Biotechnology processing to produce high-quality single cell proteins from renewable biomass – an update

生物科技对可再生生物质转化为优质单细胞蛋白的进展评估

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Chinese Swine Industry Symposium (CSIS), Shanghai, China, 2018

中国猪营养国际论坛，上海，2018




Outline 纲要

- Food security and need for sustainable feeds
  粮食安全和对可持续饲料的需求
- Opportunities and challenges in the Aquaculture industry
  在水产养殖行业的机遇与挑战
- Development of salmon feed
  鲑鱼饲料的发展
- Microbial feed resources
  微生物饲料资源
- Biotechnology processing of protein from natural gas
  利用天然气进行生物加工生产蛋白

Outline 纲要

- Nutritional value and health beneficial effects of bacterial meal
  菌粉的营养价值和保健功效
- Biotechnology processing of novel feed resources from trees and seaweed
  利用树木和海藻进行生物加工，生产新型饲料
- Nutritional value and health beneficial effects of yeast
  酵母的营养价值和保健功效
- Future perspective
  发展前景

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sfi Centre for Research-based Innovation
The Research Council of Norway

NMBU

Three faculties at NMBU:

- Biosciences
- Chemistry, Biotechnology and Food Science
- Veterinary Medicine

挪威生命科学大学的三大学院

- 生命科学
- 化学、生物技术和食品科学
- 兽医学

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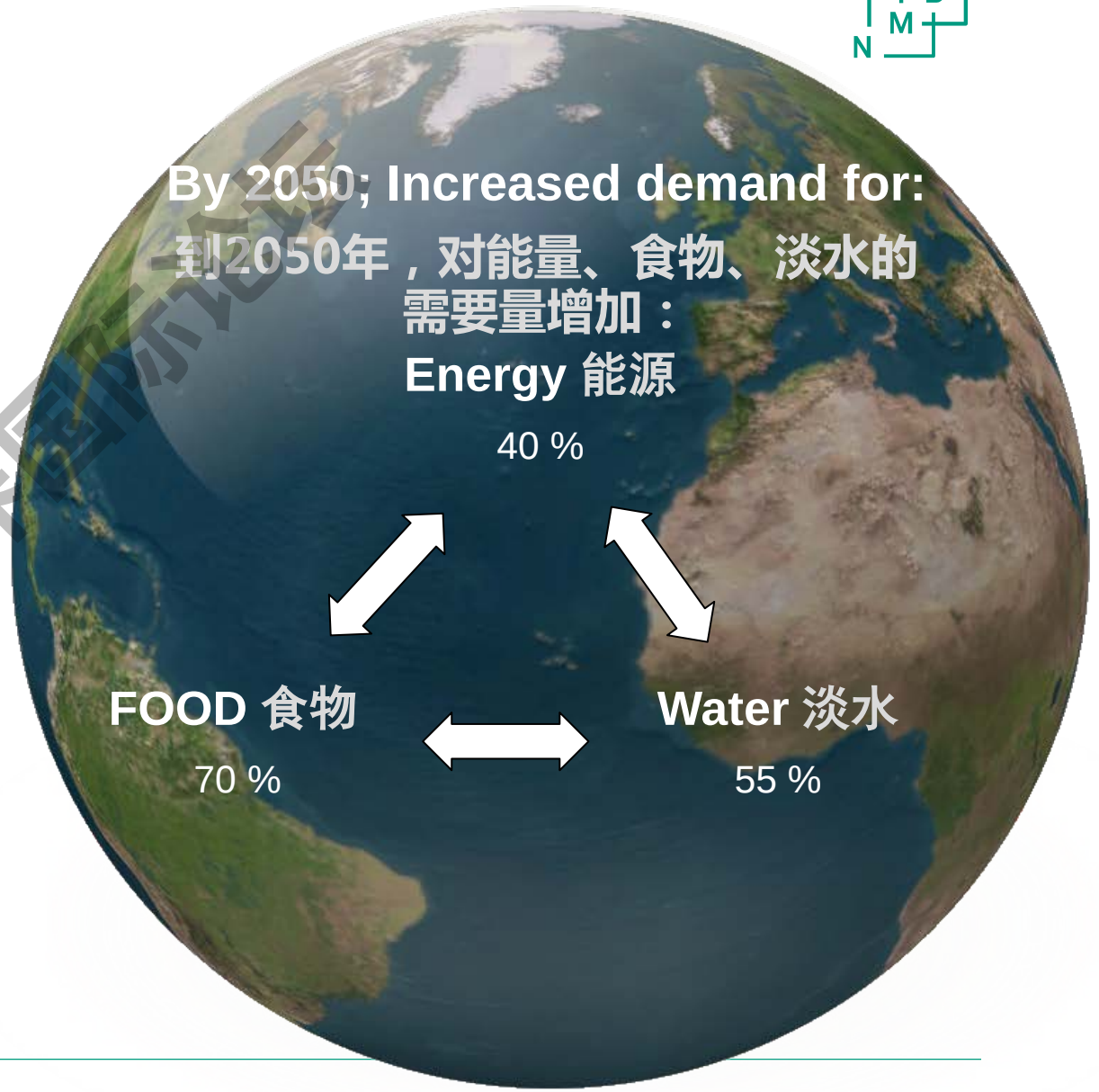
Summer in Norway 2018
2018年夏天，挪威

Food security and self-sufficiency
粮食安全&粮食自给

Challenges:

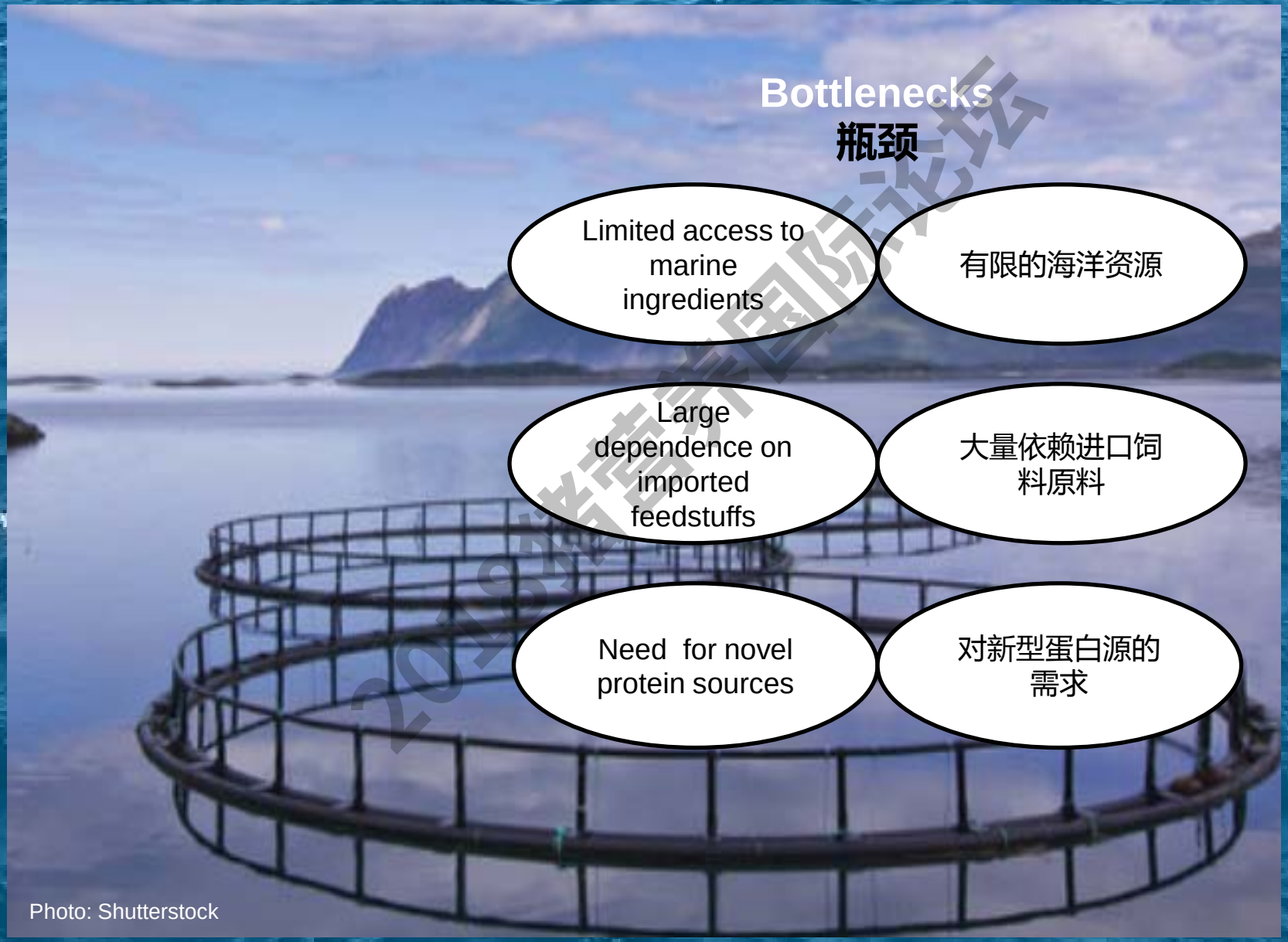
面临的挑战：

- 2050 ~9 billion people
  到2050年，90亿人口
- Climatic changes
  气候变化
- Disruption of feed supply chains
  饲料供应链中断
- Overreliance on imported feed resoruces
  过度依赖进口饲料



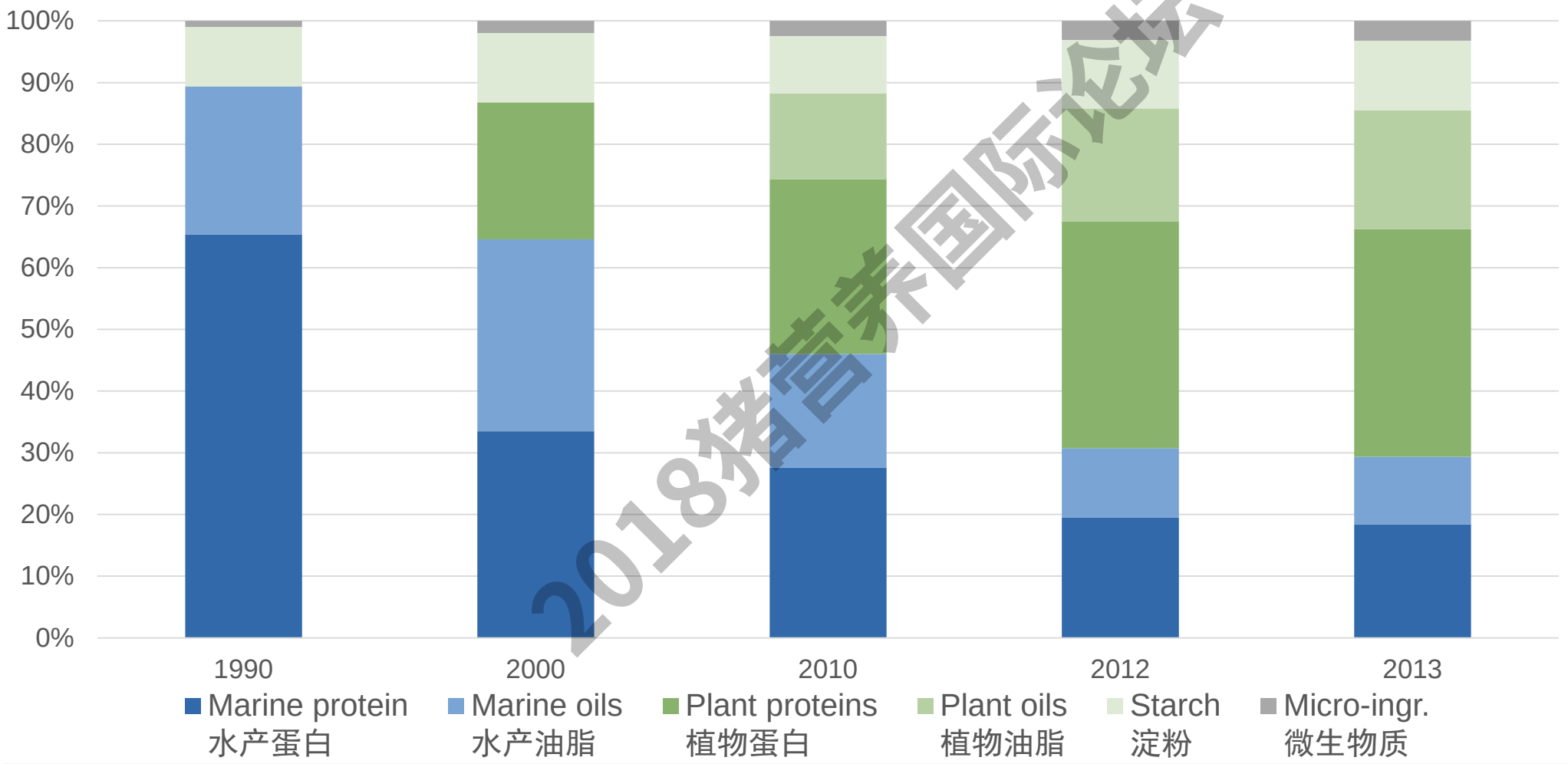
(FAO 2009; OECD 2012)

Constraints in the growth of the aquaculture industry
水产养殖业发展的限制



Development of salmon feed composition (%)

鲑鱼饲料组成的变化



Source: Ytrestøyl et al., 2015

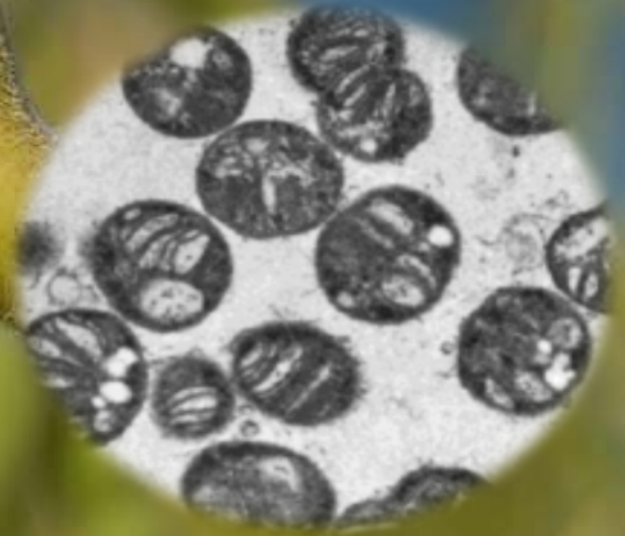
Looking ahead…展望未来...

