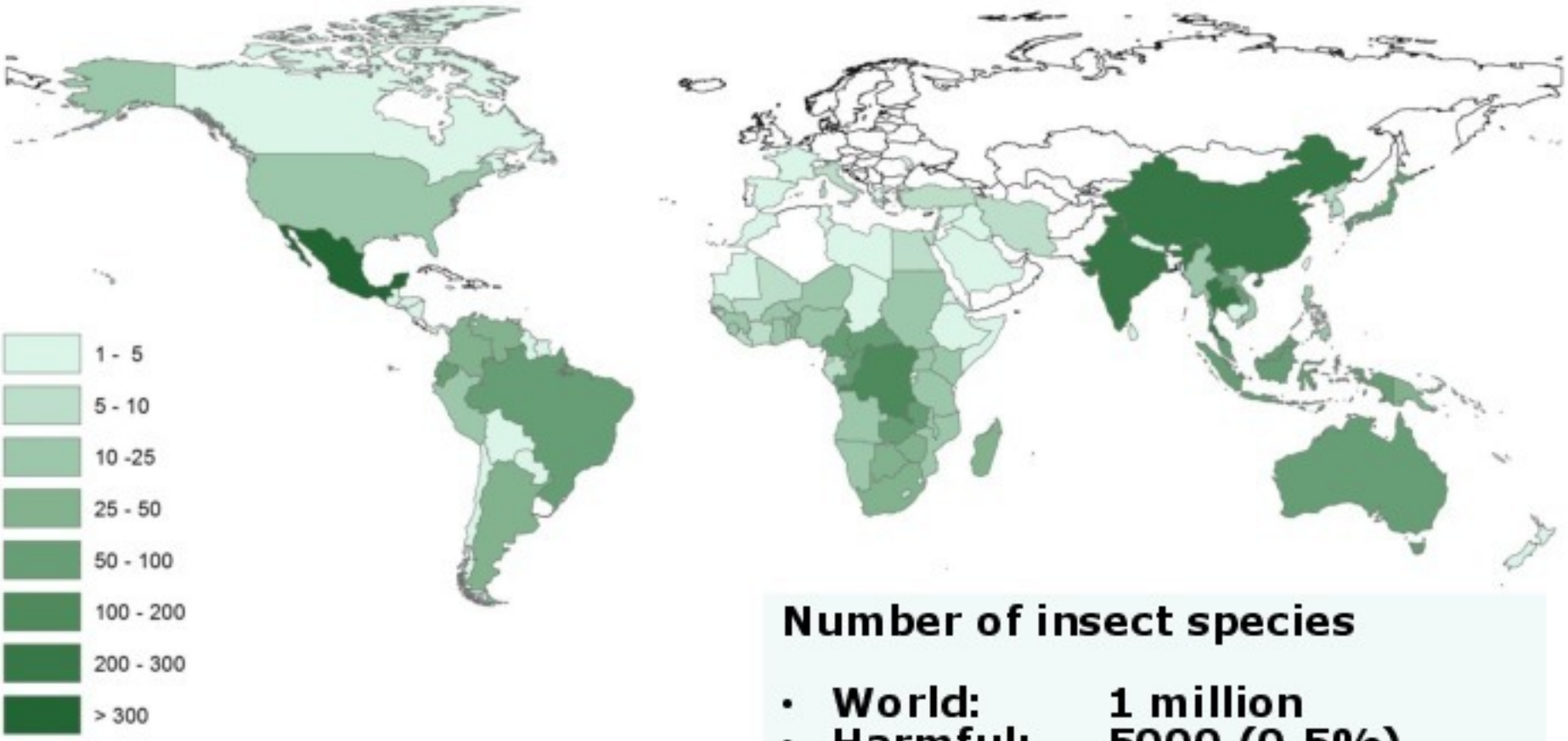
- Methane (CH_4) is produced by enteric fermentation (31% of global emissions) and released from manure (6%); N_2O is released mainly from feed crop fertilizer and manure (65%).

- 1 kg of beef has the highest environmental impact when measured in CO_2 equivalents (14.8 kg), followed by pork (3.8 kg), and chicken (1.1 kg).

- Concerning the global anthropogenic atmospheric ammonia emissions, responsible for eutrophication of surface waters and acidification of soils, almost all is emitted by the agricultural sector, of which almost two-thirds is by livestock.

（*Annu. Rev. Entomol.*, 2013）

Recorded edible insect species, by country



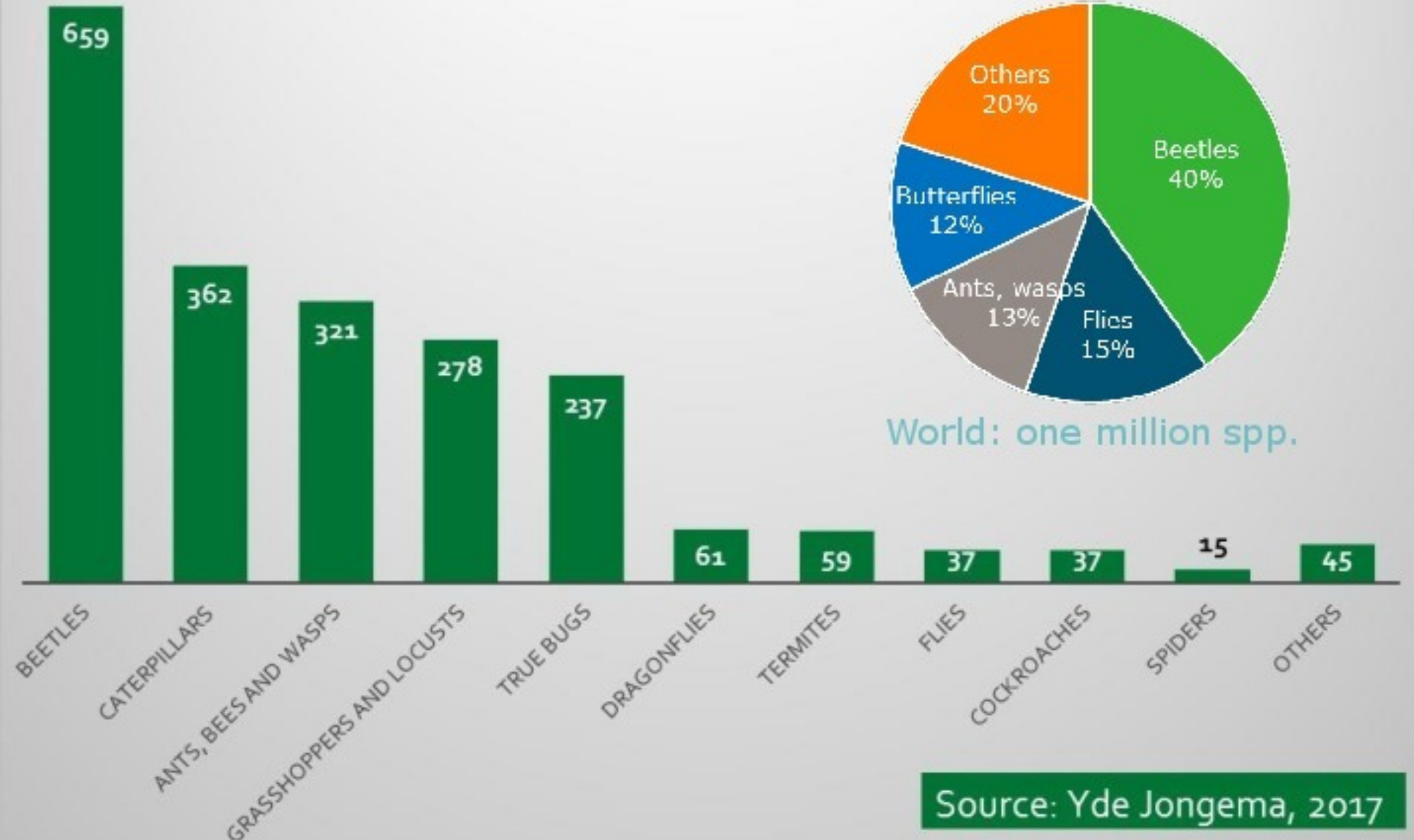
Number of insect species

- **World:** **1 million**
- **Harmful:** **5000 (0.5%)**
- **Edible:** **>2000**

Source: Centre of Geo information by Ron van Lammeren, Wageningen University, based on data compiled by Yde Jongema, 2017

version: 170402

Number of recorded edible insect species per group in the world (number: 2111)



Source: Yde Jongema, 2017

Producing more food with fewer resources may seem too good to be true, but the world's farmers have trillions of potential partners that can help achieve that ambitious goal. Those partners are microbes.

The United Nations estimated that global food production will need to increase by 70% by 2050.

Under the title of "Can Science Feed The World," a 2010 issue of *Nature* assessed the contributions that plant scientists can make toward reaching the goal of substantially increasing food production.

"roots are the key to the second green revolution"

"植物的根是第二次绿色革命的重中之重"

http://www.classhelp.info/Biology/Soldier%20Fly.jpg

Hermetia illucens (Diptera: Stratiomyidae)

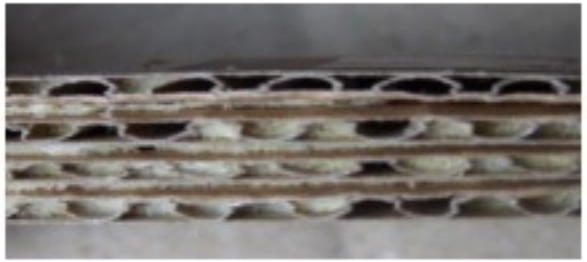
- Known as the "black soldier fly"
- Temperate/Tropics
- Colonizes variety of wastes
- April through October

亮斑扁角水虻

http://www.dipterra.com/website_images/image_of_mating_BSF.jpg

BSF life history

Egg



3-4 days

Larvae

2-3 weeks

Pupa

7-14 days

Adults

7-14 days

Black soldier fly- waste reduction, animal feed, bioenergy

Crude protein：>42%

- Reduce waste accumulation
- Control house fly
- Inhibit or kill pathogens

Dry matter：42%-43%

Insect biomass for feedstuff，coproducts

Crude fat：~35%

Insect fat, biodiesel
Special fatty acids

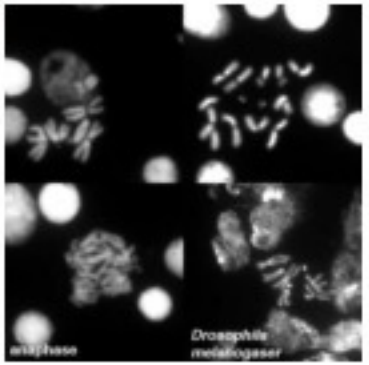
Outline

1. Brief introduction of black soldier fly (BSF) project
2. Mass breeding technology of BSF
3. BSF conversion tech of organic wastes
4. Feeding value of BSF
5. BSF biodiesel: From waste to bioenergy
6. BSF biology and microbial ecology

Retrospect on project research process

More……

•Technological design
•Industrialization
•Basic research



2012 2014

•Wuhan strain
•Mass breeding
•Co-conversion
•Basic research

2008 2011

•BSF breeding techs
•Key technology
•Conversion techs

2004 2007

FAO

- 3 Postdoc.
- 7 PhD
- 27 Master
- 30 Articles
- 14 Patents

Collaborate with Texas A&M University, USA

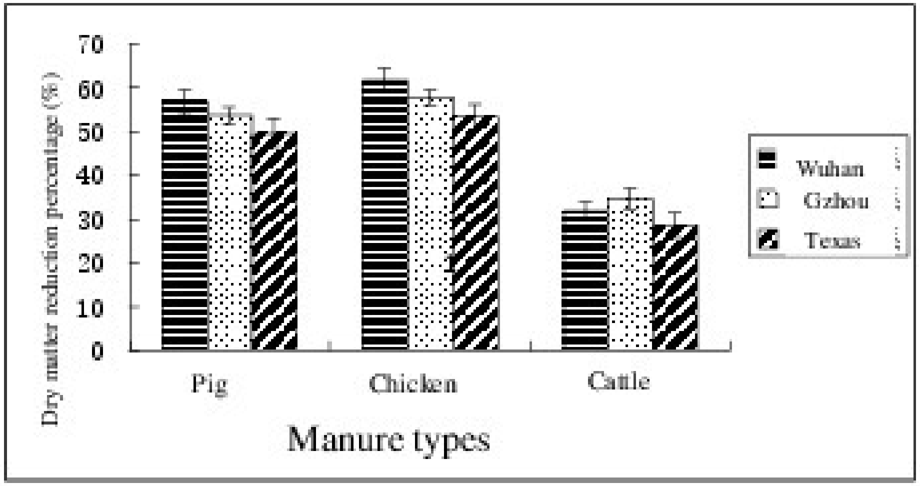
2 Mass rearing technology of BSF(规模化人工繁育）