Opportunities:
机会：

- Blue and green biomass
  蓝色和绿色的生物质资源
- Infrastructure & technology
  生产条件&专业技术
- Unique interdisciplinary research team
  独特的跨学科研发团队

Microbial feed resources 微生物饲料资源

- 500 kg soybeans produce ca. 5-10 kg protein per day.
  500kg大豆种子生产的蛋白质约5-10kg/天。
- 500 kg yeast cells produce ca. 50 tonnes protein per day.
  500kg酵母菌种可以生产的蛋白质约50吨/天。



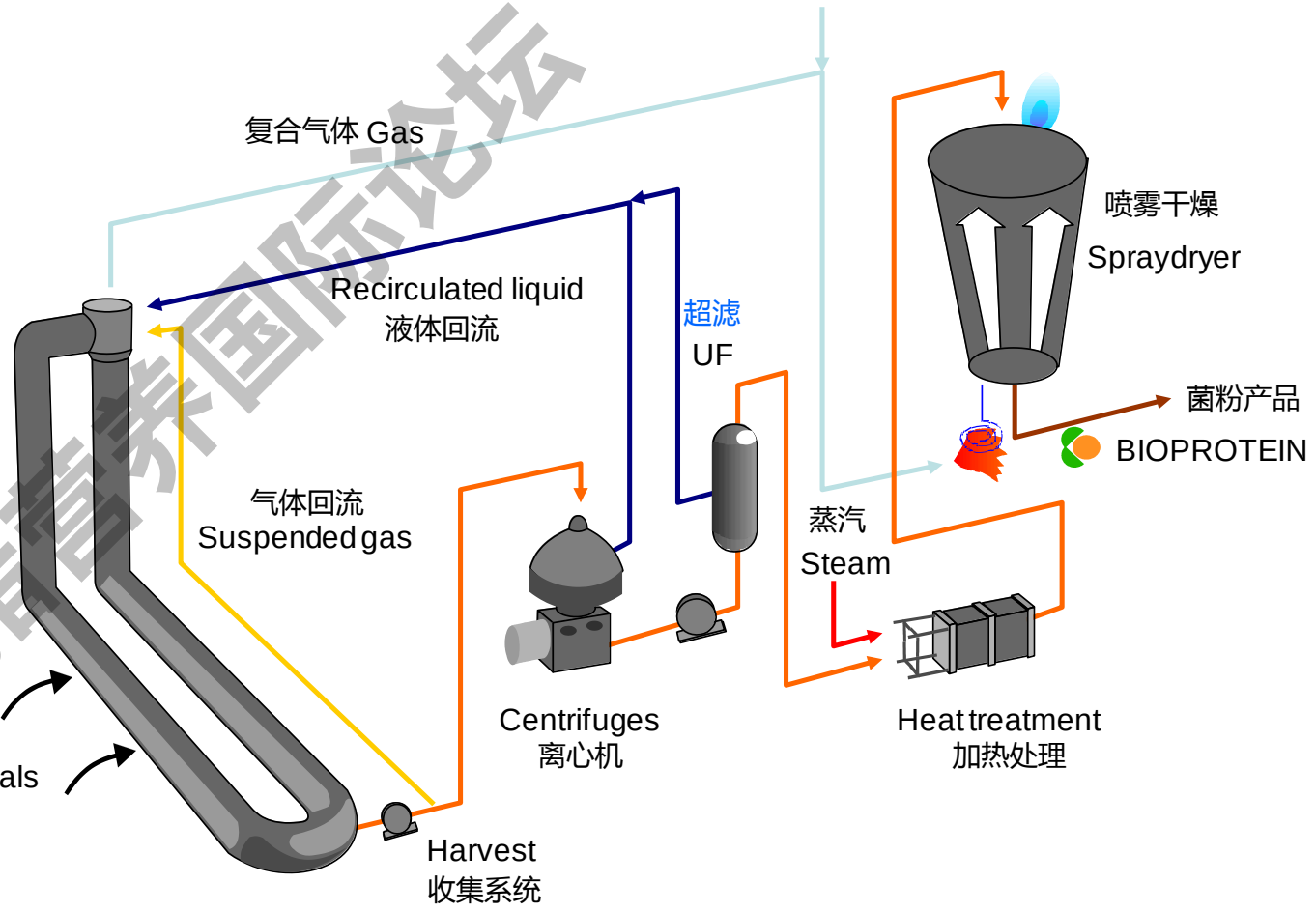
Production of protein from natural gas
利用天然气生产蛋白质的加工工艺

Natural gas
天然气

Oxygen
氧气

Ammonia
氨气

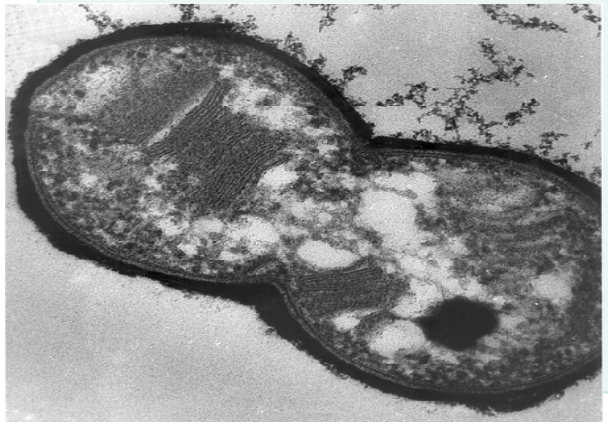
Minerals
矿物质



Methylococcus capsulatus 荚膜甲基球菌

Bacterial meal 菌粉

Methylococcus capsulatus 荚膜甲基球菌



- Methanotroph bacteria （甲烷氧化菌）
- High protein content （高蛋白含量）

Crude protein（粗蛋白）	70%
Fat （脂肪）	10%
Carbohydrates（糖类）	12%
Ash （灰分）	7%

Other traits 其它性状：

- favorable AA composition 良好的氨基酸组成
- 10% nucleic acids 核酸含量10%
- Bioactive components, e.g. 生物活性成分，例如

  Coenzyme Q8 辅酶Q8
  Methanobactin 甲烷氧化菌素
  Mop E

Production efficiency 生产效率:

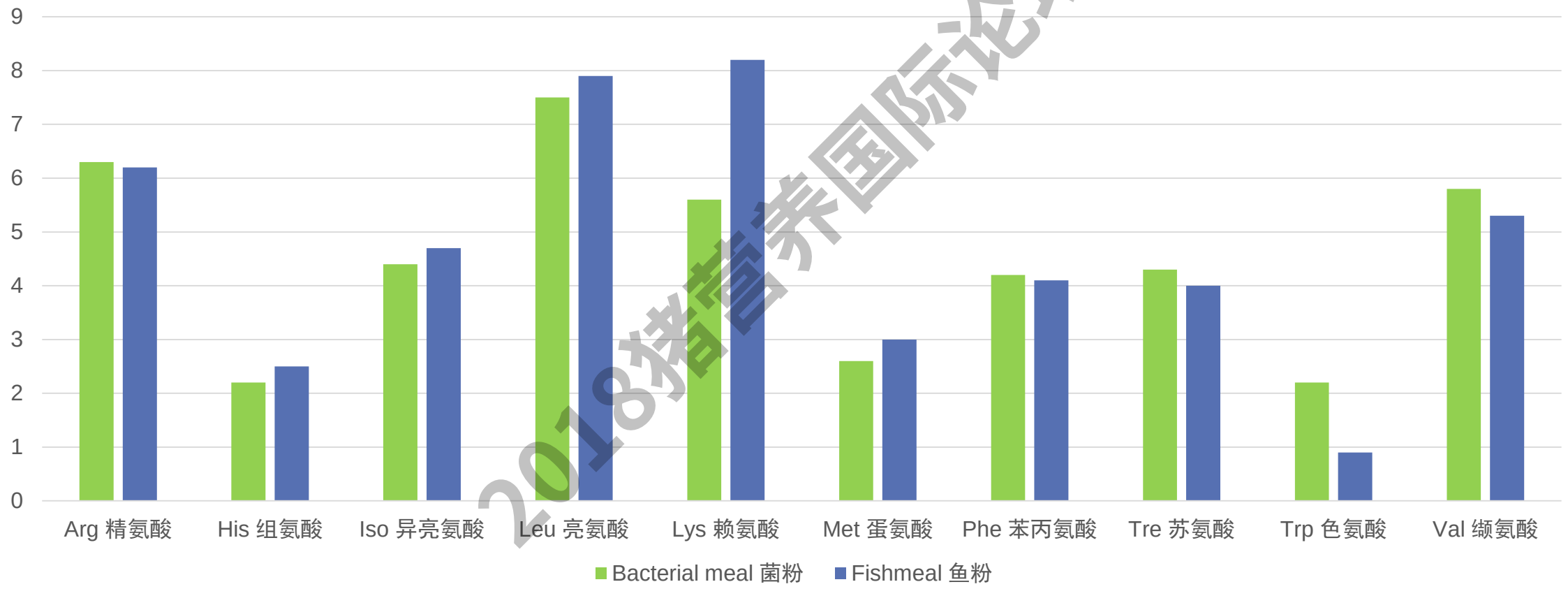
- 1.7 kg methane gas per kg BM biomass
  生产每公斤菌粉需要1.7kg甲烷气体
- 2.0 m^3 methane per kg crude protein

生产每公斤粗蛋白需要2.0m^3甲烷气体

Source: Øverland et al., 2010, Review in Archives of Anim. Nutr

The content of essential AA (g/16 g N) in bacterial meal in comparison to fishmeal

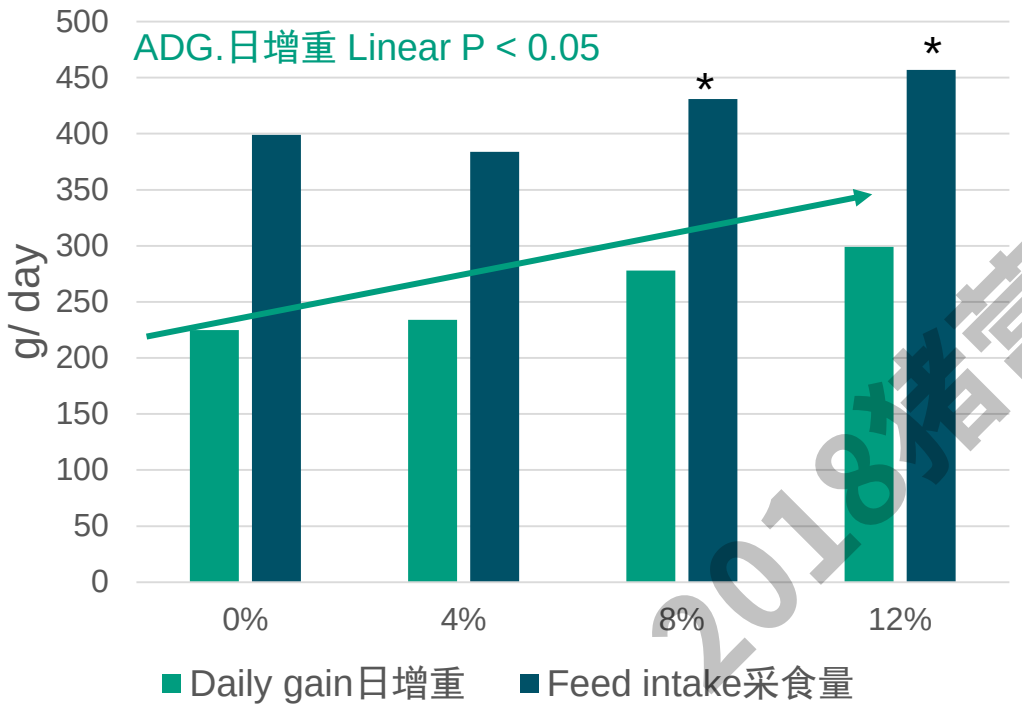
菌粉和鱼粉的必需氨基酸(g/16g 氮)组成的比较



Source: Øverland et al. 2010 Review in Archives Anim Nutr.

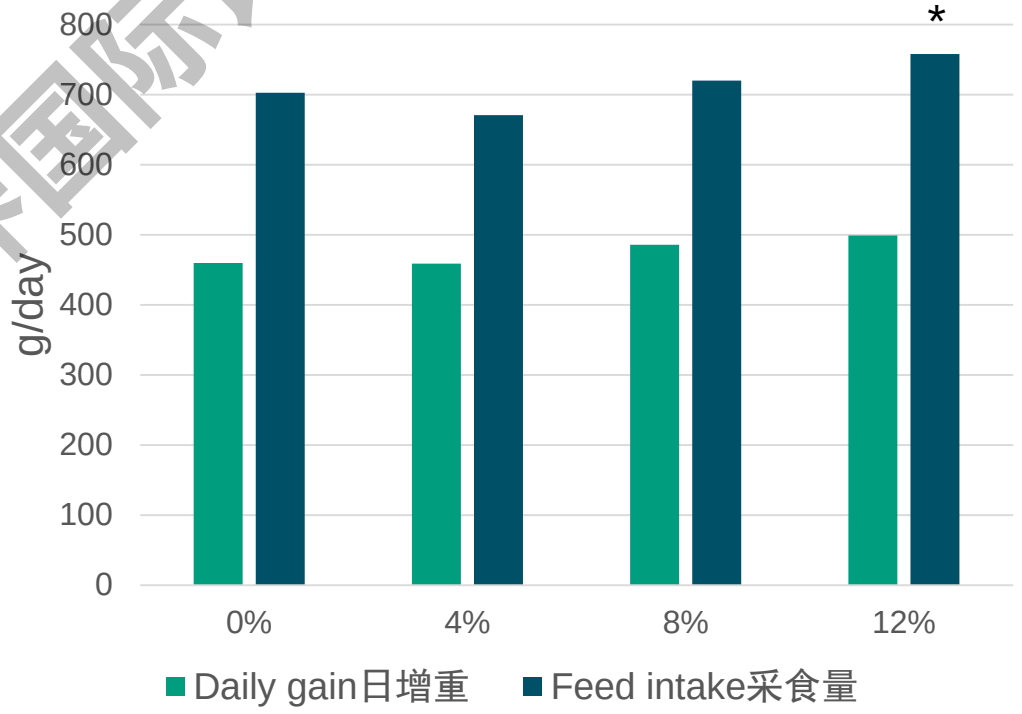
Bacterial meal in piglet diets 菌粉在仔猪料中的效果
—Effect on growth performance ——生长性能效果

Week 0-2 周



Bacterial meal level (%)
菌粉添加量（%）

Week 0-4 周



Bacterial meal level (%)
菌粉添加量（%）

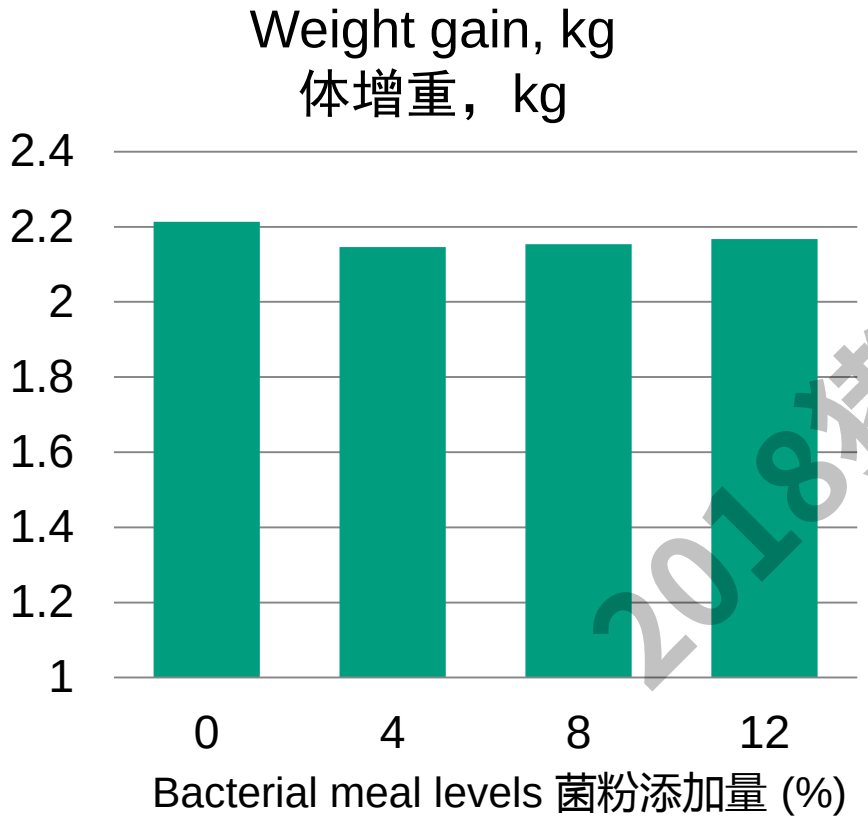
Source: Øverland et al. 2010 Review in Archives Anim Nutr.