Mass Production system

- **Wuhan strain**（better characteristics）
- Improved egg hatching rate
- Standard rearing process for larvae
- Optimized operating conditions of adult cages
- Continuous breeding tech
- Lower cost diet for BSF

- Technical team

BSF strains: developmental and waste reduction plasticity



- Wuhan strain is more efficient for pig and chicken manure
- Guangzhou stain performed the best for cattle manure

Strain	Sex	Final larval wt (g)	Larval development (d)	Sample size
Wuhan, China	F	[illegible]	[illegible]	[illegible]
	M		[illegible]	[illegible]
	Combined		[illegible]	[illegible]
Guangzhou, China	F	[illegible]	[illegible]	[illegible]
	M		[illegible]	[illegible]
	Combined		[illegible]	[illegible]
Texas	F	[illegible]	[illegible]	[illegible]
	M		[illegible]	[illegible]
	Combined		[illegible]	[illegible]

[illegible]

- Larval weight of Wuhan strain is significantly higher
- Larval developmental time of Wuhan strain is significantly short

Black soldier fly Wuhan strain: higher conversion rate, grow faster, bigger size, higher biomass yields

華中農業大學
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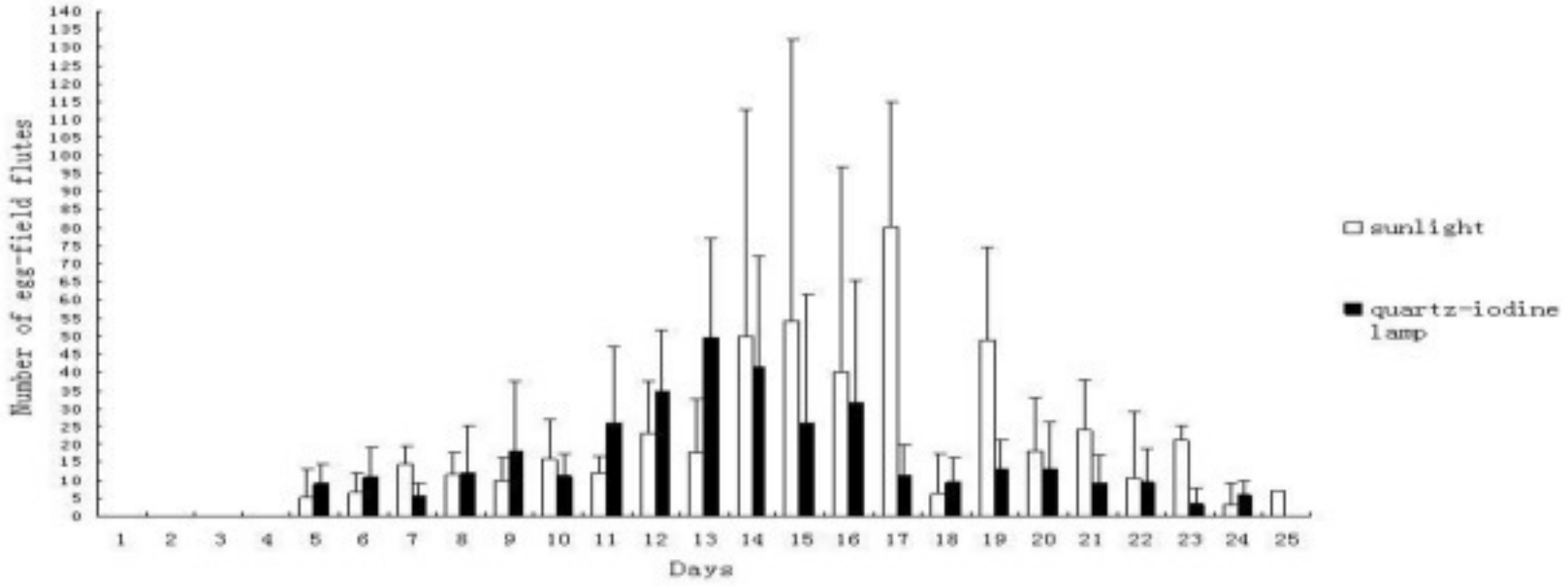
Mating and oviposition without opulent sunlight

no longer a problem!



The amount of egg-filled flutes collected under the sunlight and the quartz-iodine lamp treatments in a cage during a 25 day period. (Zhang et al., 2010)

Patent（ZL200820066774.4）


2009/02/25

1 Brief introduction of black soldier fly (BSF) project

- ~5 kg eggs per day, 400 m^2
- 100-200 ton waste / day

3 BSF conversion tech of organic wastes

BSF conversion tech _ animal manure, restaurant waste

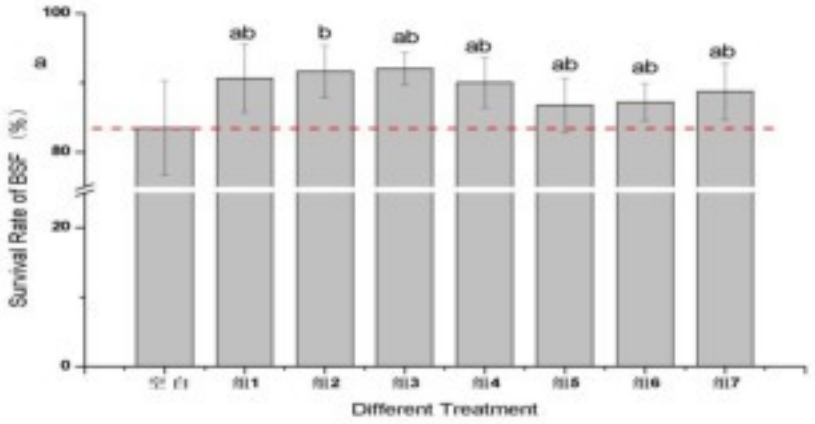
- Optimal larval age
- Optimal larval density
- Material condition and pretreatment
- Combination and material ratio
  (swine, dairy and chicken manure, etc.)

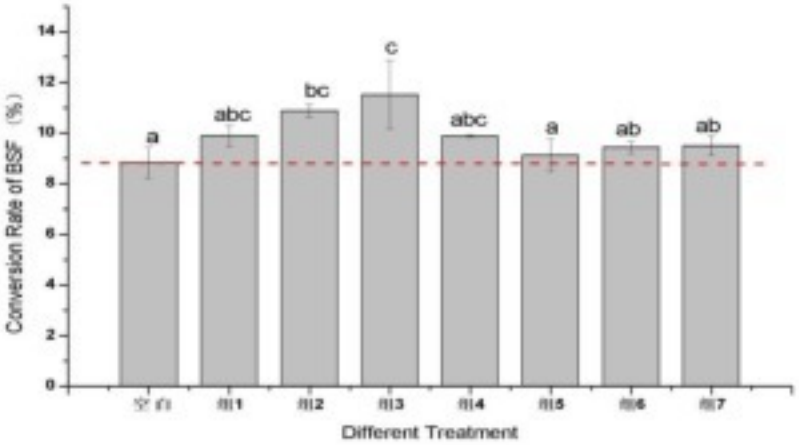
- Probiotics:

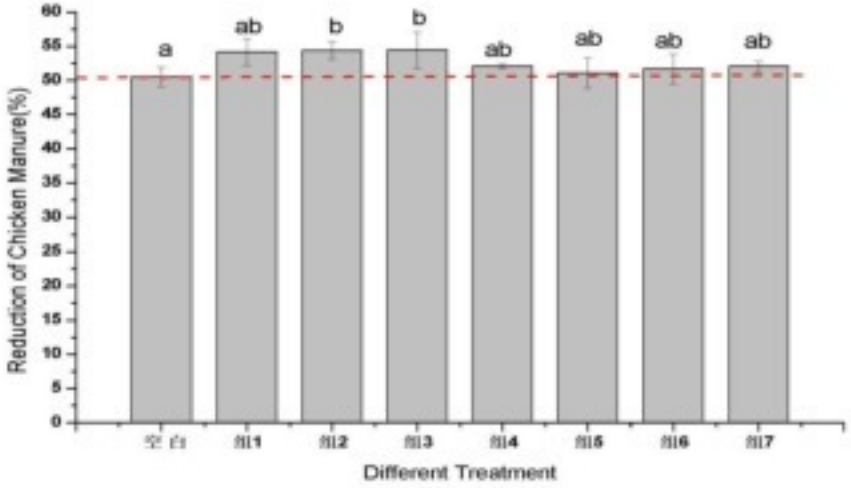
  Co-conversion = Microbes + BSF

  enhanced conversion rate, shorter period, higher yield

Chicken manure co-conversion: BSF+ Bacterial Complex I

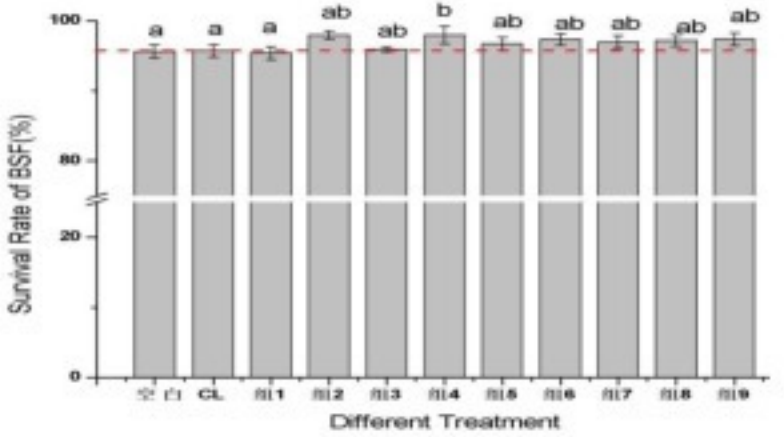


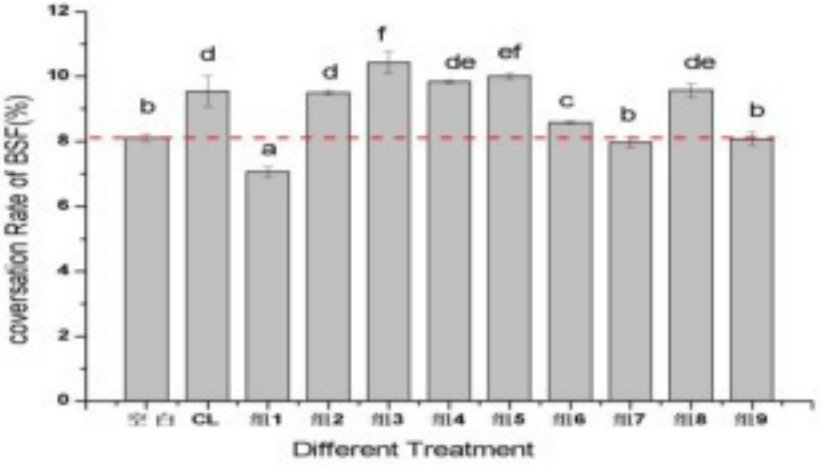


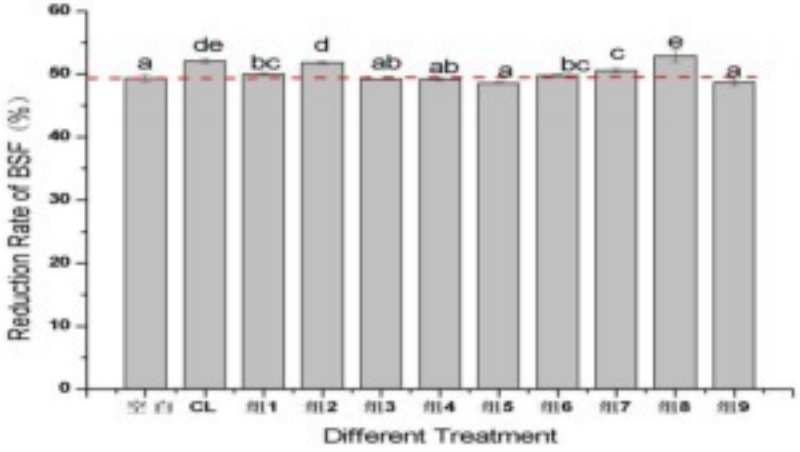


- 组3的复配菌剂的添加对水虻生长和转化的促进效果最好，组3的复配比例为R-07: R-09: F-03: F-06=4:1:1:1
- 水虻转化率提高了30.46%，鸡粪减少率增加了7.69%，水虻增重28.41%。

Chicken manure co-conversion: BSF+ Bacterial Complex II







- 组3（FE01：FE04：FE08：CL =4：1：1：1）显著提高水虻转化率，比对照组的提高了28.57%，可获得更高的水虻生物量
- 组8（FE01：FE04：FE08：CL=1：1：1：2）可显著提高鸡粪的减少率

World Population Growth

World Resources Institute

Sources: United Nations Population Division and Population Reference Bureau, 1993.

Black soldier fly products samples

Skimmed BSF Powder

(1) Co-conversion demonstration base in Wuhan

Chicken manure : BSF + Microbes

超拓®

水虻与微生物联合转化鸡粪的示范基地

400, 000 laying hen = > 40 tons manure/day


2017-09-29 10:25

(2) Co-conversion Restaurant waste: BSF + Microbes

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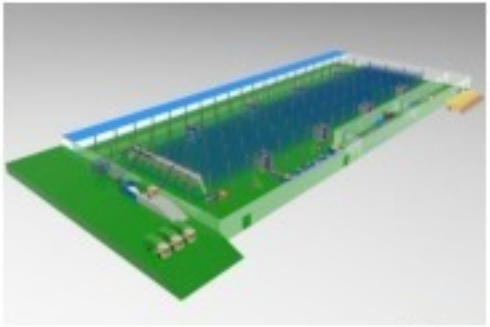
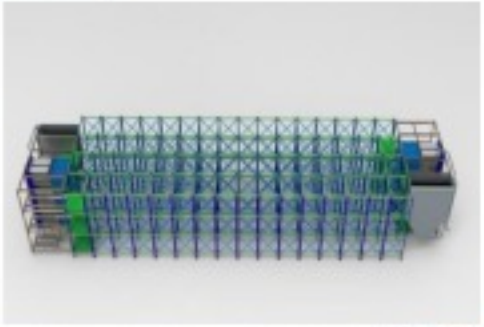
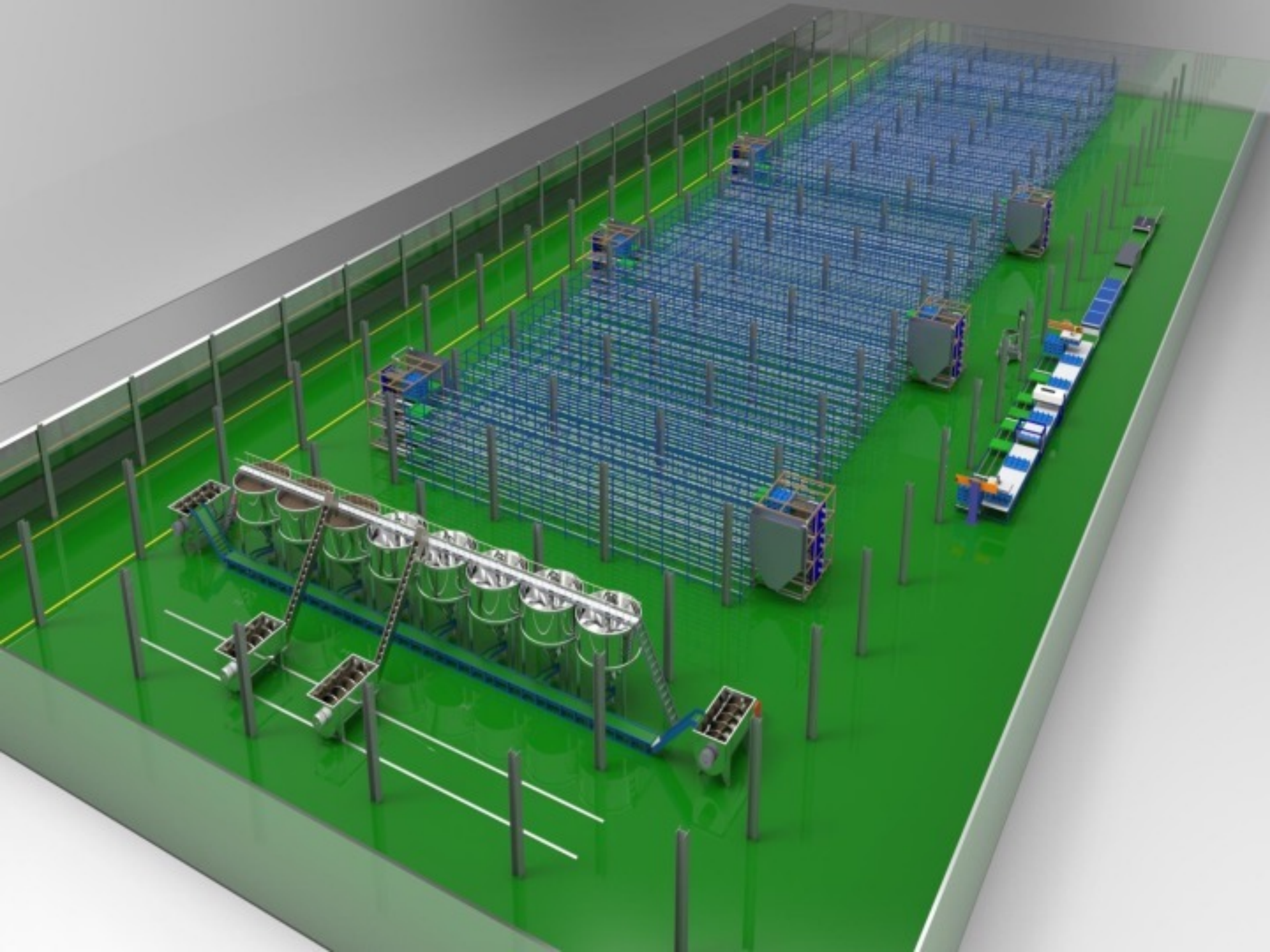
~ 200 tons food waste / day





- 目标日处理量200吨餐厨剩余物
- 年产5400吨昆虫蛋白饲料
- 年产18000吨微生物肥料
- 年销售收入7200万元
- 预计每年可获得利润3000万元

By the year 2050, there will be more than 9-11 billion people on earth to feed using the same amount or less land and water than is now available for agricultural production.

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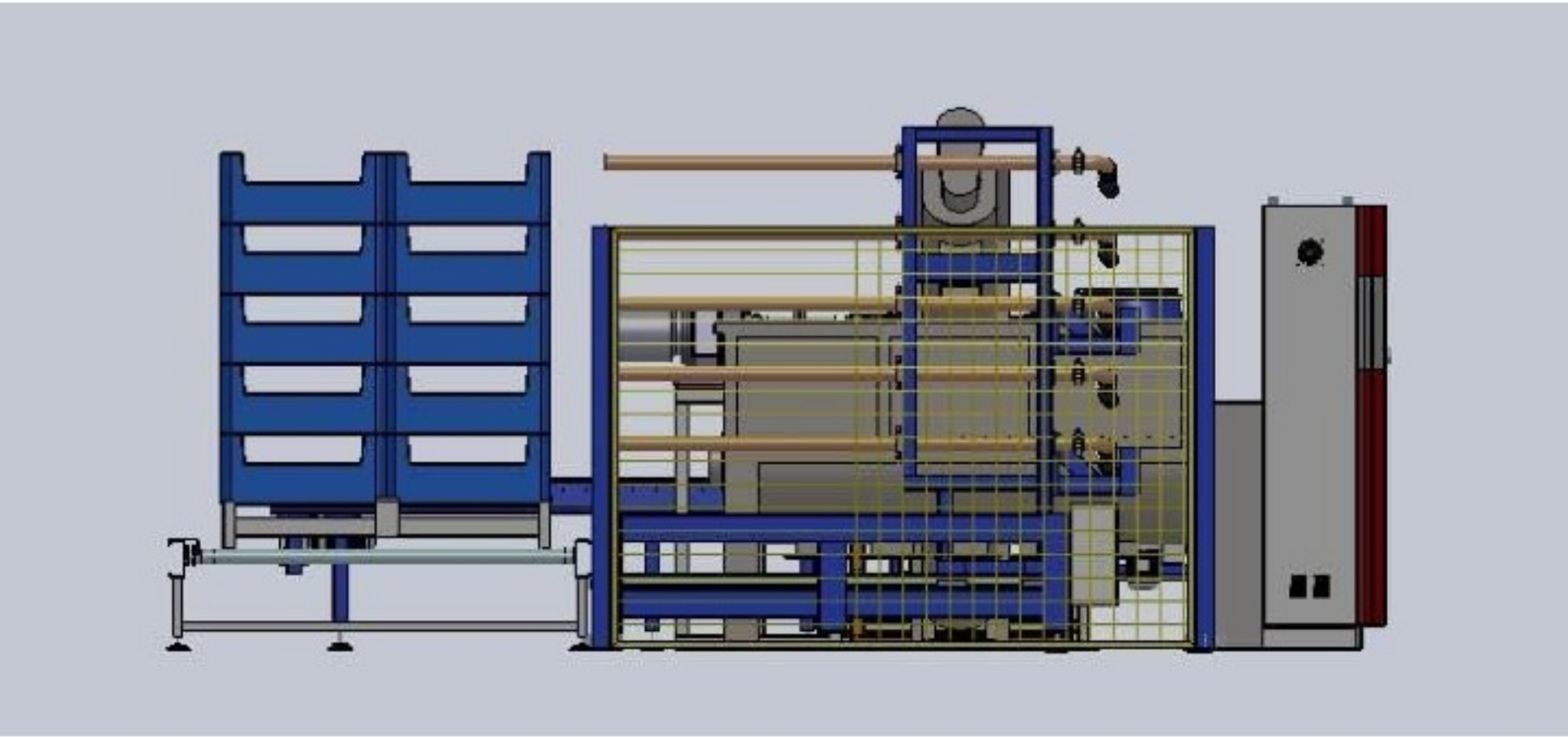

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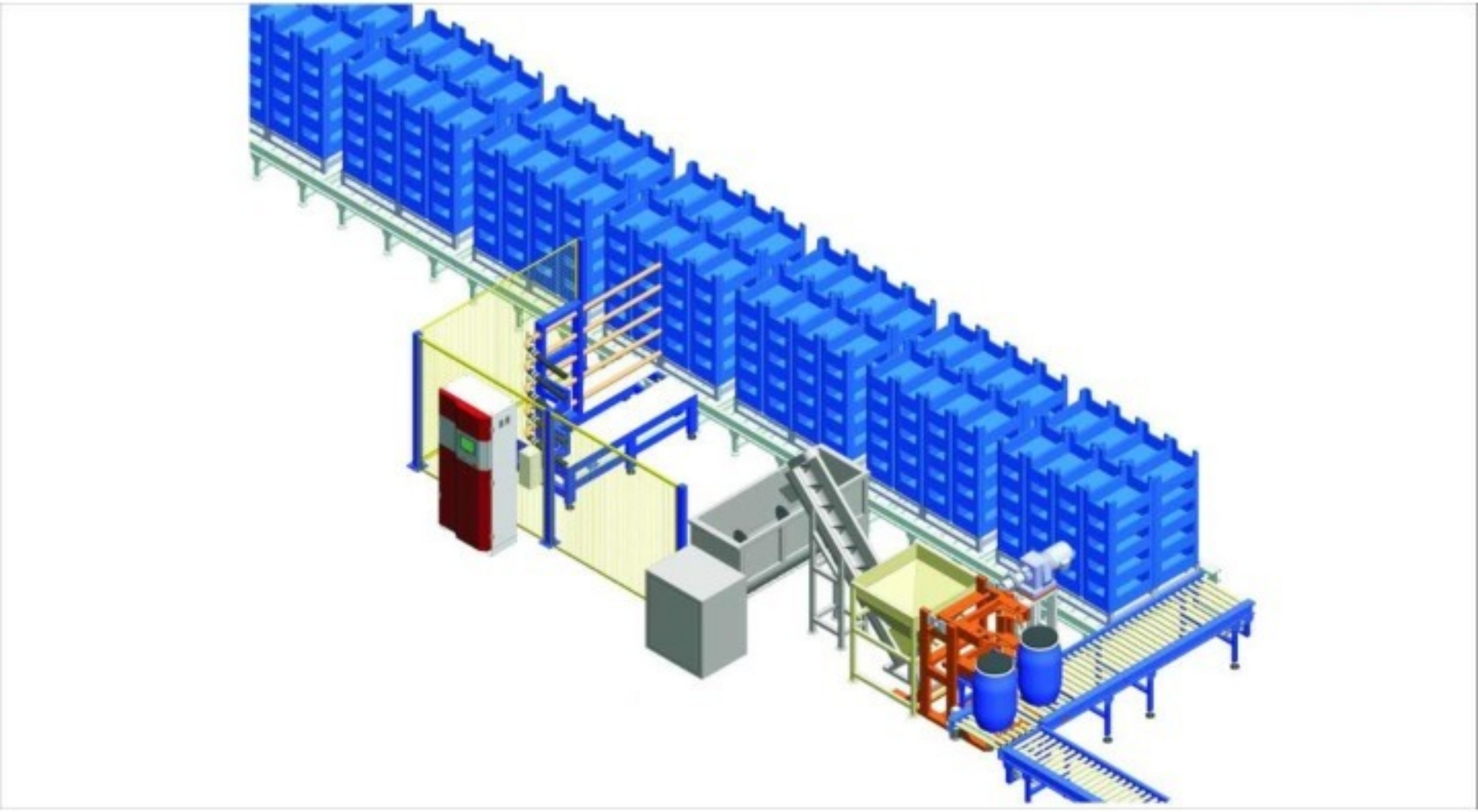