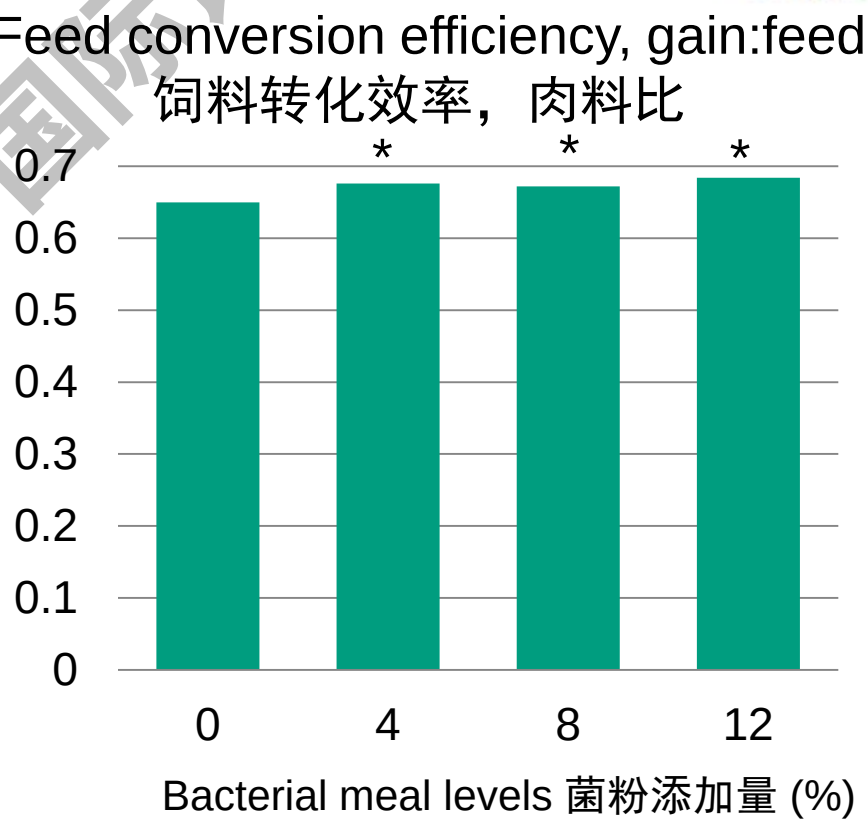
Bacterial meal in diets for broilers (day 0-36)

partial replacement of soybean meal

菌粉在肉鸡料（0-36天）中的效率

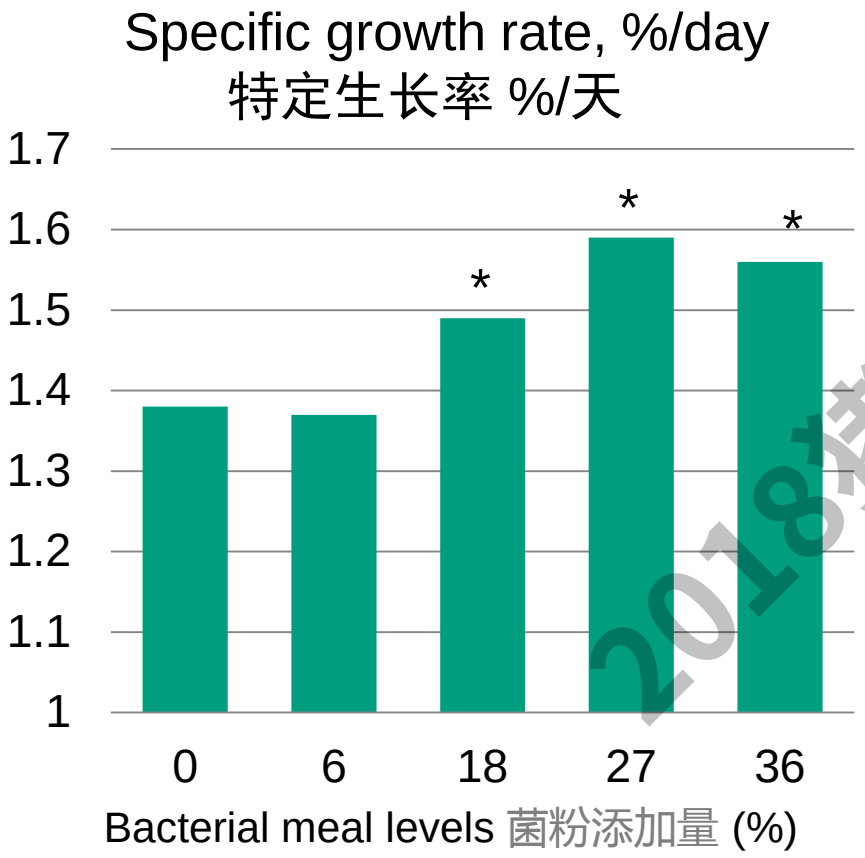
菌粉部分替代豆粕

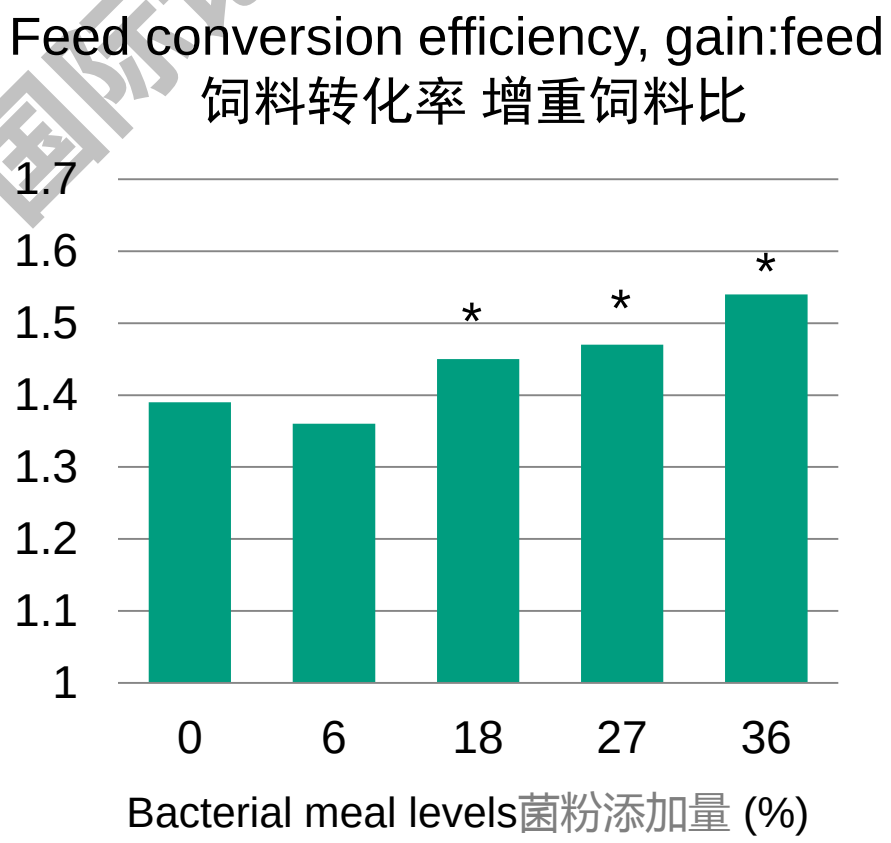




Source: Øverland et al. 2010, Br. J. Poultry Sci

Growth rate and feed efficiency of salmon fed increasing levels of bacterial meal
菌粉对鲑鱼增长率和饲料转换率的影响



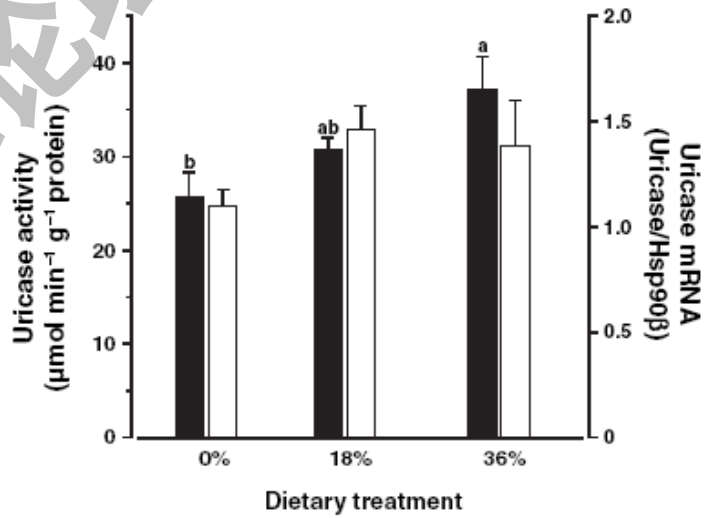


Source: Aas et al. 2006, Aquaculture; Øverland et al., 2010, Review, Archives Animal Nutr.

Purine metabolism
嘌呤的新陈代谢

Uricase oxidase
尿酸氧化酶

- No problem in salmon
  对鲑鱼没有影响
- No problem in pigs
  对猪没有影响
- Some problem in birds
  对禽类有一定影响
- Gout in primates
  对灵长目动物有痛风风险



Atlantic salmon: 大西洋鲑鱼

- Liver uricase oxidase activity increased
  肝脏中尿酸氧化酶活性增加
- Liver expression of uricase oxidase was up-regulated
  肝脏中尿酸氧化酶活性上调

Source: Andersen et al., 2006; Hellwing et al., 2007; Øverland et al., 2010, Archives Anim Nutr.

Effect of bacterial meal on fish health
菌粉对鱼健康的影响

Bacterial meal strengthens the gut barrier function and prevents inflammation in the gut
菌粉增强了肠道屏障功能，并防止肠道内的炎症

The soybean meal challenge model 豆粕攻毒模型

High levels of soybean meal in diets for Atlantic salmon leads to a inflammation (enteritis) in the distal intestine

大西洋鲑鱼饲料中，大量的使用豆粕会导致后肠道内的炎症

Source: Romarheim et al. 2011, J Nutr; Romarheim et al. 2013 Br. J Nutr; Romarheim et al., 2013, Aquaculture

Bacterial meal prevents inflammation in the gut 菌粉可以预防肠炎
Results from immunohistochemistry 免疫组织化学染色法结果

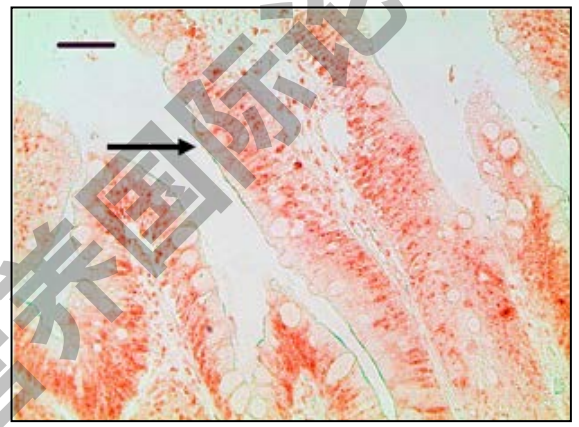
PCNA
增殖细胞核抗原

Carbonic acid 12
碳酸12

CD8α⁺ T lymphocytes
CD8α⁺ T淋巴细胞

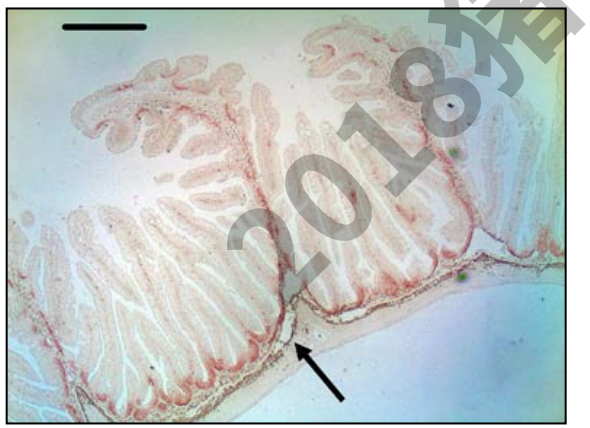
Inflammed intestine
SBM diet
肠炎

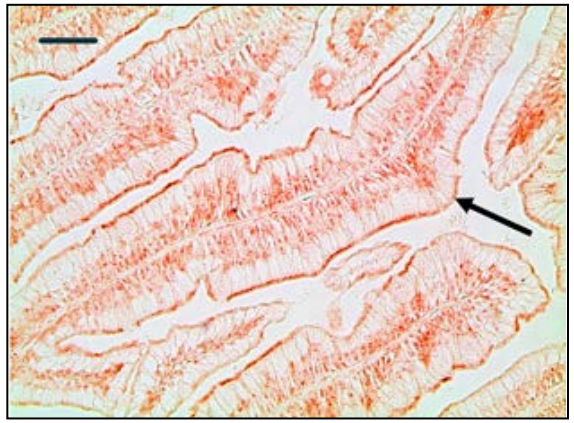
豆粕日粮



Healthy intestine
SBM & BM diet
健康的肠道

豆粕&菌粉饲料

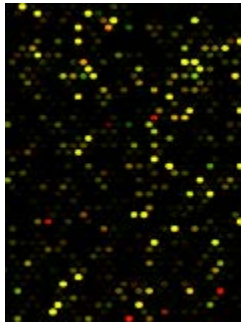




Source: Romarheim et al. 2011; J Nutrition

BM prevents inflammation in the distal intestine 菌粉预防小肠后端炎症
Results from transcriptomics 基因转录结果

Microarray chip 微阵列芯片
Hybridisation 混合淡化技术

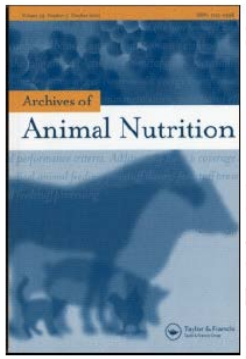


Gene expression profiling by 21K microarray of genes related to gut barrier function and health
由21K基因微阵列组成的基因表达谱可以用于显示肠道屏障功能和肠道健康