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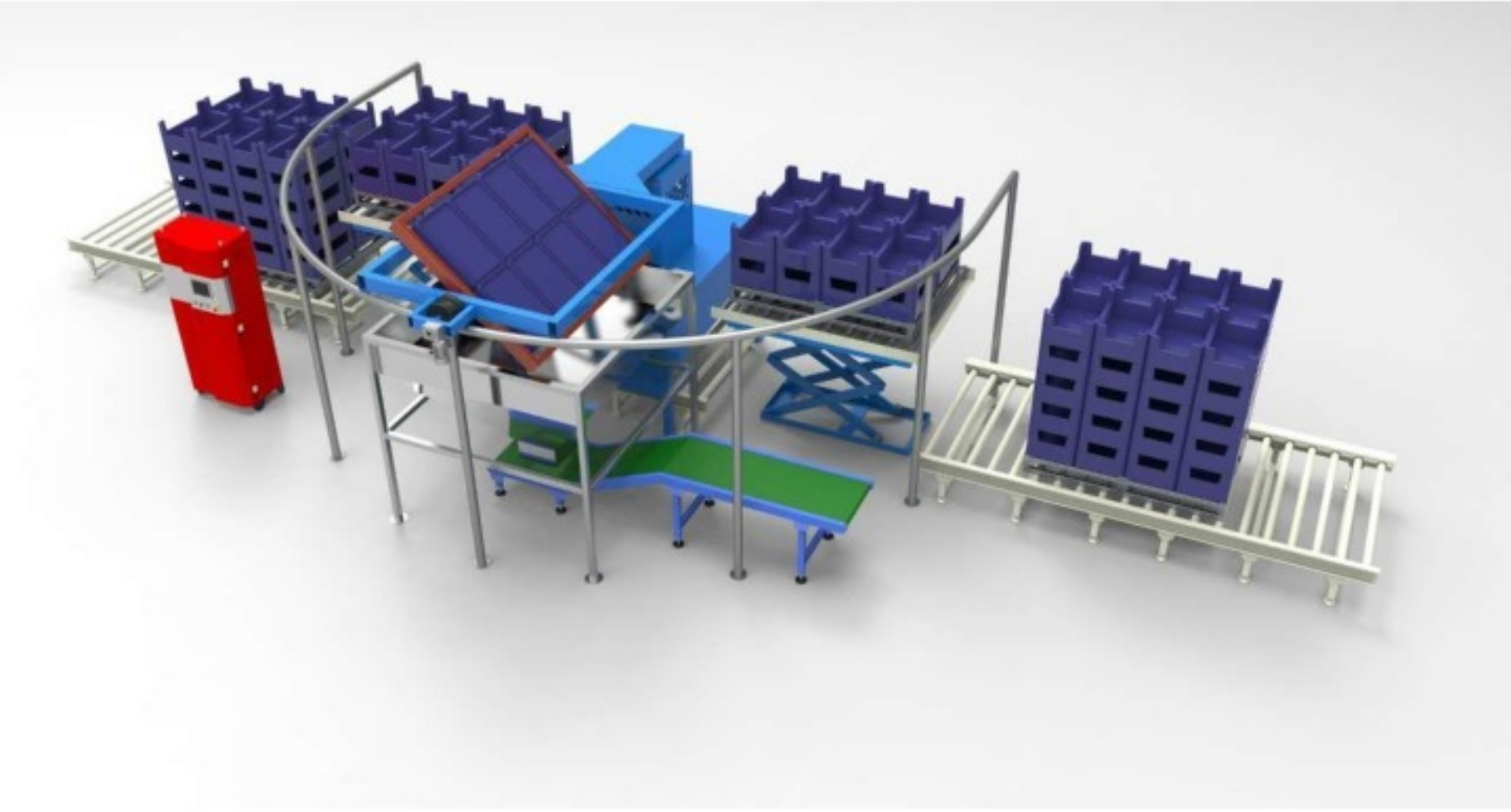

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Organic Waste Pollution in China

Animal husbandry has become the No. 1 in agricultural non-point source pollution!

- Water pollution—eutrophication
- Land pollution
- Odor pollution—NH_3, H_2S, etc.
- Pathogen, parasite

Long-term National Science and Technology Development Program (2006-2020) has put pollution and waste recycling as the key area and the theme of the priority in agriculture.

鱼粉代替品（黑水虻烘干产品）：

用亮斑扁角水虻，经去油、脱水、粉碎加工后的高蛋白质饲料原料。我国每年大约进口70万吨鱼粉，约80%来自秘鲁，从智利进口量不足10%，此外从美国、日本、东南亚国家也有少量进口。虽然迄今鱼粉仍为重要的动物性蛋白质添加饲料，但是我国饲料工作者一直研究探索低鱼粉日粮和无鱼粉日粮，黑水虻虫干是目前最好的替代产品。

有机肥（虫粪）：

主要来源于植物和（或）动物，施于土壤以提供植物营养为其主要功能的含碳物料。经生物物质、动植物废弃物、植物残体加工而来，消除了其中的有毒有害物质，富含大量有益物质，包括：多种有机酸、肽类以及包括氮、磷、钾在内的丰富的营养元素。不仅能为农作物提供全面营养，而且肥效长，可增加和更新土壤有机质，促进微生物繁殖，改善土壤的理化性质和生物活性，是绿色食品生产的主要养分。

蛋白粉：

亮斑扁角水虻活性蛋白，不仅富含人体不可缺少的蛋白质、游离氨基酸、维生素、矿物质元素、不饱和脂肪酸，还含有大量的、独特的、宝贵的、其他任何产品没有的、对人体非常有益的几丁质、抗菌肽、防御素、外源性凝集素等多种宝贵物质。

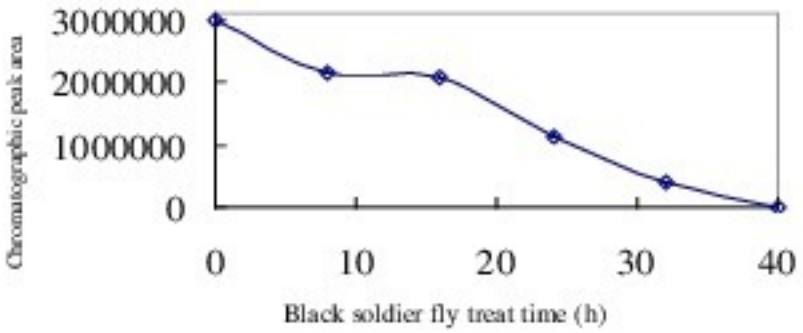
昆虫脂肪：

"黑水虻"学名亮斑扁角水虻，用"黑水虻"提取"昆虫油"的方法跟提取豆油的方法相近，但提取出来的油却比其他食用油更健康，不仅能补充人体不能合成的油酸、亚油酸和亚麻酸，还具有抗氧化防衰老、预防疾病的功效。

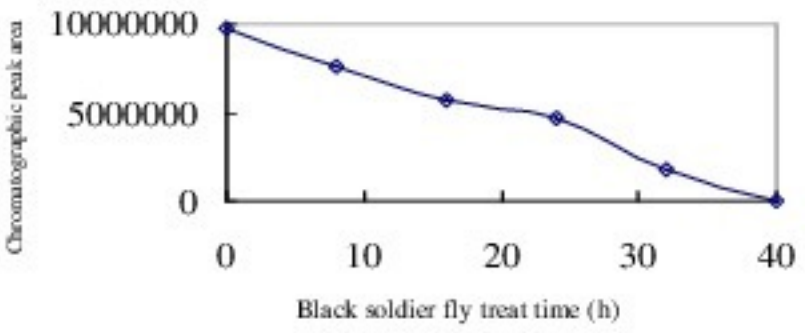
Additional benefits from BSFL bioconversion

- **Deodorization of the waste（除臭）**
- **Eliminate pathogenic bacteria in the waste（杀灭病原微生物）**
- **Innate immunity: antimicrobial peptides (AMPs)（自然免疫：抗菌肽类）**
- **Attenuates antibiotics and ARGs（消减残留抗生素和抗性基因）**

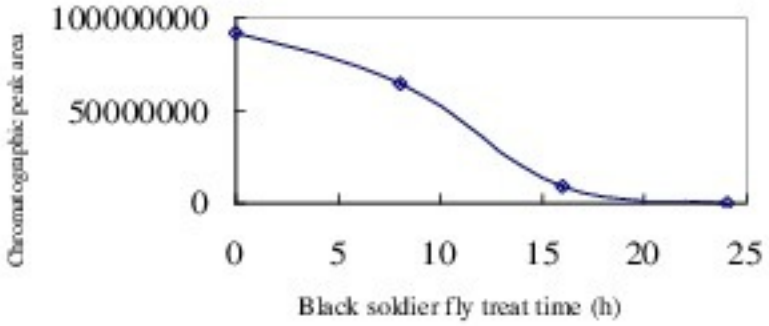
Deodorization of the animal manure (SPME-GCMS)



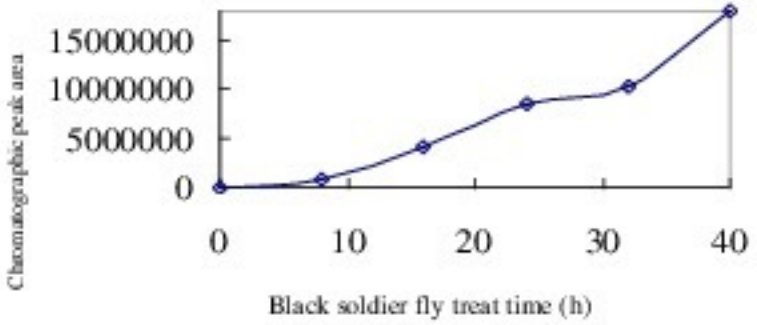
Variation of skatole in manure



Variation of indole in manure

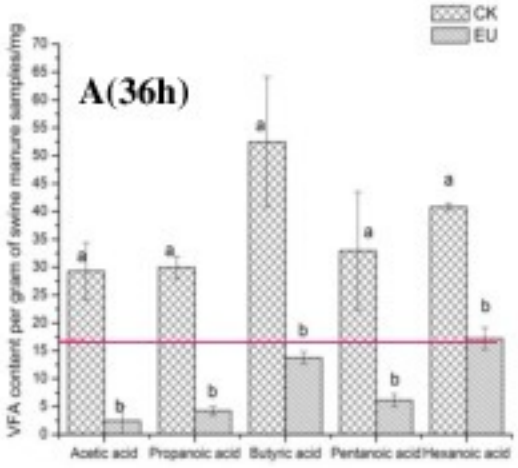


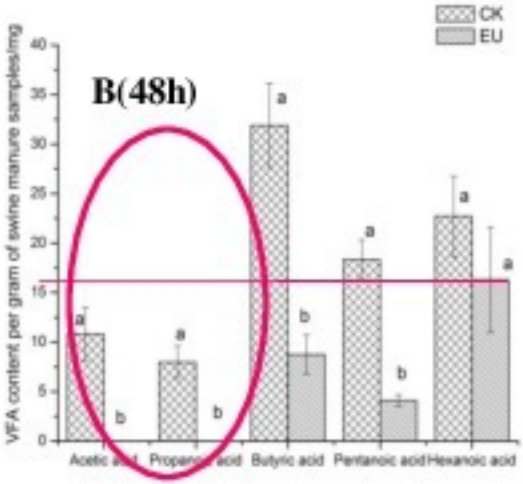
Variation of p-Cresol in manure

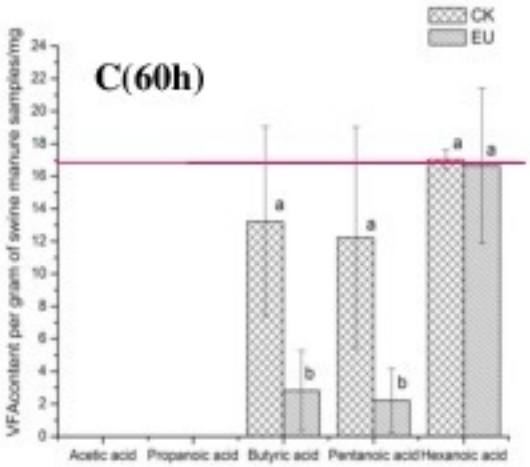


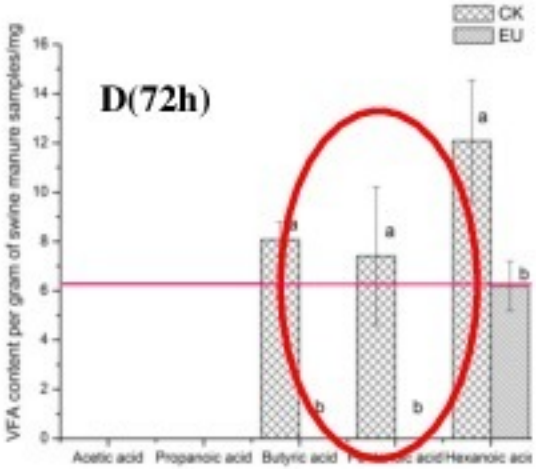
Variation of 2-nonyl ketone in manure

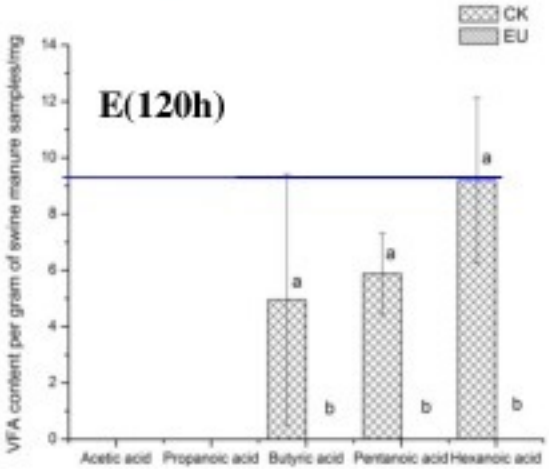
Deodorization: volatile fatty acids (VFA)