	Fish meal 鱼粉						Bacterial meal 菌粉						BM & Soy 菌粉&豆粕						Soybean meal 豆粕				
	BPMSBM_I1	BPMSBM_I3	BPMSBM_V1	BPMSBM_V2	BPMSBM_V3	BPMSBM_XII2	BPM_I1	BPM_I2	BPM_VI1	BPM_VI2	BPM_XXII1	BPM_XXII3	FM_III1	FM_III2	FM_VIII1	FM_VIII2	FM_XXI2	FM_XXI3	SBM_IV1	SBM_IV2	SBM_VII2	SBM_VII4	SBM_XI1
E3 ubiquitin-protein ligase LINCR	-0,79	-0,81	-1,05	-1,15	-0,49	-1,77	-0,78	-1,39	-0,80	-1,15	-1,18	-1,80	-0,88	-1,19	-1,72	-1,64	-1,02	-2,03	3,68	4,24	4,43	4,34	4,95
TNF decoy receptor	-0,38	0,75	-0,19	-0,77	0,52	-0,10	-0,55	0,17	0,22	-0,72	0,78	0,32	-0,90	-1,05	-0,29	-0,88	-0,97	-0,59	0,29	1,42	0,90	1,10	0,92
Arginase-2, mitochondrial precursor	-0,16	-0,78	-0,91	-0,90	-0,78	-1,16	-0,82	-0,19	-0,54	-0,54	-0,64	-0,86	-0,50	-0,81	-0,37	-1,22	-0,82	-0,81	2,13	3,13	2,68	2,96	1,91
Annexin A1	-0,38	-2,91	-0,46	-0,16	-0,07	-1,72	-0,52	0,52	0,38	0,25	-1,77	-0,24	-0,86	-1,84	0,66	-3,19	-0,61	-0,11	2,50	2,59	3,24	2,34	2,39
non-specific cytotoxic cell receptor protein-1	-0,05	0,02	-0,19	-0,40	0,10	-0,07	-0,24	-0,07	-0,29	-0,46	-0,25	-0,31	-0,56	-0,36	-0,41	-0,48	-0,61	-0,26	0,76	1,04	0,95	1,02	1,12
Transcription factor AP-1	0,09	-0,01	0,57	-0,32	-0,32	0,18	-0,41	-0,16	-0,54	-0,50	-0,34	-0,53	-0,16	-0,24	-0,31	-0,63	-0,29	-0,11	1,09	-0,02	1,19	0,75	1,01
Cysteine dioxygenase type 1	1,27	0,12	0,81	1,72	-1,93	2,41	0,28	0,96	-0,12	1,33	1,10	1,38	2,56	2,03	1,58	1,47	2,28	1,81	-3,53	-4,90	-3,96	-4,30	-4,36
Fatty acid-binding protein, intestinal	1,05	0,69	0,65	0,99	0,64	2,18	0,44	0,88	0,27	0,63	-0,06	0,23	1,67	1,04	0,53	0,42	1,52	1,25	-1,79	-2,81	-4,24	-1,64	-4,53
Apolipoprotein A-IV precursor	0,72	0,02	0,28	1,31	0,76	2,20	0,51	1,03	0,75	1,45	-0,12	0,53	1,70	1,15	0,83	1,21	1,48	1,72	-2,25	-3,15	-4,40	-4,60	-3,11
epidermal retinal dehydrogenase 2	0,21	0,08	0,22	0,09	1,22	0,10	0,56	0,56	0,45	1,20	1,27	1,19	-0,95	1,01	0,58	1,49	0,67	-0,23	-2,93	-0,58	-2,12	-1,77	-2,30
H-2 class I histocompatibility antigen, LDa chain	0,96	1,31	-0,27	1,46	0,79	1,92	1,55	1,81	1,33	2,11	1,68	2,44	1,57	1,31	0,52	1,83	0,46	0,92	-5,17	-2,13	-5,37	-4,88	-6,16

Source: Skugur et al., 2018, Reveco-Urzua et al., 2018, manuscripts in prep

Bacterial meal from natural gas
利用天然气生产菌粉



Archives of Animal Nutrition
Vol. 64, No. 3, June 2010, 171–189

Taylor & Francis
Taylor & Francis Group

REVIEW

Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals

Margareth Øverland[a]*, Anne-Helene Tauson[a,b], Karl Shearer[a] and Anders Skrede[a]

甲烷为原料生产的菌粉作为单胃动物饲料成分的评估

- **Nutritional aspect 营养价值**
  - High quality protein source 高品质蛋白源
  - No health risk 没有健康风险
- **Pigs 猪**
  - High growth rate and feed efficiency
    生长率和饲料利用率高
  - Positive effect on product quality
- **提高产品质量**
- **Broiler chickens 肉鸡**
  - High growth rate and feed efficiency
    生长率和饲料利用率高
  - Positive effect on product quality
- **提高产品质量**
- **Salmonids 鲑鱼**
  - High growth rate and feed efficiency
    生长率和饲料利用率高
  - Positive effect on product quality
    提高产品质量
- **2009: EU approval (Regulation (EC) No 767/2009)**
  2009：欧盟认证（No 767/2009)

Biomass from the ocean 海洋生物

Harvesting 捕捞

Cultivation 养殖

Ongoing research in Foods of Norway
挪威食品公司正在进行的研发工作





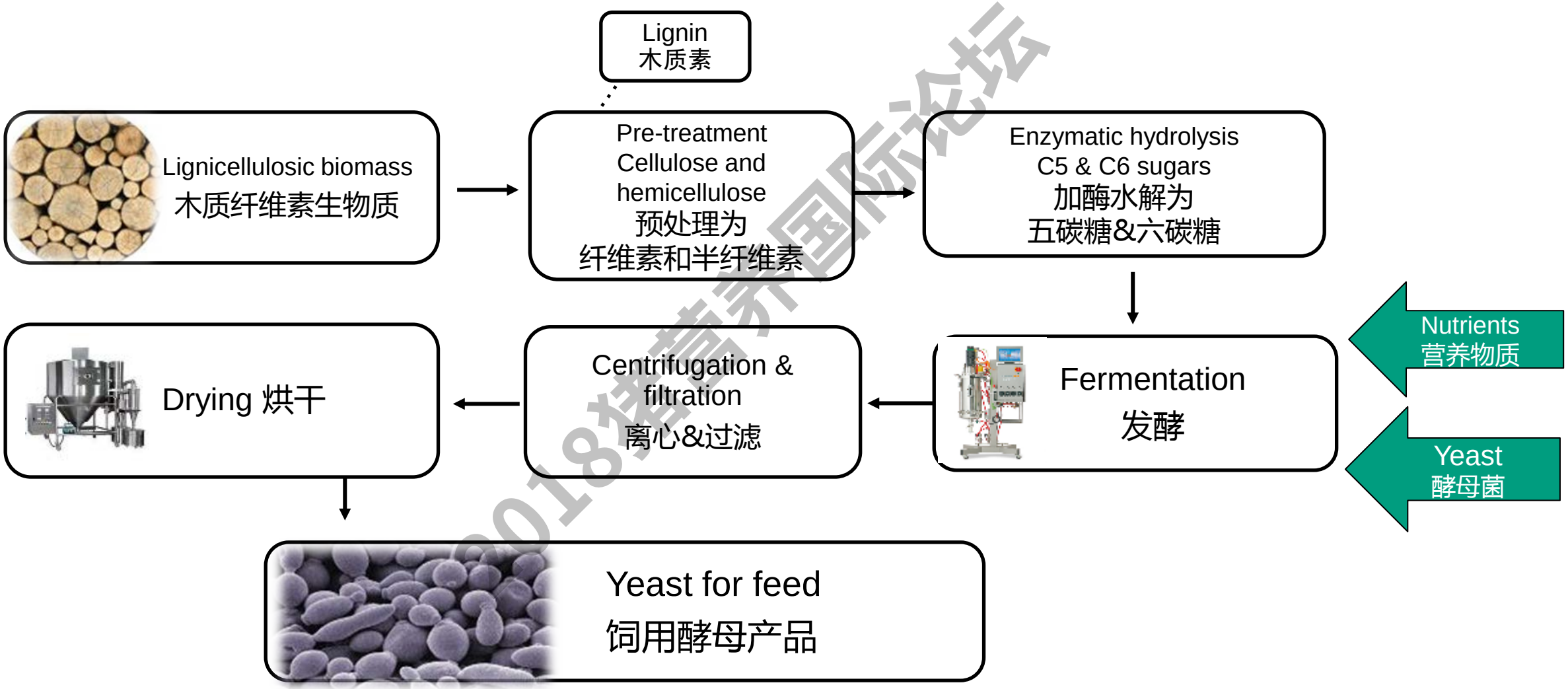




rees as a feed resource 树木作为饲料原料

Norwegian forest is our largest bioresource
挪威的森林是我们最大的生物质资源

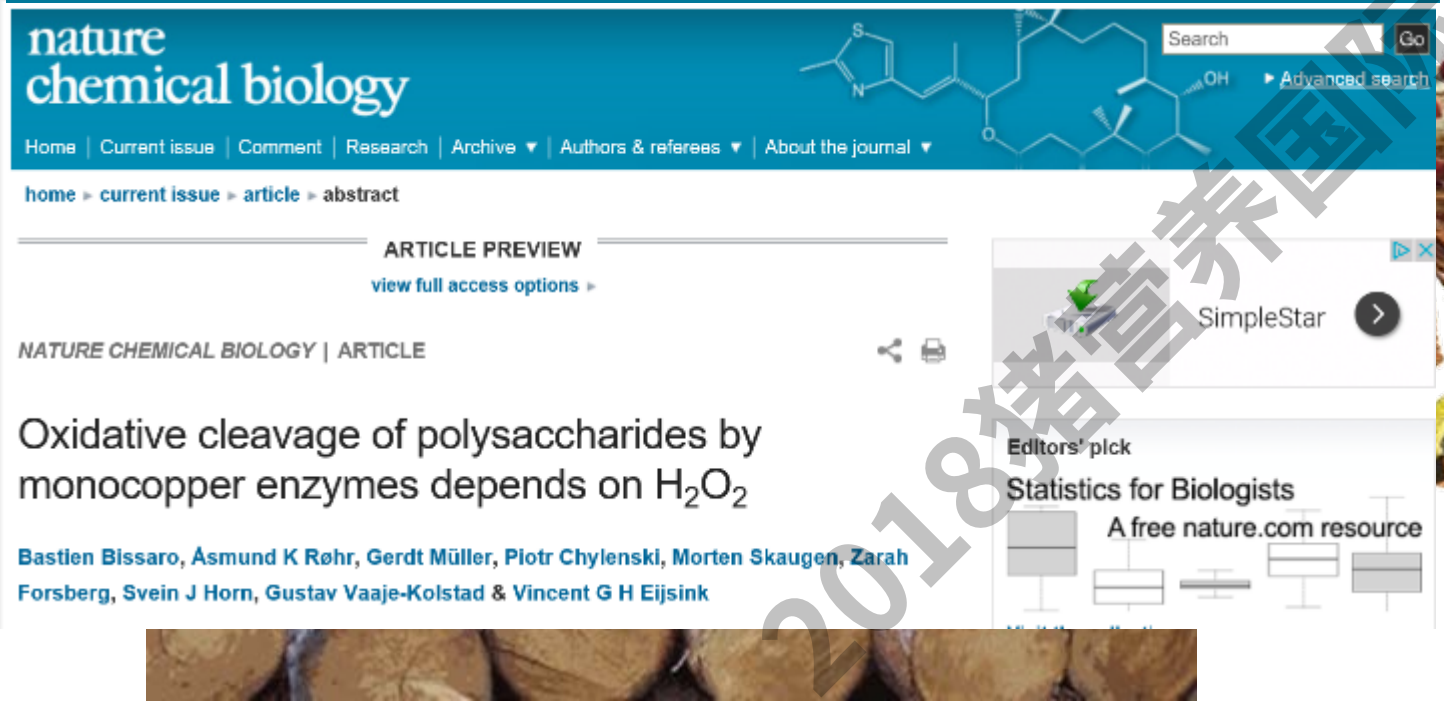
Flow chart of yeast production from lignocellulosic biomass
木质纤维素生物质生产酵母的流程图



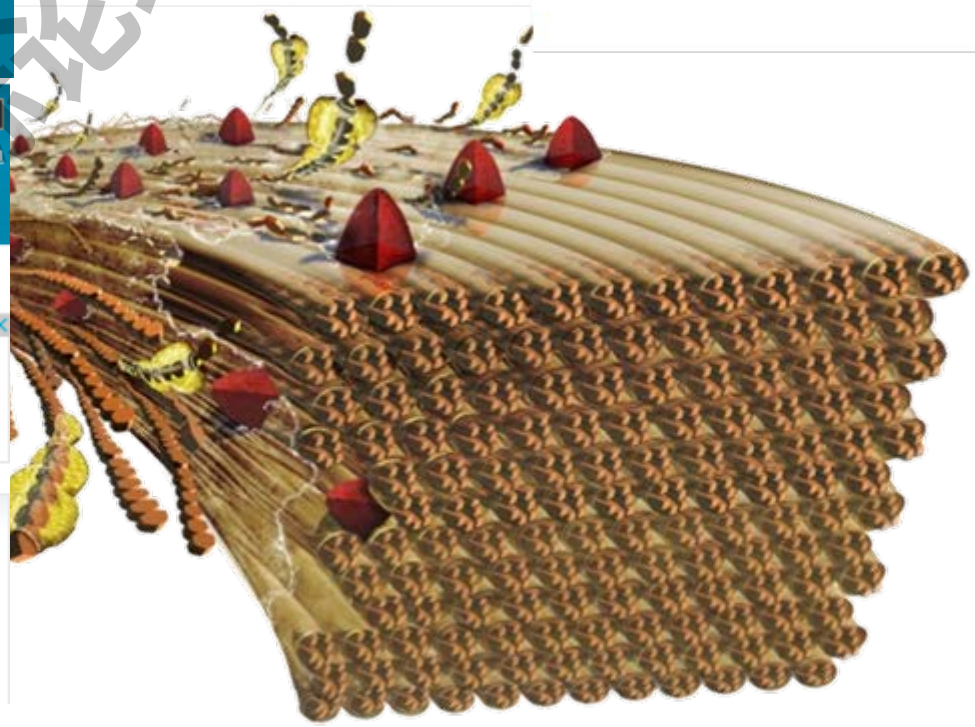
Source: Øverland & Skrede 2016, J. Sci. of Foods and Agriculture

New enzymes make the green resources available
一种新的酶制剂使得该绿色资源可以被利用

New discovery – to improve enzyme efficiency at NMBU
重大发现—挪威生命科学大学提高酶解效率



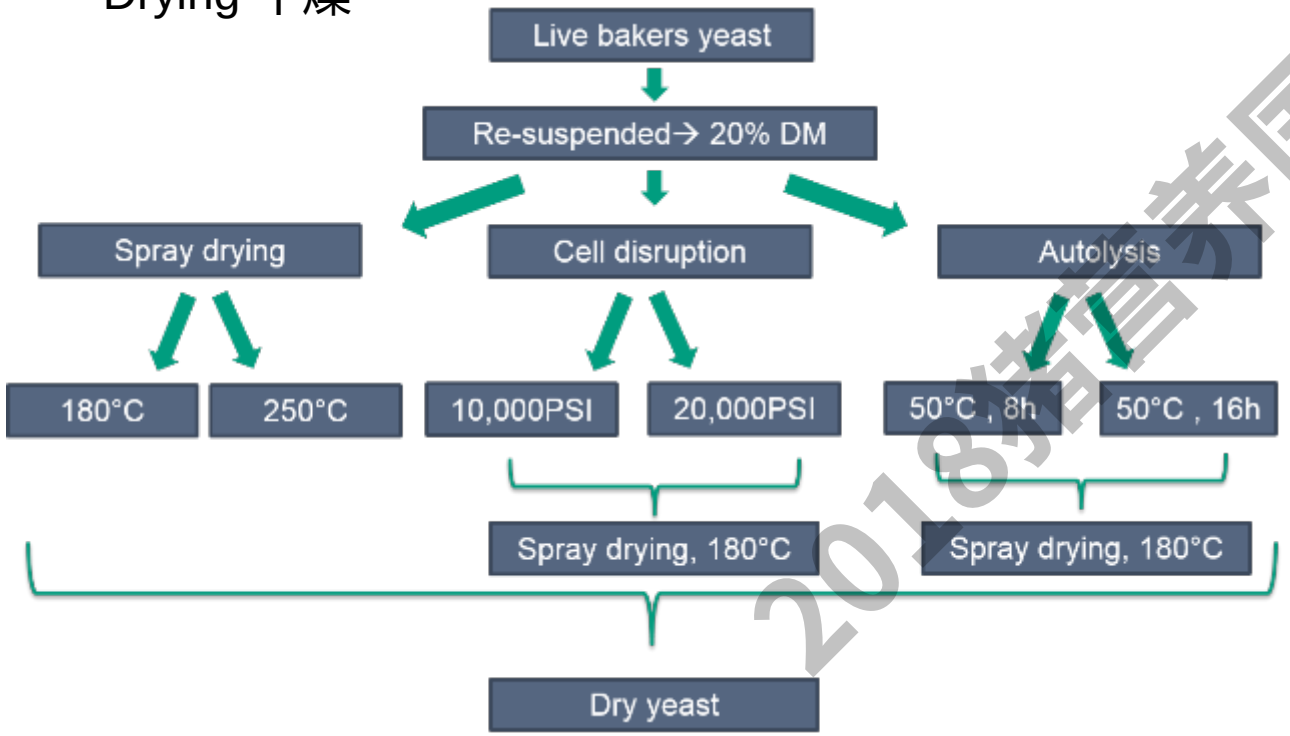
Lytic Polysaccharide Monooxygenases
裂解多糖的单氧酶