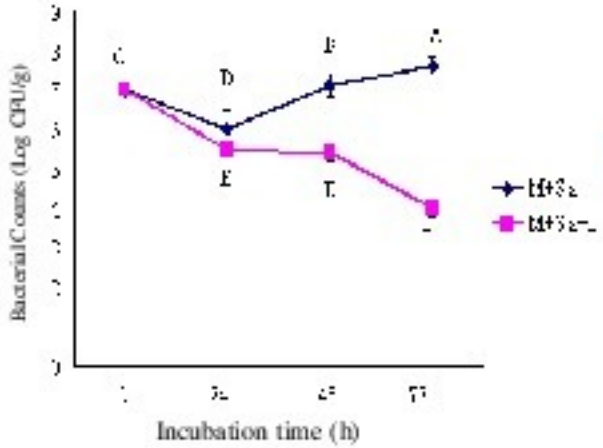






- Most VFA were significantly reduced after digested for 36h
- Short chain VFA could be detected after digested for 48 or 72h.
- Long chain VFA need longer time to eliminate.

BSFL inhibited pathogens in animal manure

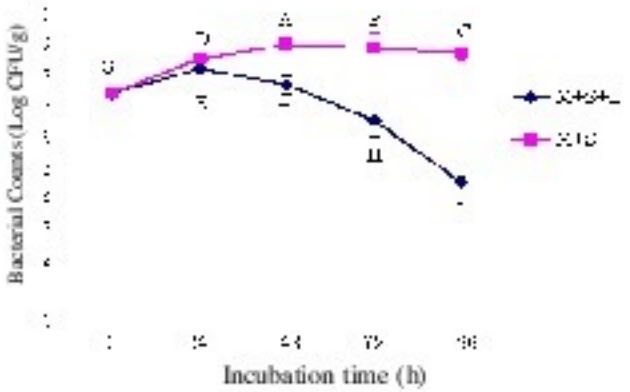


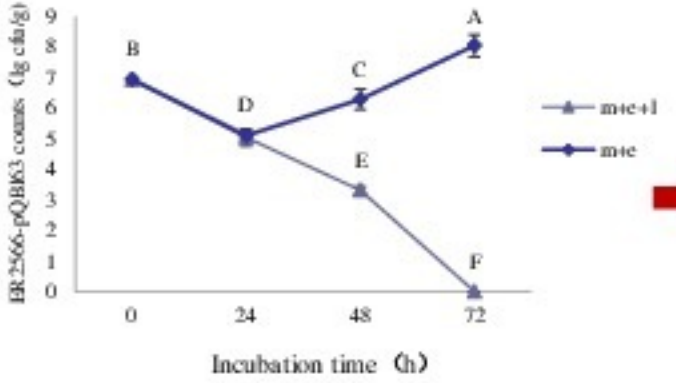
➢Swine manure

➢When larvae present, *Staphylococcus aureus* was significantly reduced after incubated for 72 h.

➢Swine manure

➢When larvae present, *Salmonella enteritidis* was significantly reduced after incubated for 96 h.



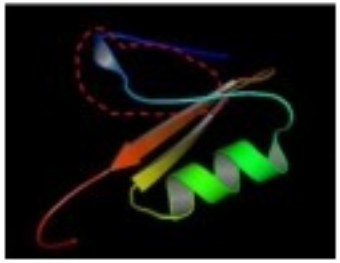


➢Dairy manure

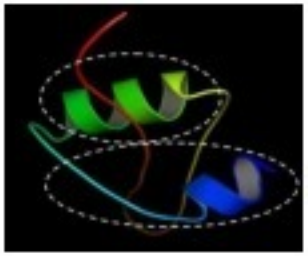
➢When larvae present , *Escherichia coli* ER2566 was significantly reduced and could not be detected after incubated for 72h.

BSF antibacterial peptides (AMPs) gene screening and gene cloning

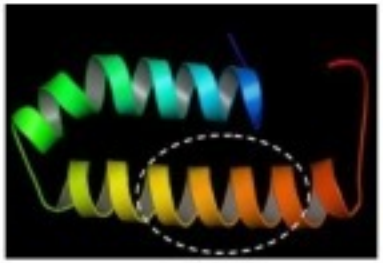
Gene sequence	Number of amino acids (aa)	Optimal Tm	Identity (%)	Remarks
Sarcotoxin1	53	54.8	44.44	--
Sarcotoxin1(a)	53	54.8	46.29	99% identity to sarcotoxin1; different bases at No. 26 and 57
Sarcotoxin1(b)	53	54.8	46.29	98% identity to sarcotoxin1; different bases at No. 26, 57 and 59
Sarcotoxin3	47	54.8	42.55	--
Cecropin	53	56.8	46.29	--
Stomoxyn	63	50.6	46.29	--
Stomoxyn(a)	63	50.6	46.29	99% identity to Stomoxyn; different base at No. 21



Sarcotoxin 1

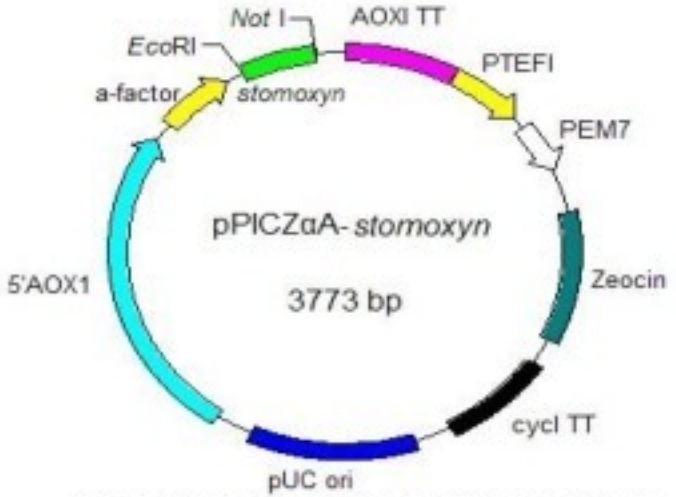


Cecropin

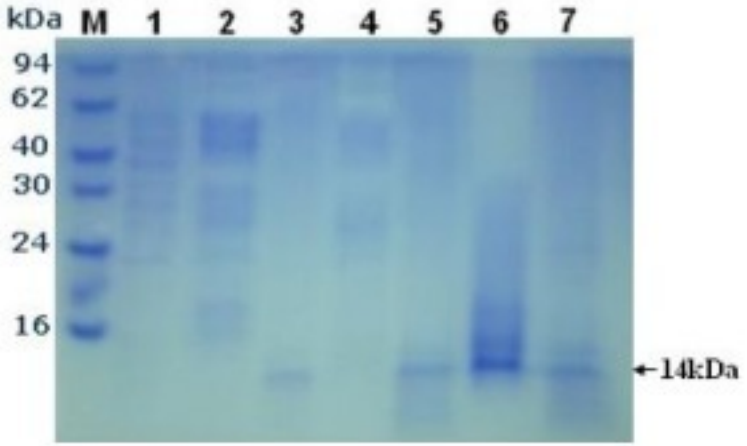


Stomoxyn

Stomoxyn: recombinant expression

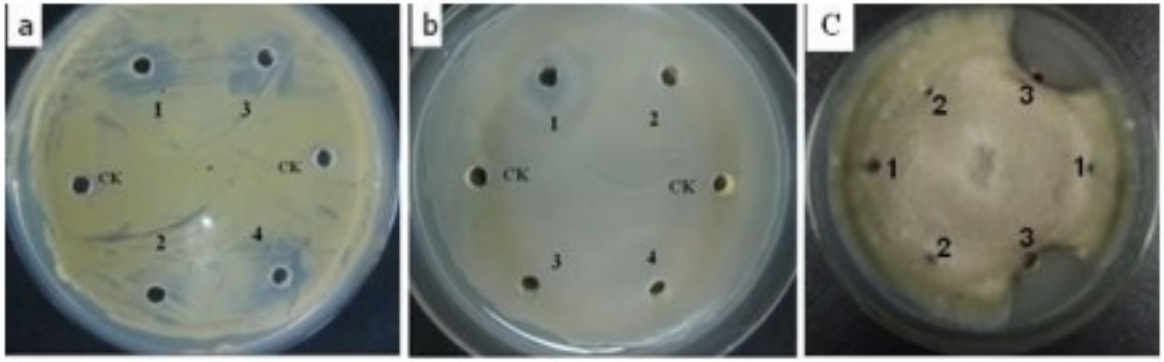


Construction of the recombinant expression vector pPICZαA/stomoxyn



SDS-PAGE analysis of the pPICZαA-stomoxyn products expression in *Pichia pastoris* GS115

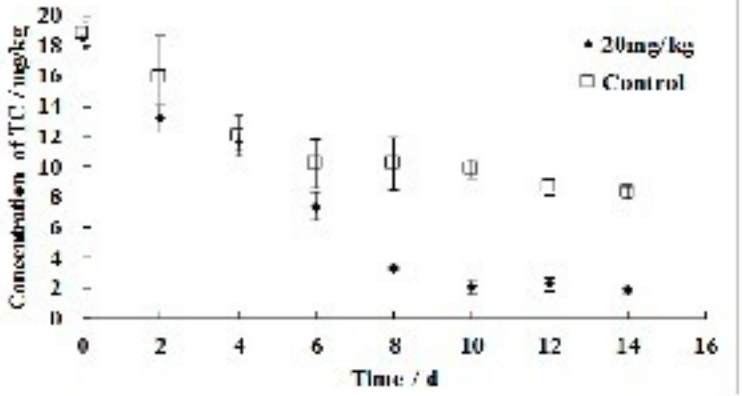
new antimicrobial agents???
feed additives???

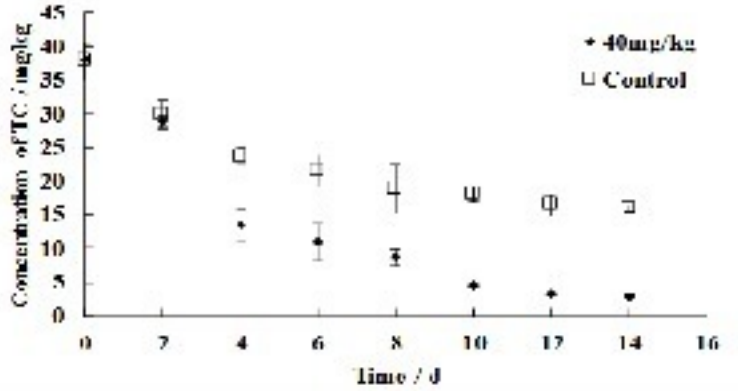


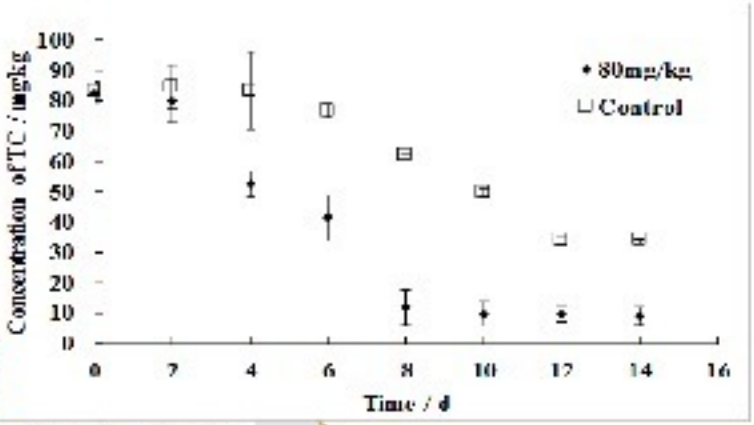
Antibacterial detection of expressed stomoxyn sample