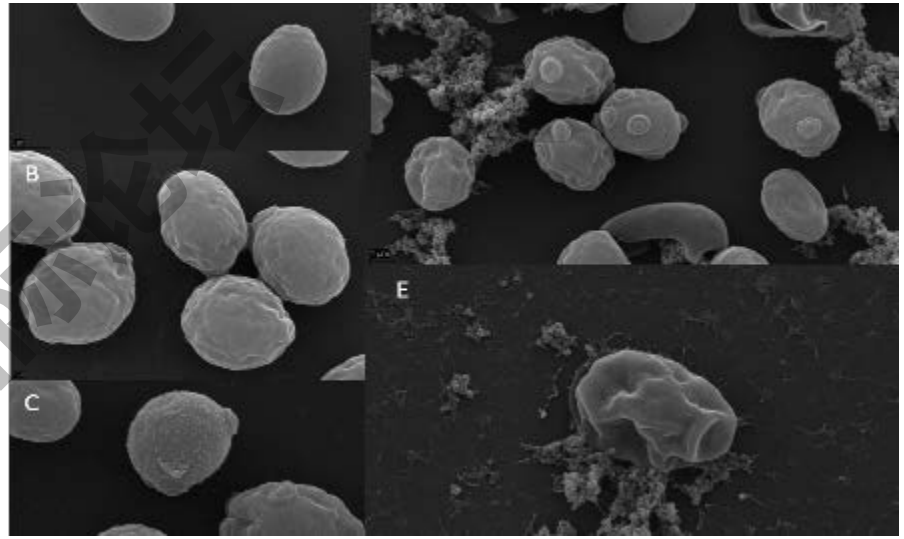


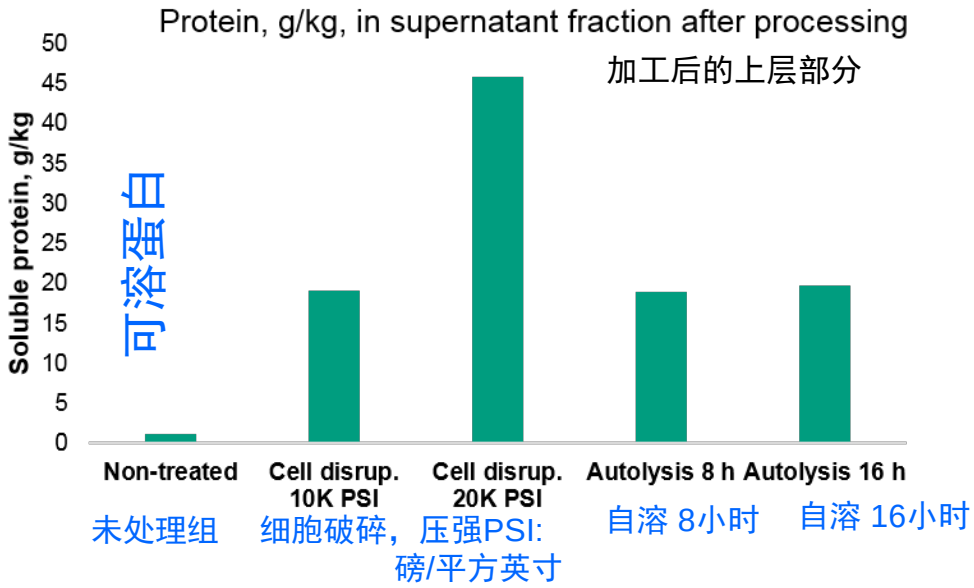
Source: Vaaje-Kolstad, Westereng, Horn, Liu, Zhai, Sørlie, Eijsink. Science, 2010

Optimizing down stream processing of yeast
优化酵母的下游加工技术（后续加工技术）

- Methods 方法:
  - Cell crushing 细胞破碎
  - Autolyses 自溶
  - Drying 干燥



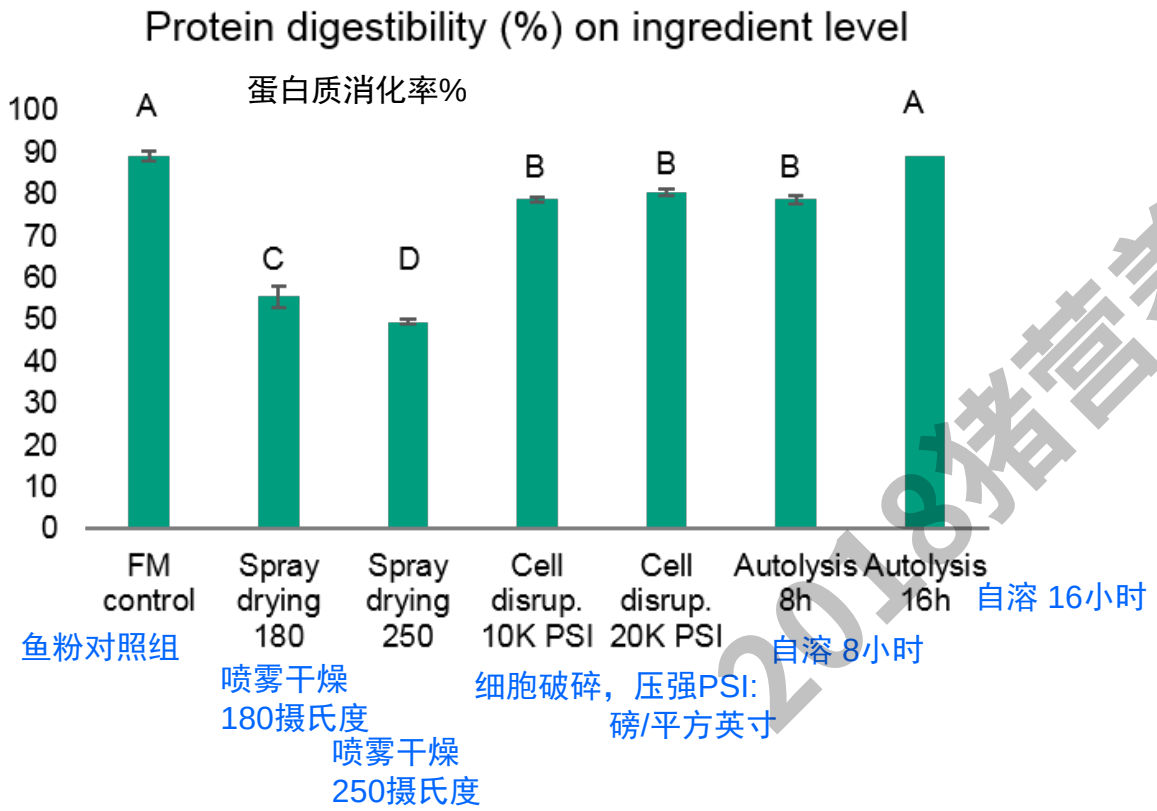


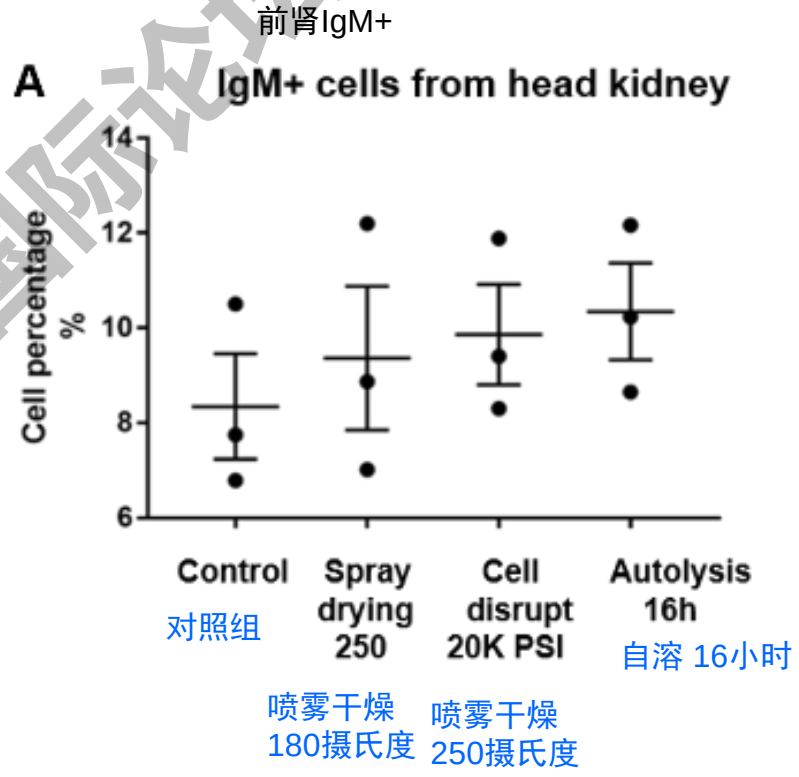


Source: Øvrum-Hansen et al., 2018, ISNFN2018 Las Palmas

Optimizing down stream processing of yeast
优化酵母的下游加工技术（后续加工技术）

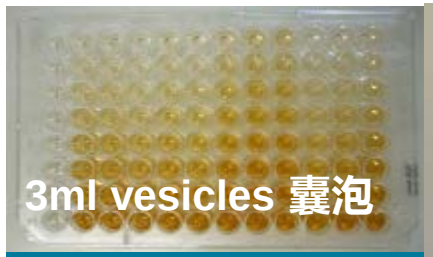
- Protein digestibility of yeast, 酵母蛋白的消化率%

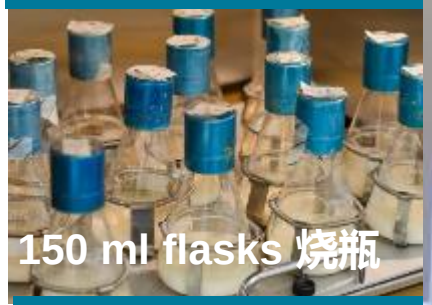




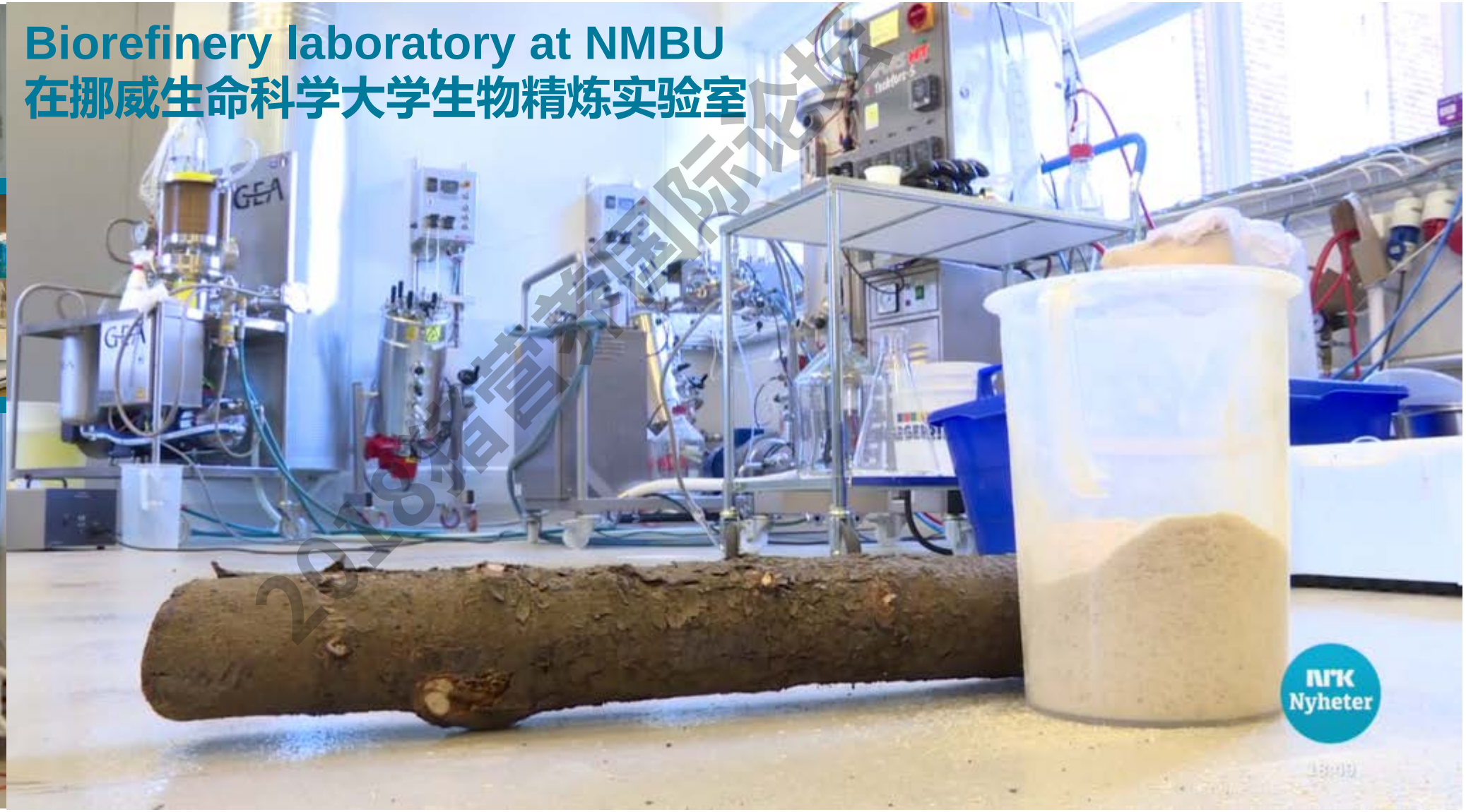
Source: Øvrum-Hansen, 2018, ISNFN2018, Las Palmas

Yeast fermentation in small scale
酵母发酵小试


3ml vesicles 囊泡


150 ml flasks 烧瓶

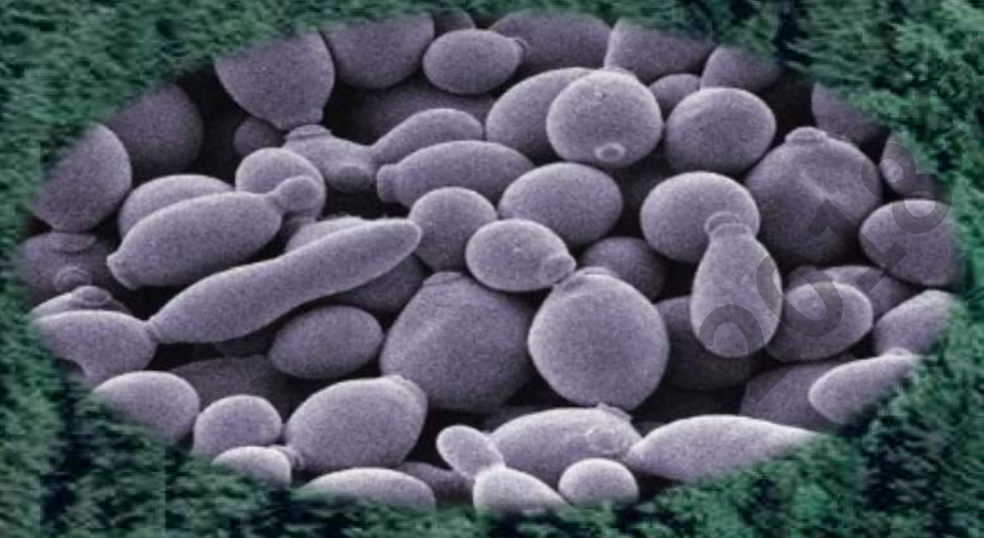

3-30 L fermenters
发酵罐



Yeast fermentation in large-scale Biorefinery factory, Borregaard,Norway
酵母发酵中试
挪威Borrogaard的生物精炼工厂