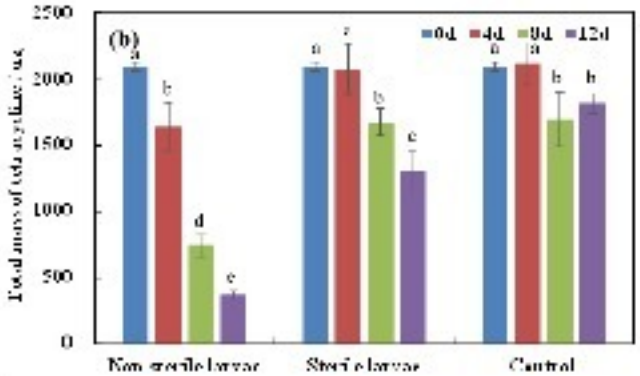
水虻生物转化有效降解畜禽粪便中残留抗生素

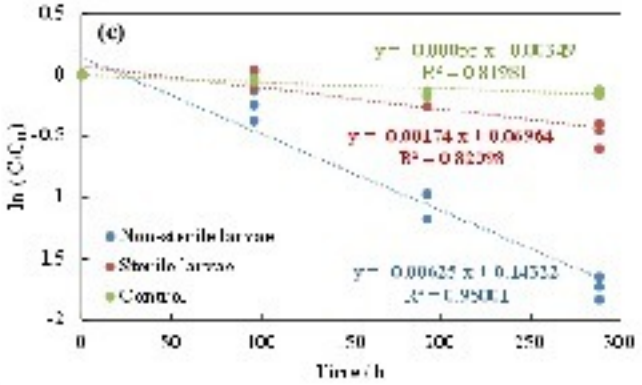
BSFL digestion of TC at different concentrations in an open conversion system





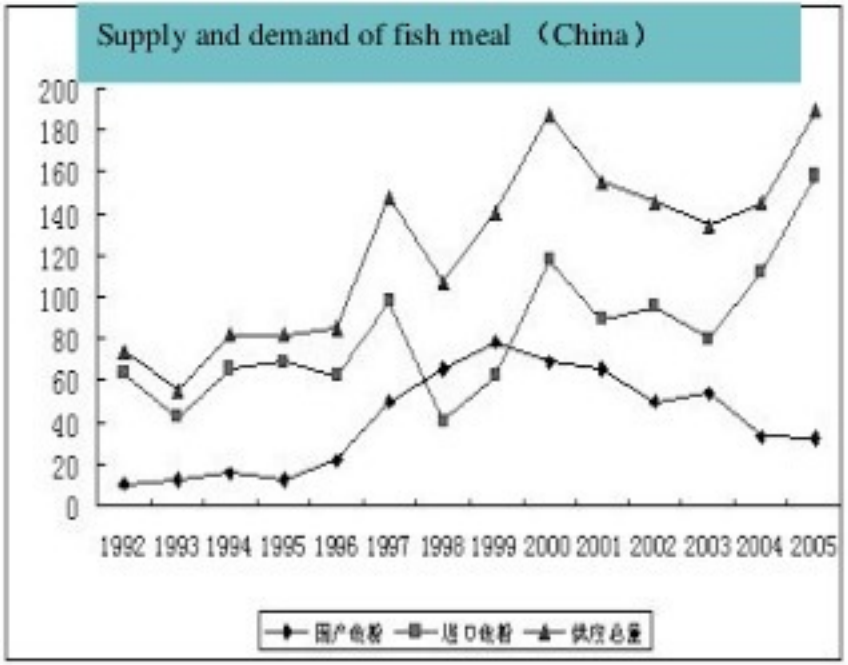


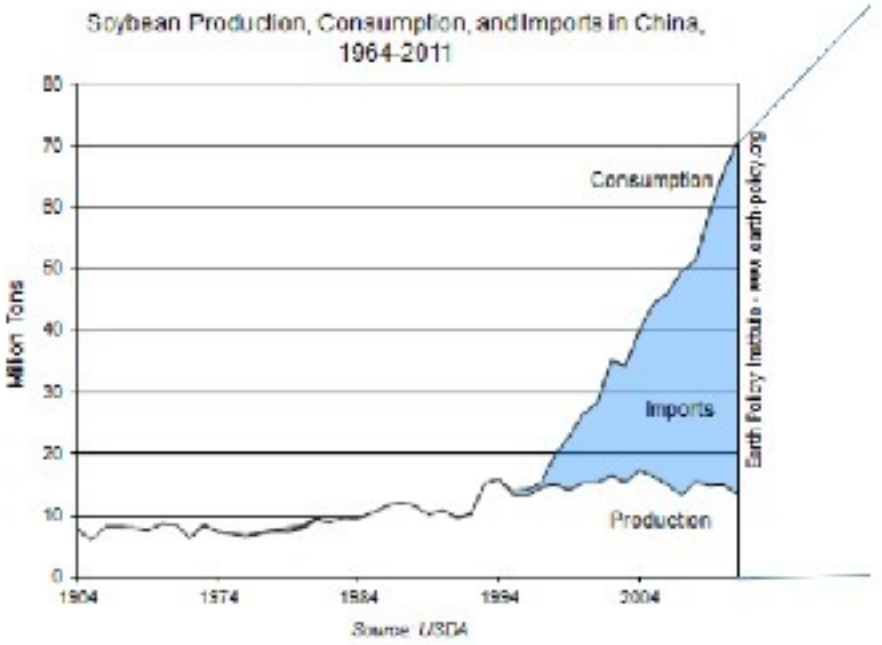




- 水虻转化系统对多种抗生素（以四环素为例）具有显著的降解效果。
- 在相同处理时间内，比单纯的微生物处理效率要高3倍以上。

China :No.1 importer of protein material





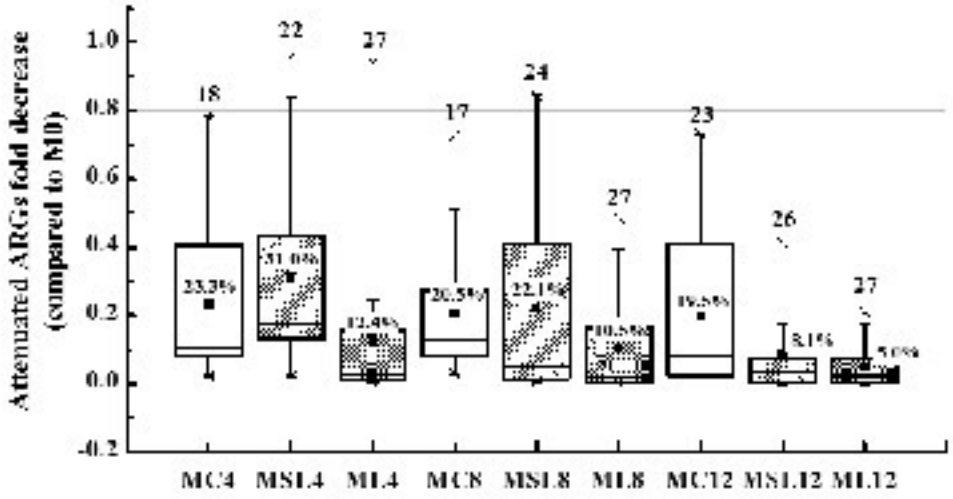
China bought a record 70 million tonnes of soy (FAO, 2013)

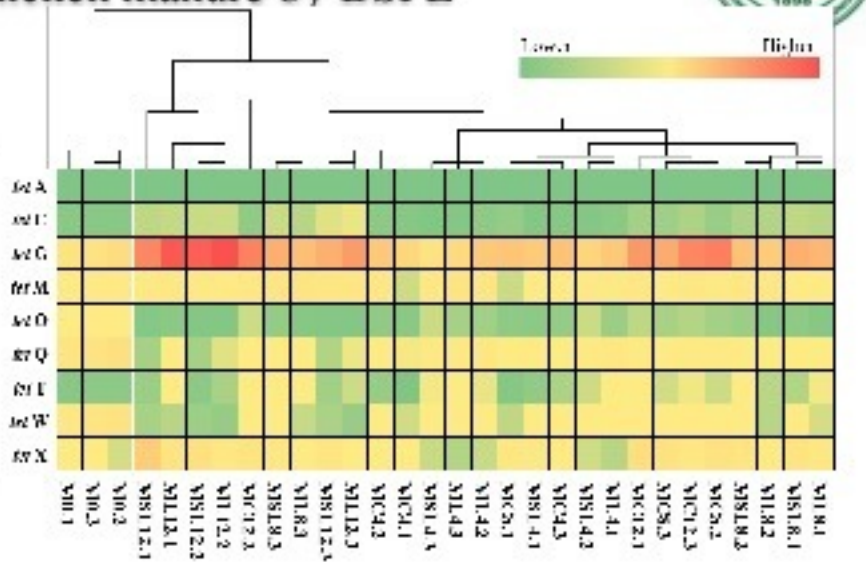
- 1, 200, 000 tons of fish meal imported.
- 70, 000, 000 tons of soy imported.

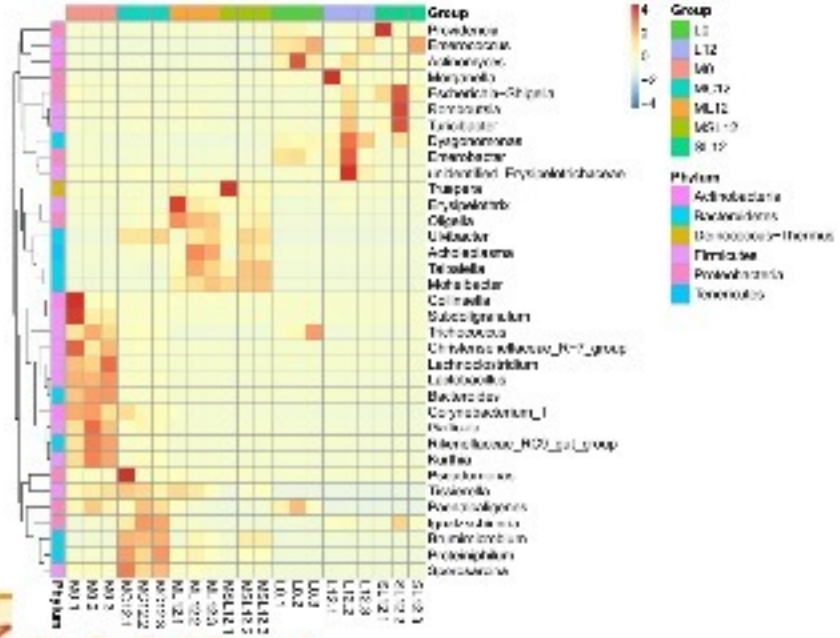
Foreign-trade Dependence >85%

水虻转化消减抗性基因丰度及其传播

Attenuates antibiotic resistance genes in chicken manure by BSFL







- 水虻生物转化对粪便中的抗性基因的绝对丰度有良好的削减作用。
- 水虻自身的抑菌作用及肠道微生物互作是抑制抗性基因积累和迁移的主要原因。

4

Feeding value of BSF（饲用价值评价）

Feeding Value of BSF

Commercial formulations partial replacement:

- Laying hen feeding and nutritional test
- Broiler chicken feeding and nutritional test
- Fish feeding and nutritional test

(yellow-head catfish, *Pelteobagrus fulvidraco*)

Nutrition ingredient analysis of BSF larva, prepupa and comparison to fish meal and soybean meal

Nutritional ingredient	Fish meal (%)	Soybean Meal (%)	BSF dry larva (%)		BSF dry prepupa (%)	
			Dairy manure	Swine manure	Dairy manure	Swine manure
Crude protein	60.2	44.2	42.1	45.2	42.1	43.2
Crude fat	4.9	1.9	34.8	31.4	34.8	28.0
Crude fiber	0.5	5.9	7.0	6.4	7.0	-
N-free extract	11.6	28.3	1.4	4.9	1.4	-
Crude ash	12.8	6.1	14.6	8.3	14.6	-
Calcium (Ca)	4.04	0.33	5.00	-	5.00	5.36
Phosphorous	2.9	0.62	1.51	-	1.51	0.88

Amino acid composition of BSF larval meal and comparison to fish meal and soybean meal

Essential amino acids	BSF larva (%)				Non-essential amino acids	BSF larva (%)			
	Dairy manure	Swine manure	Soybean Meal	Fish meal		Dairy manure	Swine manure	Soybean Meal	Fish meal
Methionine	0.9	0.83	0.68	1.64	Tyrosine	2.5	2.38	1.57	1.96
Lysine	3.4	2.21	2.99	4.72	Aspartic acid	4.6	3.04	-	-
Leucine	3.5	2.61	3.57	4.8	Serine	0.1	1.47	-	-
Isoleucine	2	1.51	2.1	2.68	Glutamic	3.8	3.99	-	-
Histidine	1.9	0.96	1.22	1.71	Glycine	2.9	2.07	-	-
Phenylalanine	2.2	1.49	2.33	2.35	Alanine	3.7	2.55	-	-
Valine	3.4	2.23	1.53	3.17	Proline	3.3	2.12	-	-
Arginine	2.2	1.77	3.43	3.57	Cystine	0.1	0.31	0.73	0.52
Threonine	0.6	1.41	1.44	2.57					
Tryptophan	0.2	0.59	0.49	0.7					

The fatty acid composition of BSFL grease

Fatty acids	Retention time (min)	Number of carbon and unsaturated bond	Relative percentage (%)
Capric acid	5.79	C10:0	3.11
Lauric acid	7.32	C12:0	35.64
Myristic acid	8.78	C14:1	7.61
Pentadecanoic acid	9.67	C15:0	1.03
Palmitoleic acid	11.24	C16:1	3.77
Palmitic acid	11.65	C16:0	14.84
Oleic acid	15.50	C18:1	23.62
Stearic acid	16.10	C18:0	3.55
Nonadecanoic acid	17.97	C19:1	1.43
Docosenoic acid	19.93	C22:1	1.37

Laying hen feeding 蛋鸡

Effects of larvae-residue mixture on production performance of Hy-line brown (Mean ± SE).