Yeast from trees as a feed resource
利用木质纤维素发酵生产的酵母作为饲料原料

Protein source
蛋白源



- Sustainable feed resources
  可持续的饲料原料
- Contains ~ 50-60% protein
  蛋白含量 ~ 50-60%
- Favorable amino acid profile
  氨基酸组成平衡
- Good taste
  适口性好
- Positive health effect for fish
  促进鱼类健康
- GRAS
  公认的安全性

Source: Øverland & Skrede 2016, J. Sci. of Foods and Agriculture

Feeding experiments with salmon 鲑鱼饲喂实验

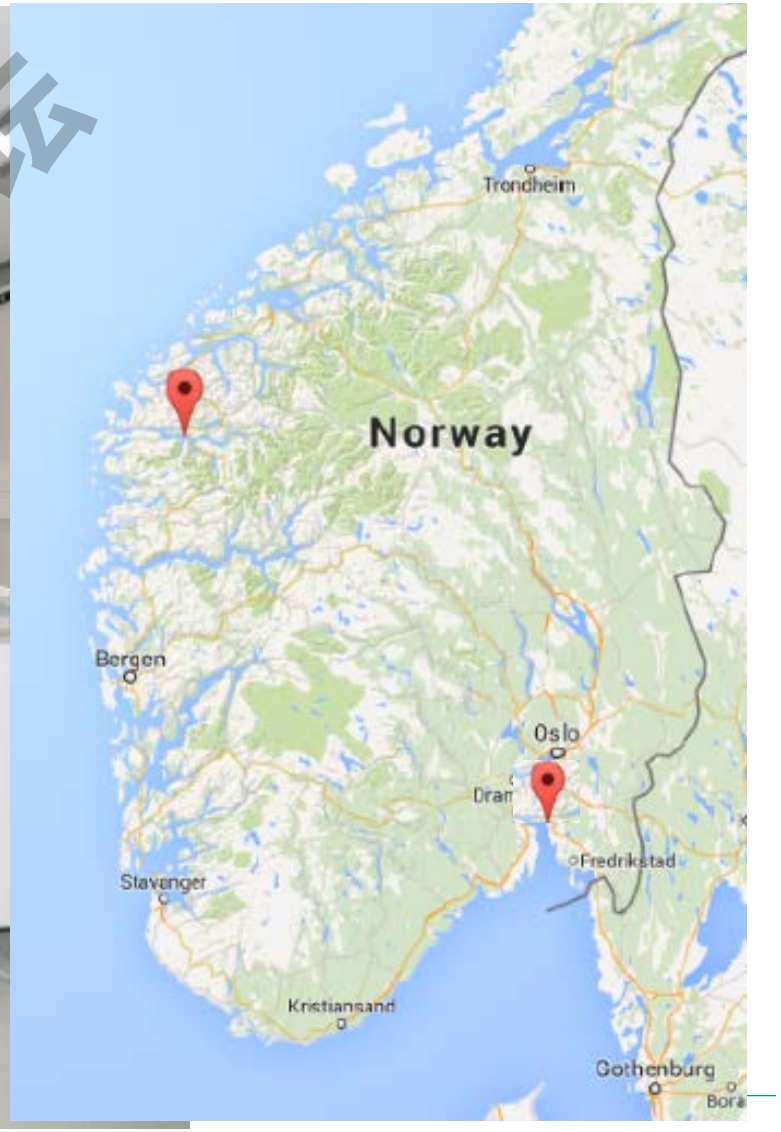
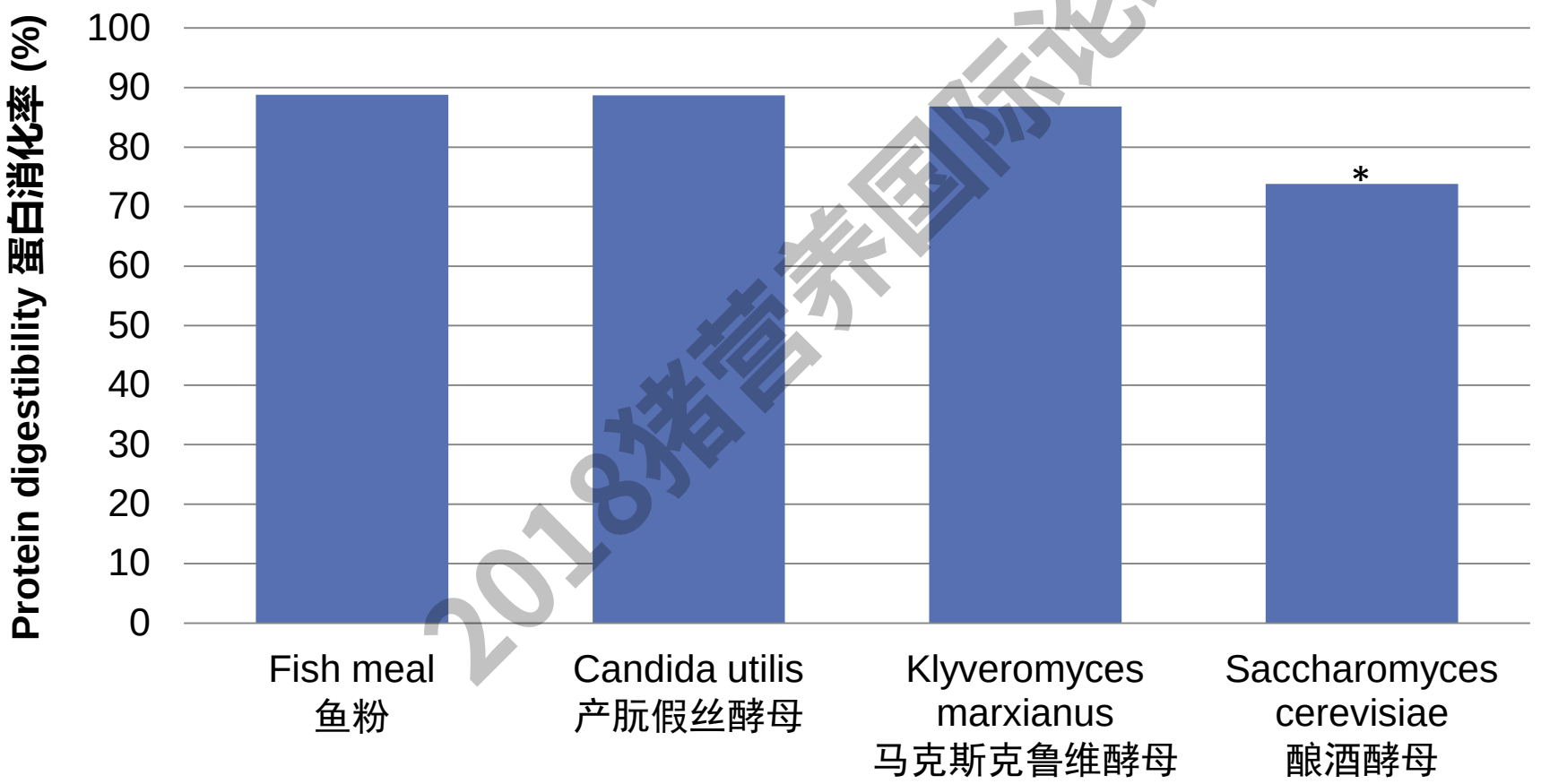


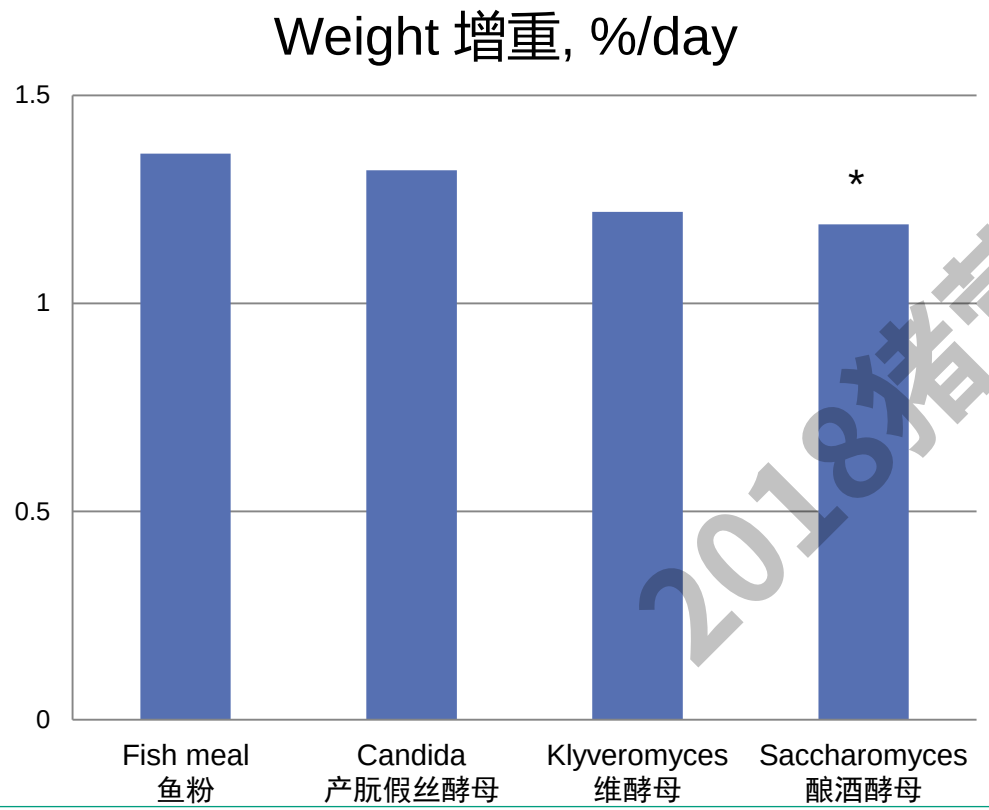
Photo: Fish laboratory at NMBU

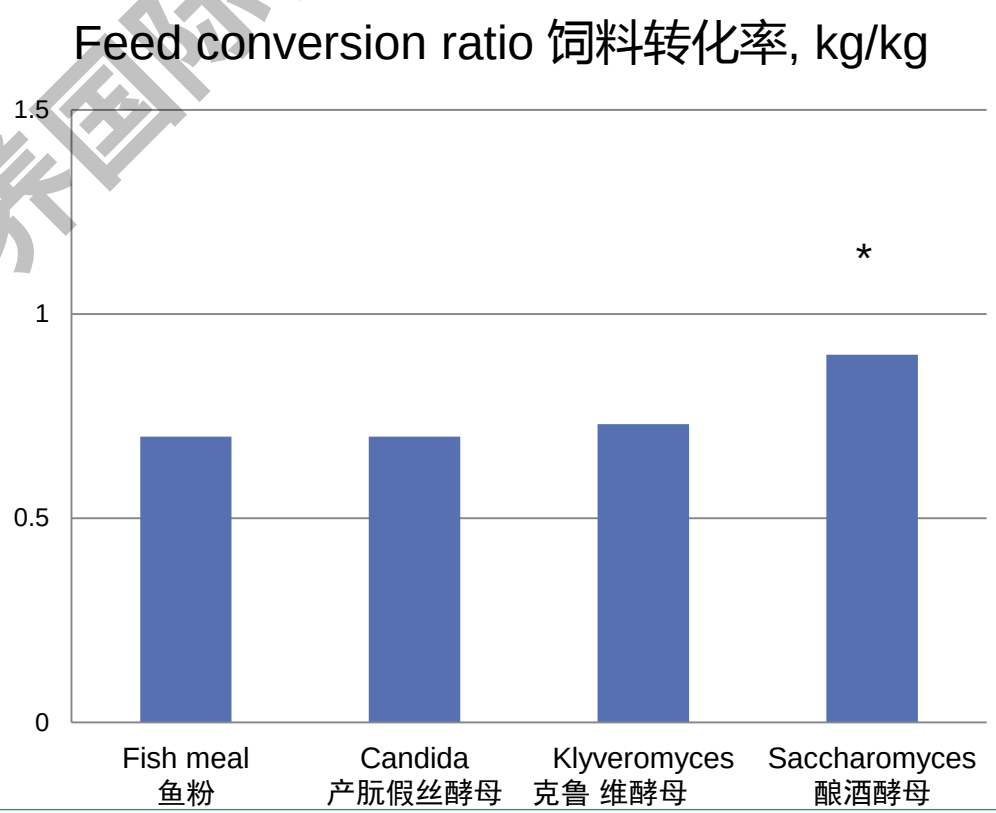
Digestibility of protein in salmon fed 30% yeasts
鲑鱼饲料中添加30%酵母的蛋白消化率



Source: Øverland et al., 2013, Aquaculture, 402-403, 1-7

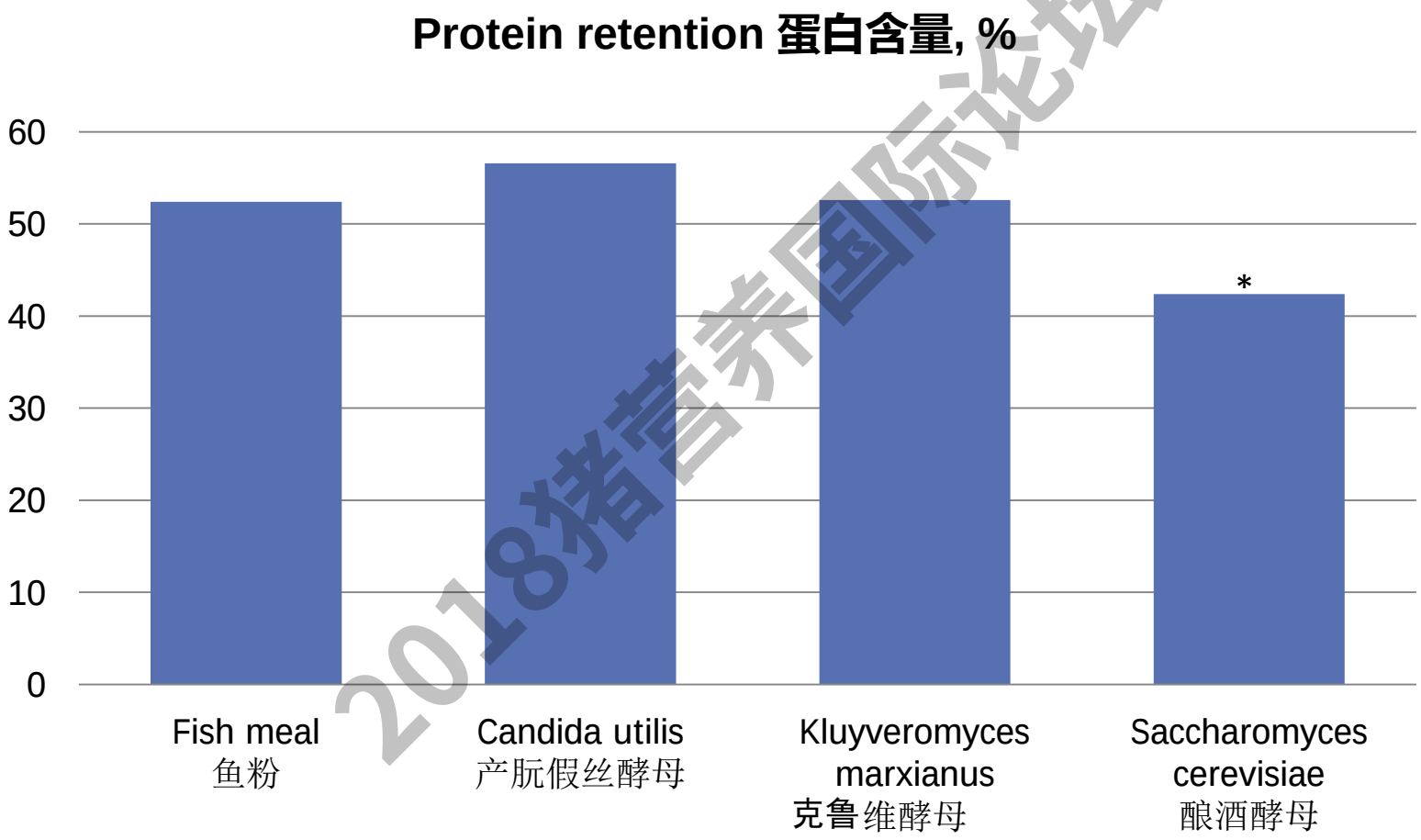
Growth and feed efficiency in Atlantic salmon fed 30% yeast
大西洋鲑鱼饲料中添加30%酵母对生长效率和饲料转化率的影响