Parameters	Levels of larvae-residue mixture in the diet, %			
	0	5	15	25
Hen-day production, %	83.2 ± 3.23a	85.7 ± 2.92a	83.7 ± 2.35a	73.8 ± 1.82b
Egg weight, g	62.2 ± 0.54a	63.8 ± 0.52b	63.5 ± 0.35b	65.3 ± 0.49c
Feed intake, g / bird / day	134.1 ± 1.68a	136.0 ± 2.21a	133.7 ± 4.56a	121.8 ± 2.96b
Kg Feed / Kg eggs	2.6 ± 0.12	2.5 ± 0.07	2.6 ± 0.03	2.6 ± 0.10

a, b, c means along the same row with different superscripts differ significantly ($p<0.05$).

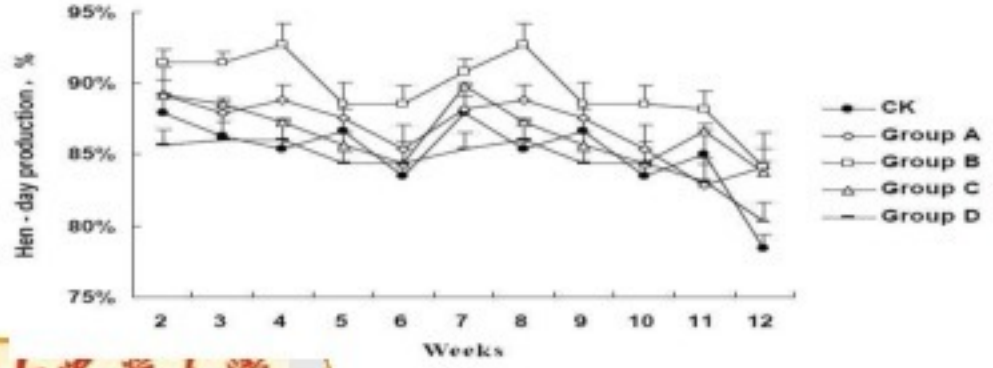
➤ Egg weight is higher

Effect of larvae-residue mixture on egg equality (Mean ± SE).

Parameters	Level of larvae-residue mixture in the diets, %			
	0	5	15	25
Yolk weight, g	15.64 ± 0.31a	16.61 ± 0.23b	15.97 ± 0.17ab	16.15 ± 0.17ab
Haugh unit	85.49 ± 1.12	84.34 ± 1.49	84.44 ± 1.04	84.27 ± 1.54
Albumen weight, g	37.59 ± 0.55a	38.54 ± 0.57a	38.55 ± 0.42ab	40.13 ± 0.51b
Egg shape index, length / width	1.29 ± 0.06a	1.28 ± 0.01b	1.28 ± 0.00ab	1.27 ± 0.01b
Albumen height, mm	7.41 ± 0.19	7.38 ± 0.23	7.35 ± 0.16	7.44 ± 0.22
Egg shell thickness, mm	0.35 ± 0.00a	0.38 ± 0.01b	0.36 ± 0.01a	0.36 ± 0.01ab

a, b means along the same row with different superscripts differ significantly ($p<0.05$).

➤Yolk and albumen weight got higher
➤Thicker egg shell
➤Egg shape index decreased



➤At 15% ration, hen-day egg production increased.

Broiler chicken feeding 肉鸡

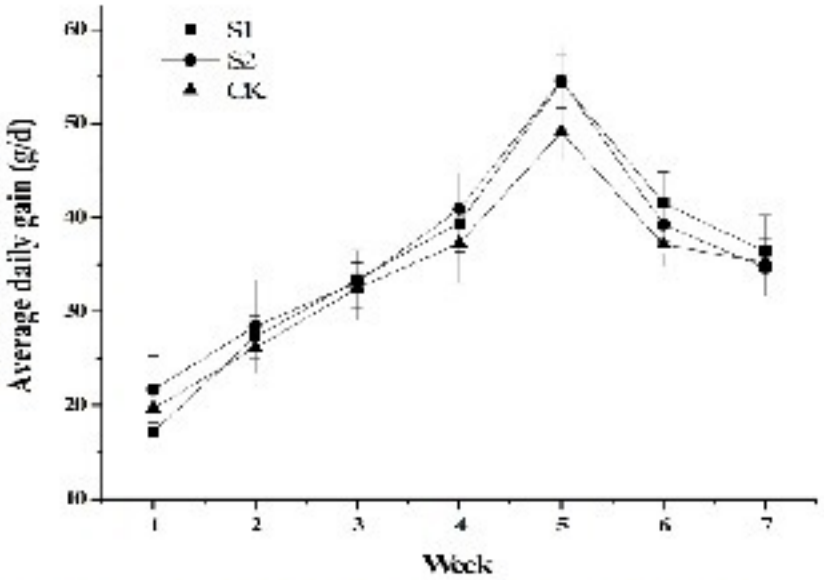


Fig. The average daily gain curves of the Broiler

- At 12% substitution rate, chicken weight gain rate was accelerated;
- After feeding of 52 days, each chicken weighed 102 g more than the control on average.

水虻虫粉替代鱼粉对黄颡鱼生长性能的影响

(Growth effects of fish meal substitution with BSFL powder on Yellow-head catfish)

Testing items	Fish meal replacement ratio							
	Control	13%	25%	29.12%	48%	68%	85%	100%
Weight gain rate（%）	668.99 ±70.18b	836.18 ±47.40a	863.77 ±17.63a	824.29	813.85 ±45.01a	633.52 ±47.17bc	533.05 ±32.13c	300.35 ±32.28d
specific growth rate（%/d）	3.13 ±0.14b	3.44 ±0.08a	3.48 ±0.03a	11.18% 3.42	3.41 ±0.08a	3.07 ±0.10bc	2.84 ±0.08c	2.13 ±0.13d
Feed coefficient	1.08 ±0.07ab	0.90 ±0.04a	0.89 ±0.03a	21.35% 0.91	0.93 ±0.04a	1.08 ±0.09ab	1.19 ±0.05b	1.66 ±0.16c
Protein Efficiency Ratio（%）	214.34 ±13.76b	252.23 ±10.08a	258.60 ±9.33a	20.56%	250.38 ±9.61a	214.21 ±18.64b	192.10 ±7.63b	139.20 ±13.13c
Protein deposition rate(%)	115.41 ±5.92cd	135.22 ±5.60ab	136.32 ±5.84a	18.12% ±0.76ab	129.16 ±2.04abc	119.32 ±9.80bcd	106.59 ±3.74d	80.79 ±7.73e
Survival rate（%）	98.89 ±1.92a	100 ±0.00a	100 ±0.00a	98.89 ±1.92a	100 ±0.00a	98.89 ±1.92a	100 ±0.00a	100 ±0.00a

水虻虫粉替代鱼粉对黄颡鱼免疫性能的影响

(Effects of fish meal Substitution with BSFL powder on immune indexes of Yellow-head catfish)

Testing items	Fish meal replacement ratio							
	Control	13%	25%	[illegible]	48%	68%	85%	100%
(Lysozyme in blood) (U/mL)	22.57 ±2.45ab	29.67 ±1.97a	29.77 ±7.82a	[illegible]	[illegible] ±4.61ab	21.59 ±1.26ab	22.98 ±0.89ab	19.36 ±2.12b
SOD in blood (U/mL)	22.76 ±4.73b	28.19 ±7.17ab	32.45 ±7.77a	[illegible]	24.18 [illegible] 4.36b	22.60 ±3.81b	22.35 ±8.75b	22.06 ±5.52b
SOD in liver-pancreas (U/mgprot)	141.03 ±18.65a	143.46 ±38.01a	145.11 ±20.20a	[illegible]	[illegible] ±41.42a	140.86 ±15.21a	134.76 ±19.00a	130.99 ±18.08a
Leukocyte phagocytic percentage (%)	28.33 ±0.58ab	33.67 ±2.31a	34.67 ±3.79a	[illegible]	[illegible] 0.58ab	25.00 ±2.00bc	21.67 ±3.06c	21.33 ±1.15c
Leukocyte phagocytic index	2.80 ±0.12a	2.84 ±0.09a	2.86 ±0.08a	2.83 ±0.20a	2.84 ±0.10a	2.78 ±0.03a	2.71 ±0.20a	2.55 ±0.13a

31.90%

42.47%

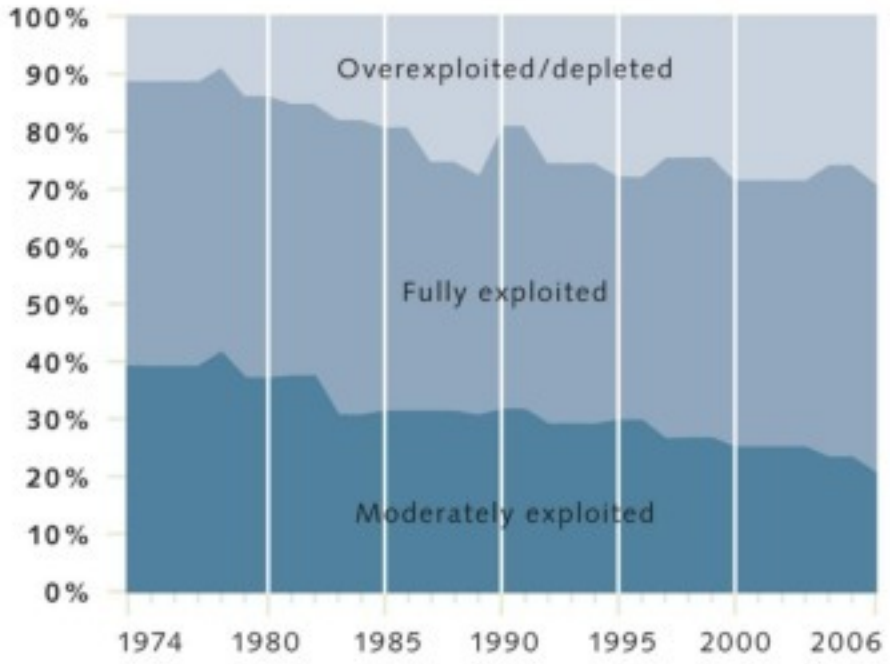
2.89%

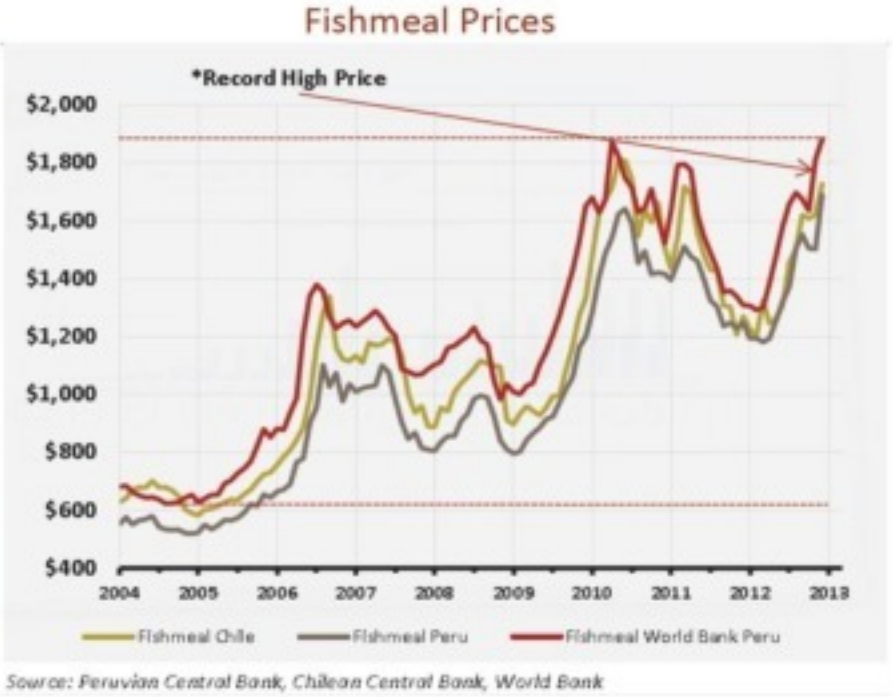
22.38%

2.14%

High Nutrition + Immune Enhancement

International Marine fisheries





- **53% of the world's fisheries are fully exploited, and 32% are overexploited**
- **Several important commercial fish populations have declined to the point where their survival is threatened**
- **Stocks of all species currently fished for food are predicted to collapse by 2048**

5 BSF biodiesel: From waste to bioenergy（生物柴油）

From organic waste to bioenergy

- black soldier fly make it feasible

Lignocellulose

Food waste

Animal manure

Comparison of fuel properties of BSFL fat-based biodiesel, rapeseed oil-based biodiesel, and the standard EN14214.