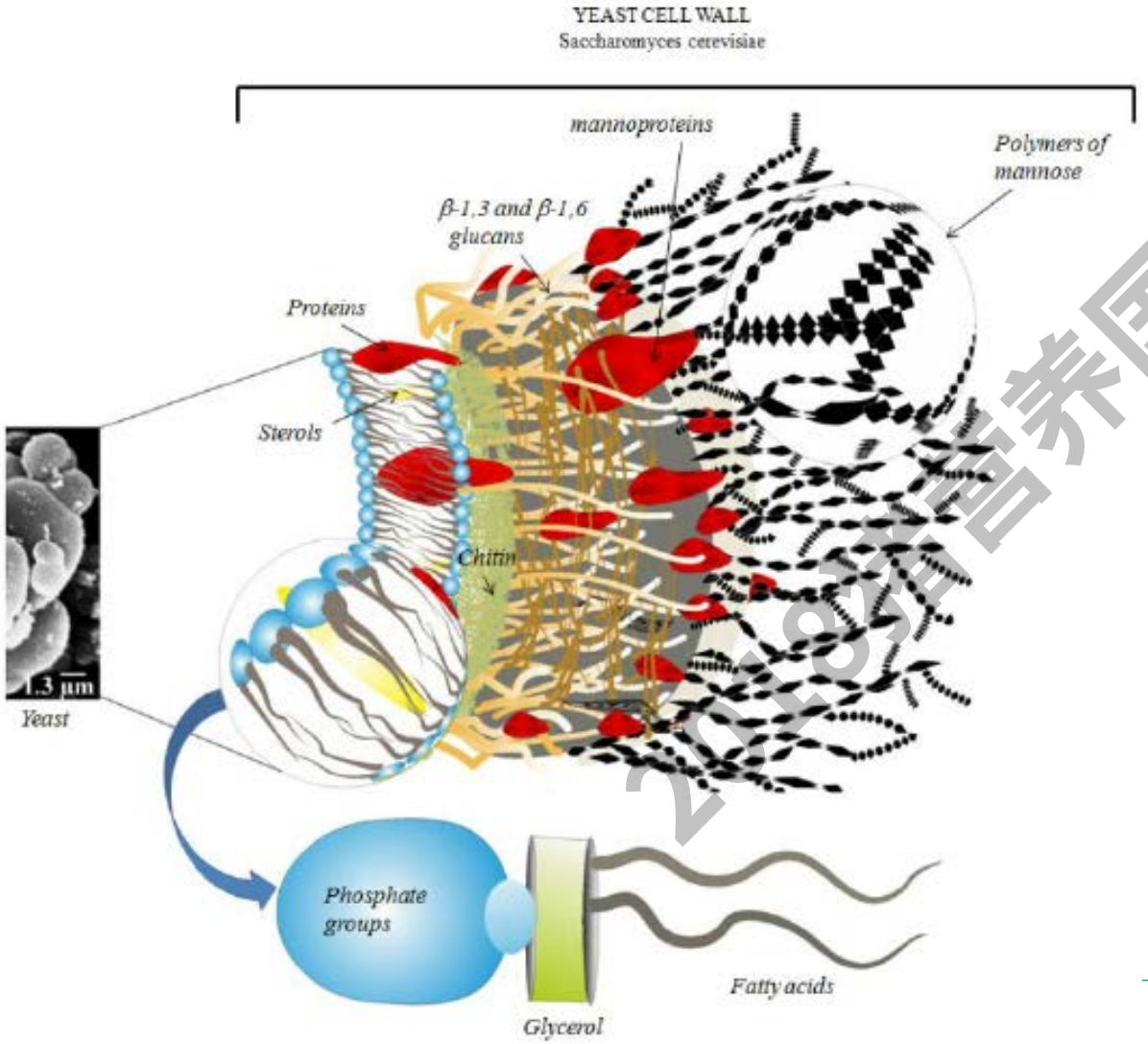
Source: Øverland et al., 2013, Aquaculture, 402-403, 1-7

Protein retention in salmon fed 30% yeast
鲑鱼饲料中添加30%酵母对蛋白沉积的影响



Source: Øverland et al., 2013, Aquaculture, 402–403 1–7

Bioactive components in yeast
酵母中的生物活性成分



Source: Nataly Talavera et al, 2013

- Manno-proteins
  甘露糖-蛋白
- β-glucans
  β-葡聚糖
- Chitin
  几丁质
- Nucleotides
  核苷酸
- Antioxidants
  抗氧化物

Effect of yeast on distal intestinal histology
酵母对肠道后端组织学上的影响

Normal intestine
正常的肠道

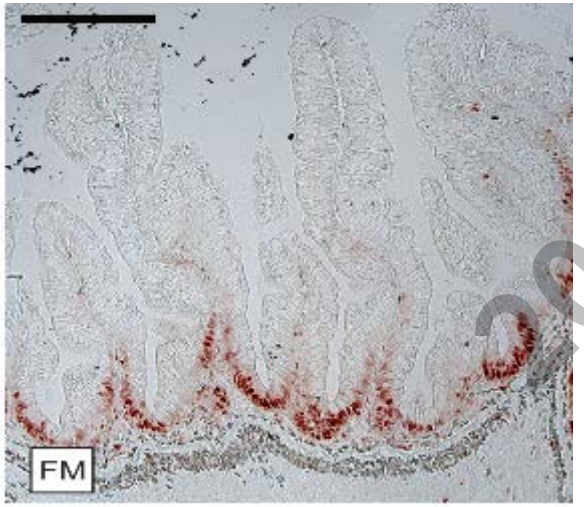
SBM-induced enteritis
豆粕诱发的肠炎

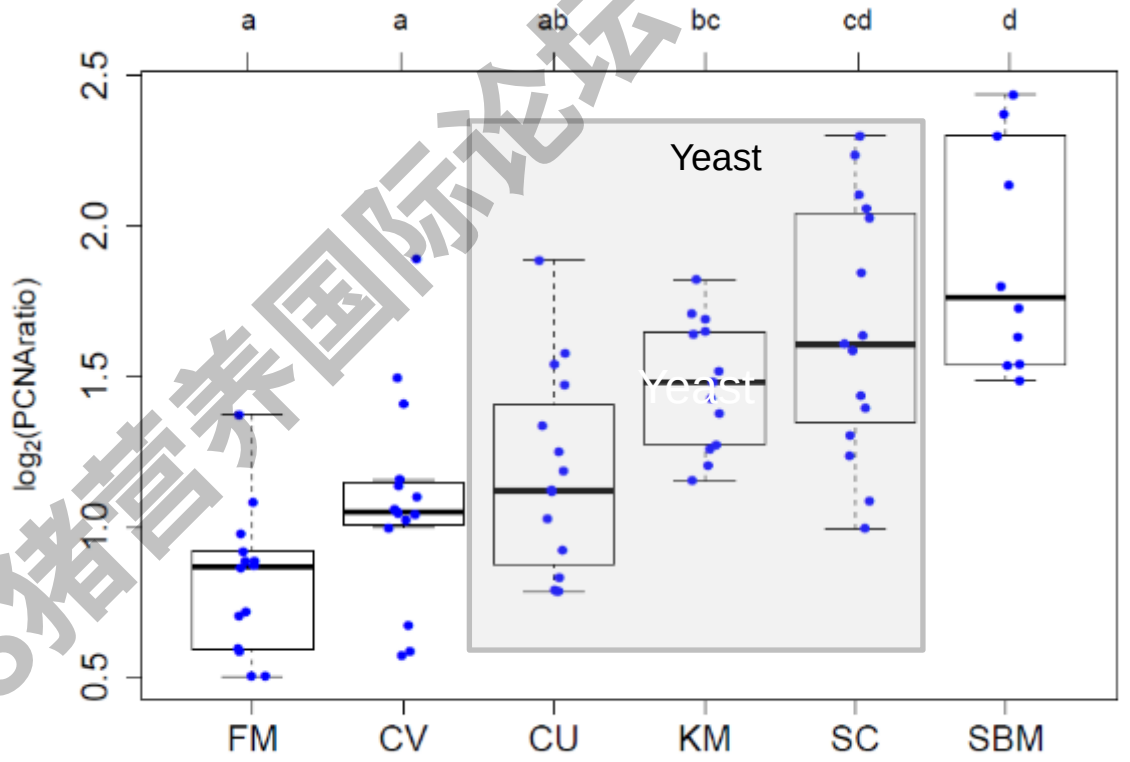
- Normal atrophy of mucosal folds
  正常的粘膜褶皱萎缩
- Normal odema score
  瘤腺体数量正常
- Normal supra nuclear vacules
  超核小体正常
- Reduced widening of the lamina propria
  固有层扩增减少

Source: Grammes et al., 2013, PlosOne, 8-12, 1-13

Immunohistochemistry of distal intestine 小肠末端的免疫组织化学

effect of yeast on cell proliferation (PCNA) 酵母对细胞增殖的影响

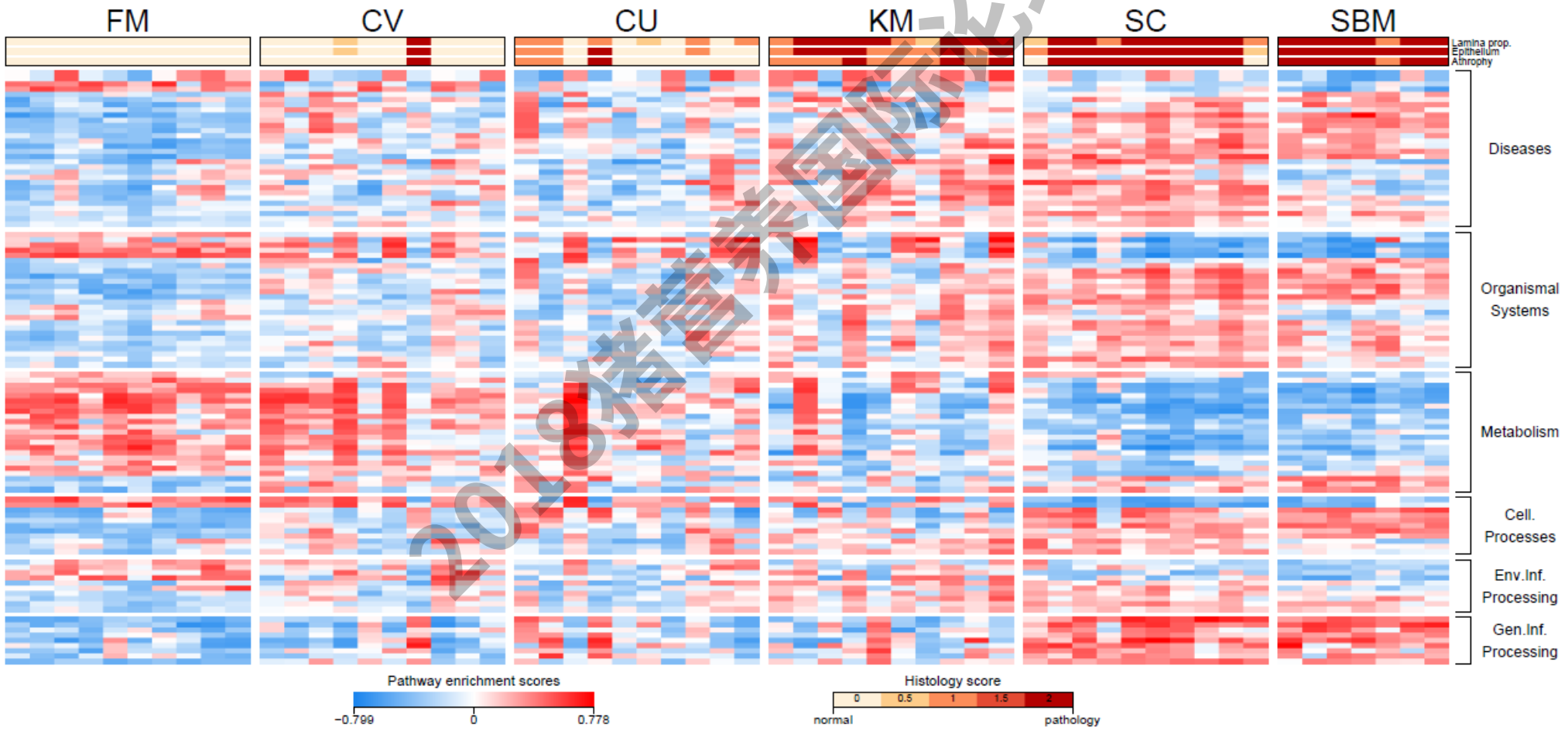




FM	Fish meal 鱼粉	KM	Kluyveromyces marxianus 克鲁维酵母
CV	Chorella vulgaris 小球藻	SC	*Saccharomyces cerevesia* 酿酒酵母
CU	*Candida utilis 产朊假丝酵母*	SBM	Soybean meal 豆粕