- **High fat BSF: waste to biodiesel**
- **Most of the properties of BSF biodiesel met the specifications of the EU standard EN 14214**
- **BSF grease could serve as a novel low cost biomass feedstock for biofuel**

Cetane index	48–60	55	45
Acid number (mg KOH/g)	<0.8	1.1	0.3
Methanol or ethanol (m/m)	0.2%	0.3%	n/a
Distillation (°C)	n/a	360	352

（Li Q et al, 2011； Zheng LY et al, 2013）

Promising future!!

有机废物
（organic waste）

水虻与微生物
生物转化
（Co-
cor n）

动物蛋白饲料
（Animal feedstuff）

功能微生物肥料
（Microbial fertilizer）

特殊脂肪酸
Special fatty acids

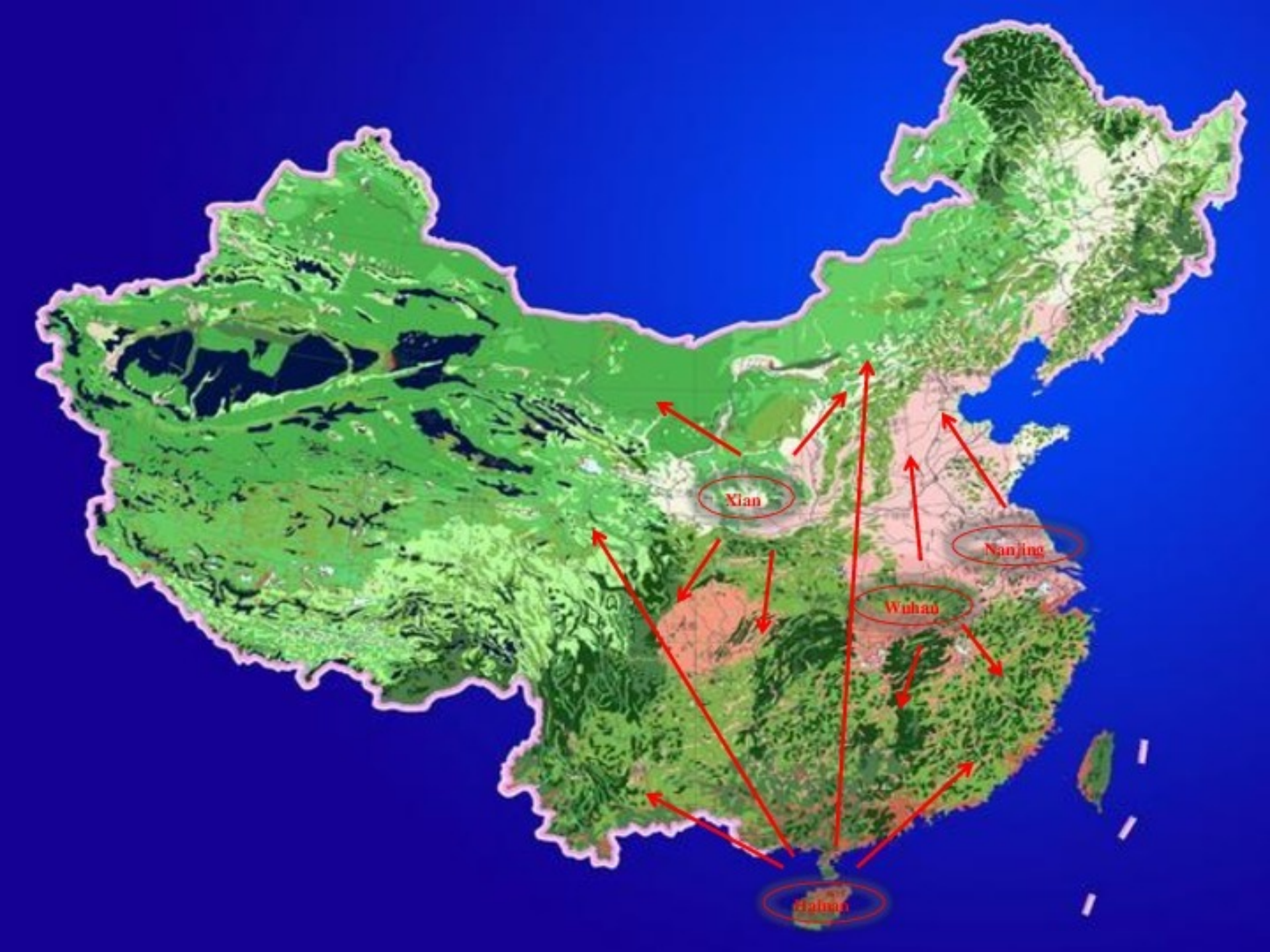
脂肪
(oil)

几丁质
(chitin)

精蛋白
(refined protein)

氨基酸产品
(Amino acids products)

壳聚糖及衍生物
Chitosan and derivative


Xian
Nanjing
Wuhan
Hainan





昆虫 – 崛起的新兴资源

Insects - Last Piece of Cake from Mother Nature





Acknowledgements

- Special Scientific Research Fund of Public Welfare Profession of China (201303094-10)
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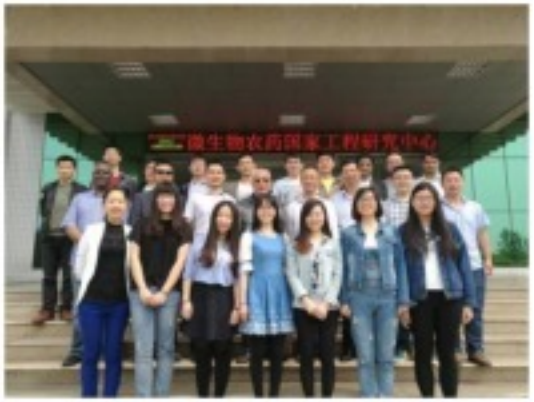
- Key Project of International Cooperation (Hubei Province) (2007-2009)



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Dr. Twani Crippen

The 2nd International Conference Insects to Feed the World

15 to 18 May 2018 Wuhan, China

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31
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Mar. 2018

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Insects not just feed the world But also heal the world!

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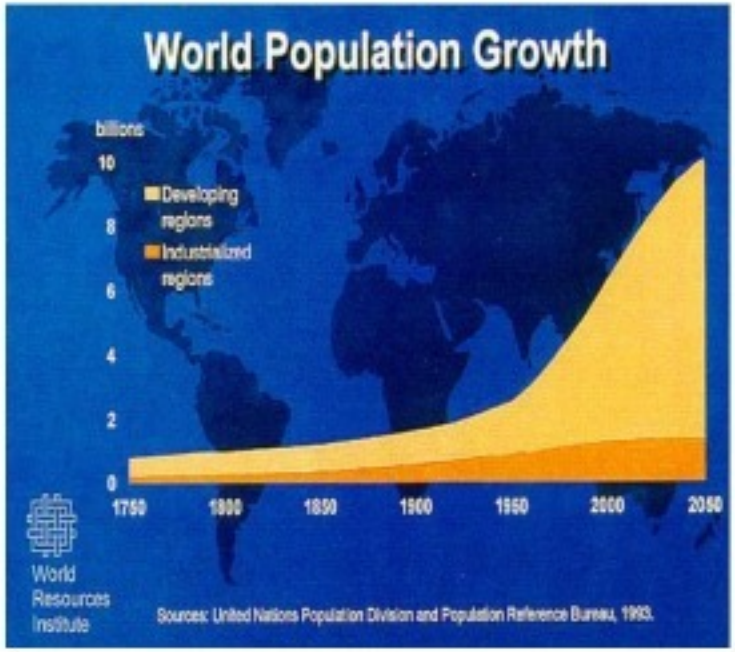
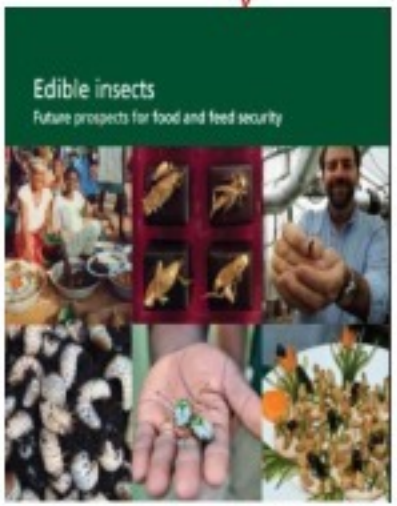
Food and feed security-Globally



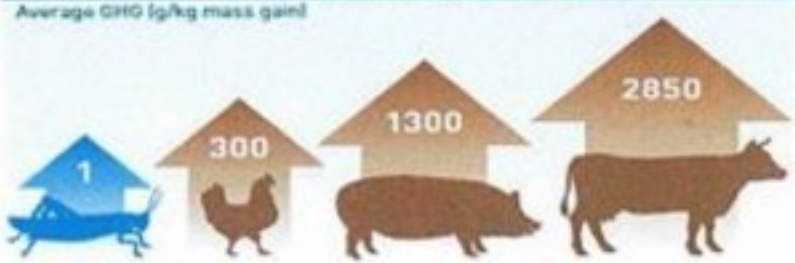
- The world will host 9 billion people by 2050
- Food production will need to almost double



ENVIRONMENTAL BENEFITS

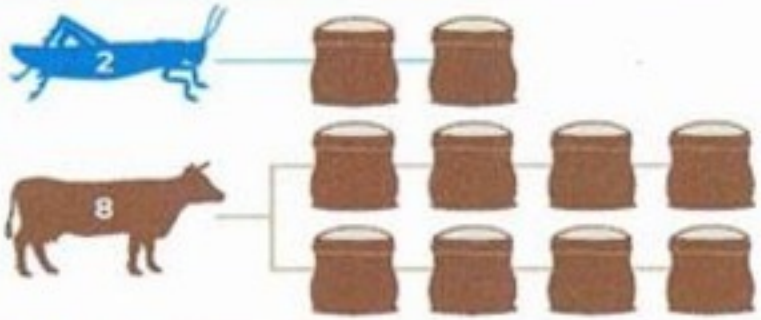
GREENHOUSE GAS PRODUCTION

Average GHG (g/kg mass gain)



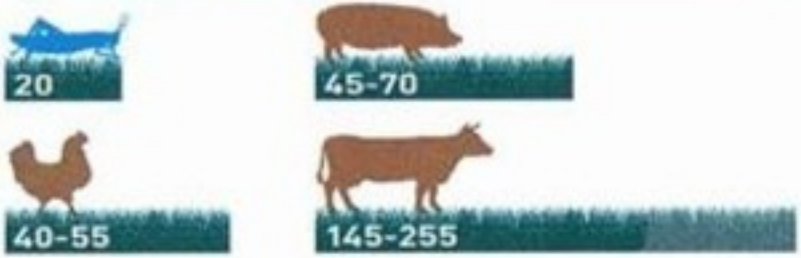
FEED CONVERSION EFFICIENCY

kg of feed required to produce 1kg of edible weight



LAND USE

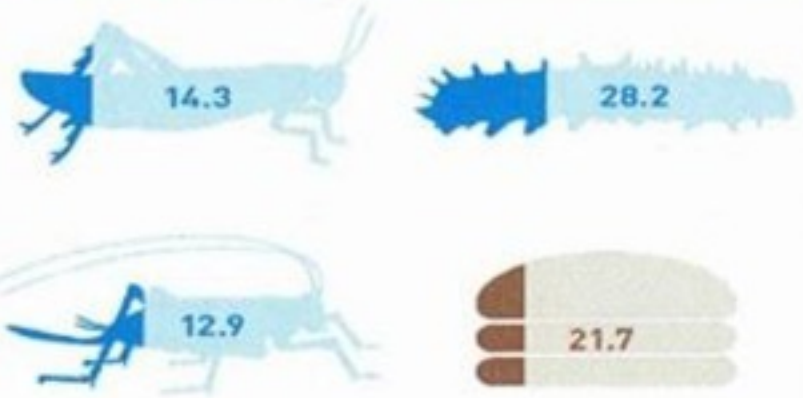
Land use m^2 for 1 kg of protein



HEALTH BENEFITS

PROTEIN CONTENT

Protein content (g/100 g fresh weight)



CALCIUM CONTENT

Calcium content (mg/100 g fresh weight)