Source: Grammes et al., 2013, PlosOne, 8-12, 1-13

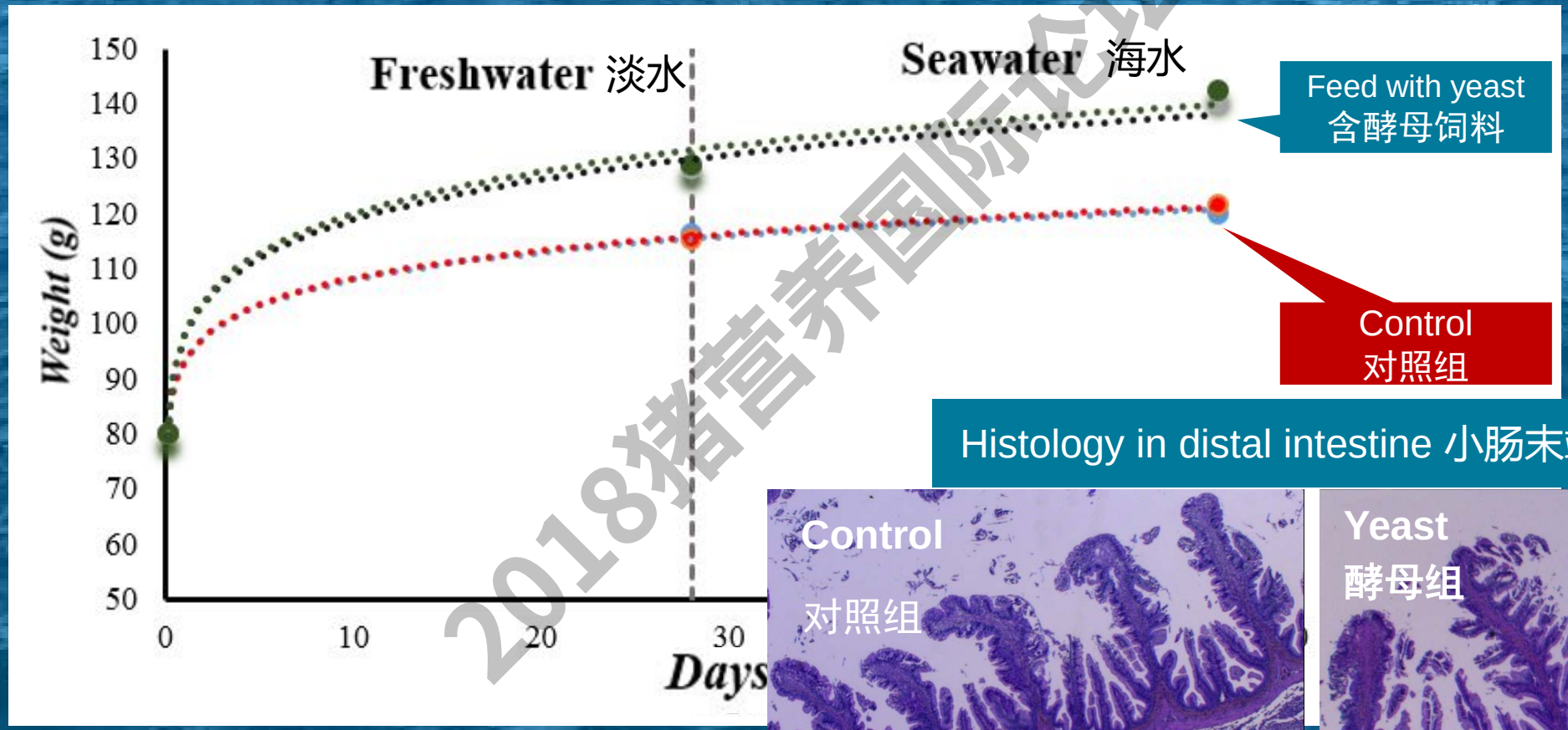
Microbial ingredients prevented inflammation in the distal intestine
微生物原料防止小肠末端的炎症
Transcriptomal aspects: Functional interpretation 转录表达：功能解析
Heat map – gene enrichment annotation, KEGG 基因分选富集图，KEGG



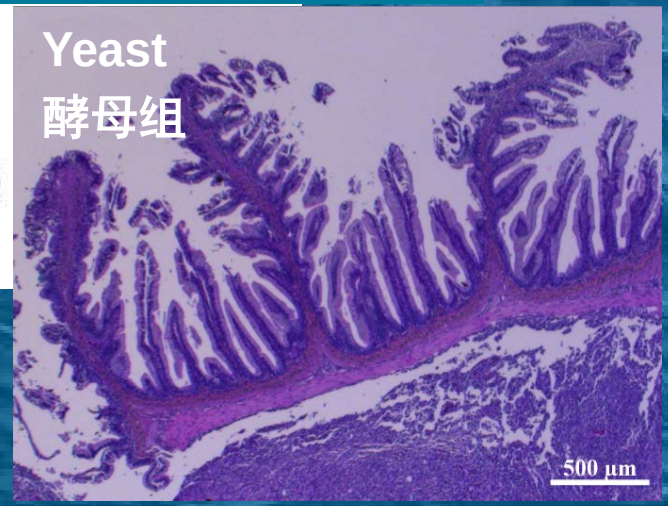
Source: Grammes et al., 2013, PlosOne, 8-12, 1-13

Yeast in diets for Atlantic salmon during seawater transfer

饲喂酵母的大西洋鲑鱼与对照组在换水前后的表现

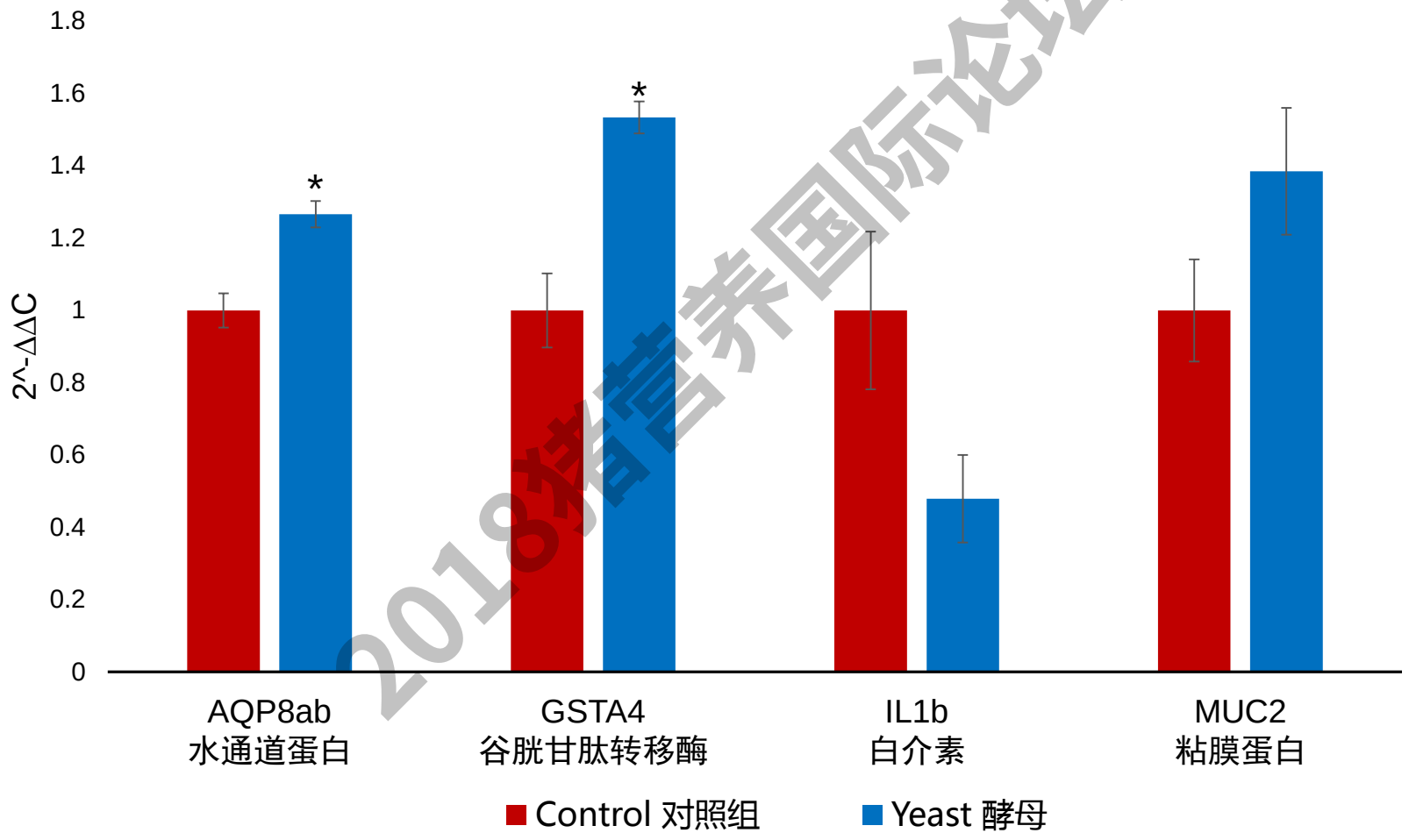


Histology in distal intestine 小肠末端组织



Source: Sahlmann et al., ISNFN, 2018 Las Palmas

Gene expression in distal intestine of salmon during seawater transfer
换水过程中鲑鱼小肠末端的基因表达



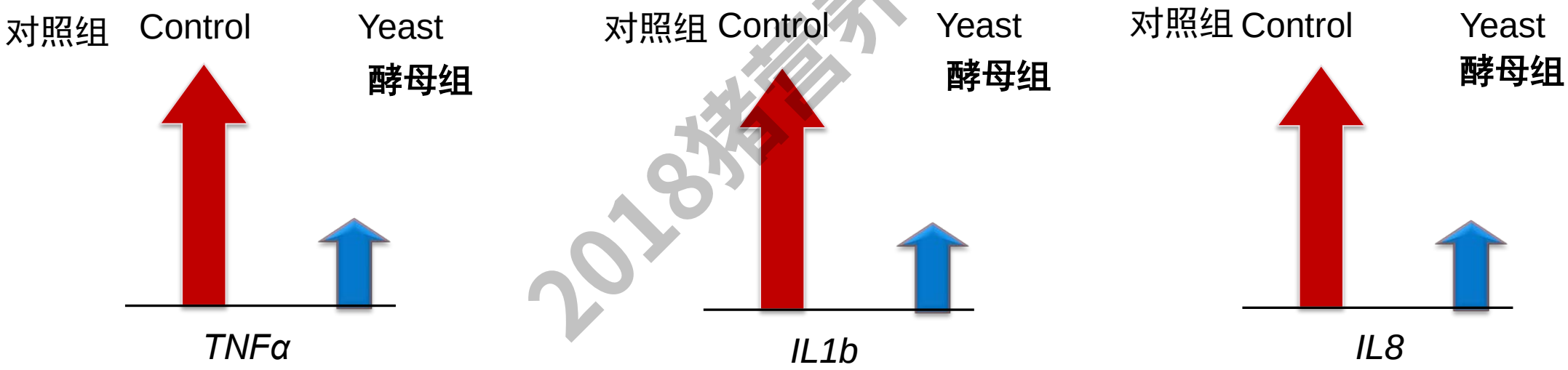
Source: Sahlmann et al., 2018, Manus in prep

Protein production in distal intestine of salmon during seawater transfer

在换水过程中，鲑鱼的小肠末端蛋白质生产量

Yeast significantly decreased the protein expression of TNFα, IL1b and IL8

添加酵母明显降低了TNFα, IL1b 和 IL8三种与免疫炎症反应相关基因的表达



Source: Foods of Norway, Sahlmann et al., 2018, Manus in prep, Morales-Lange, Pontificia Universidad Catolica de Valparaiso;

Yeast in diets for piglets, ongoing
酵母在仔猪料中的作用，试验进行中

% yeast in the diets, Replacing conventional protein sources
酵母替代传统蛋白的比例，%

- 0%
- 3.6%
- 7.3%
- 14.6%

Yeast from lignocellulosic biomass
利用木质纤维生物质生产的酵母

Review

SCI

Received: 21 June 2016 Revised: 19 August 2016 Accepted article published: 25 August 2016 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8007

Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture

Margareth Øverland* and Anders Skrede

- **Nutritional aspect 营养价值**
  - High quality protein source
    高品质蛋白源
  - No health risk
    没有健康风险

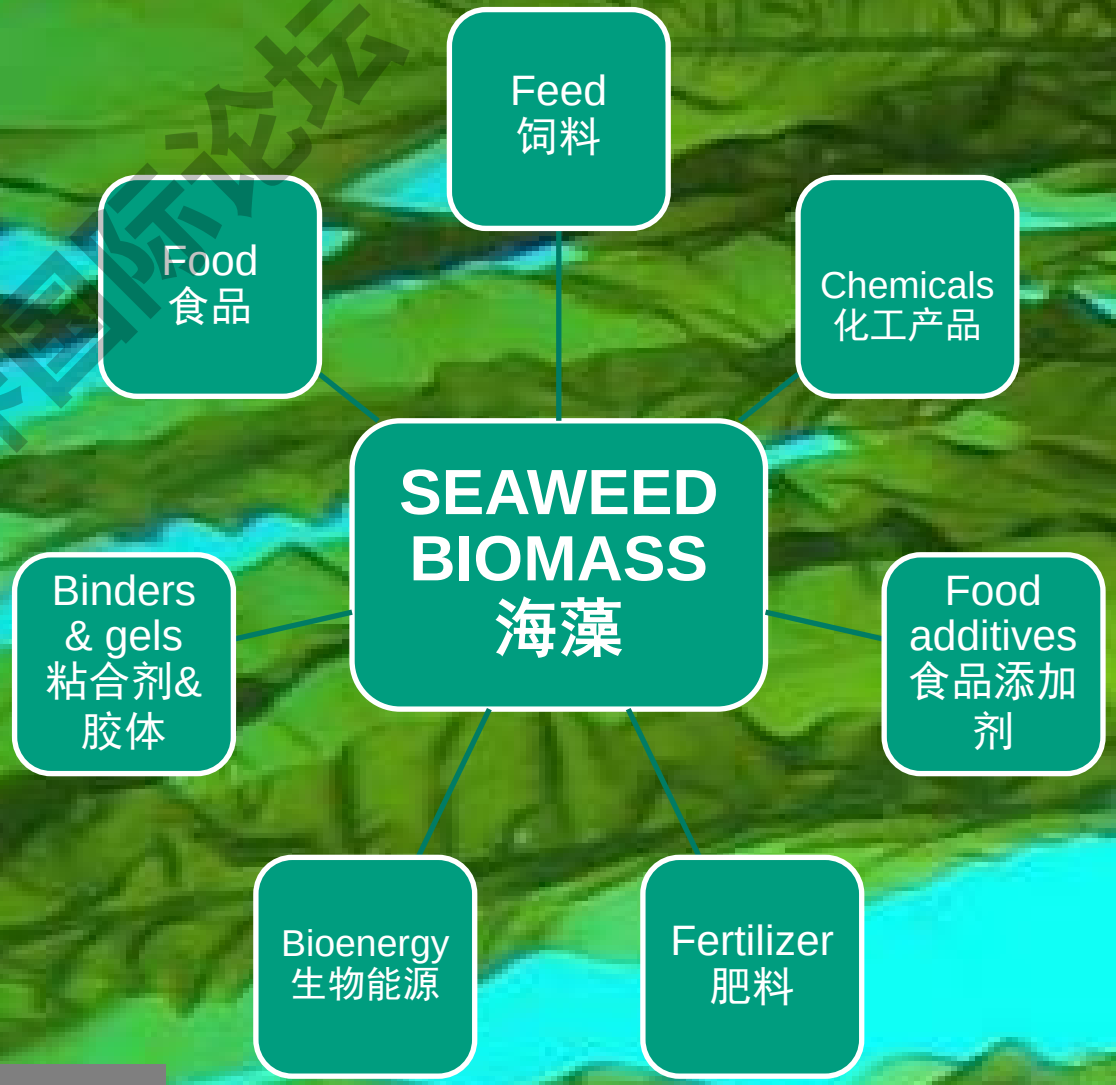
- **Piglets仔猪**
  - Preliminary results suggest positive effect on growth performance
    初步结果表明，对生长性能有积极影响
  - Improved on nutrient digestibility and gut health
    提高消化能力，促进肠道健康
  - Stimulation of innate immunesystem
    刺激先天性免疫系统

- **Salmonids 鲑鱼**
  - High growth rate, feed intake and feed efficiency
    生长速率高，采食量大，饲料转换率高
  - Strenghetens the gut barrier function
    增强肠道屏障功能
  - Favorable gut microbiota
    良好的肠道微生物群
  - Positive effect on robustness and health
    促进鲑鱼健壮生长

Cultivated seaweeds - a potential feed resource
海藻培植 – 一种潜在的饲料原料

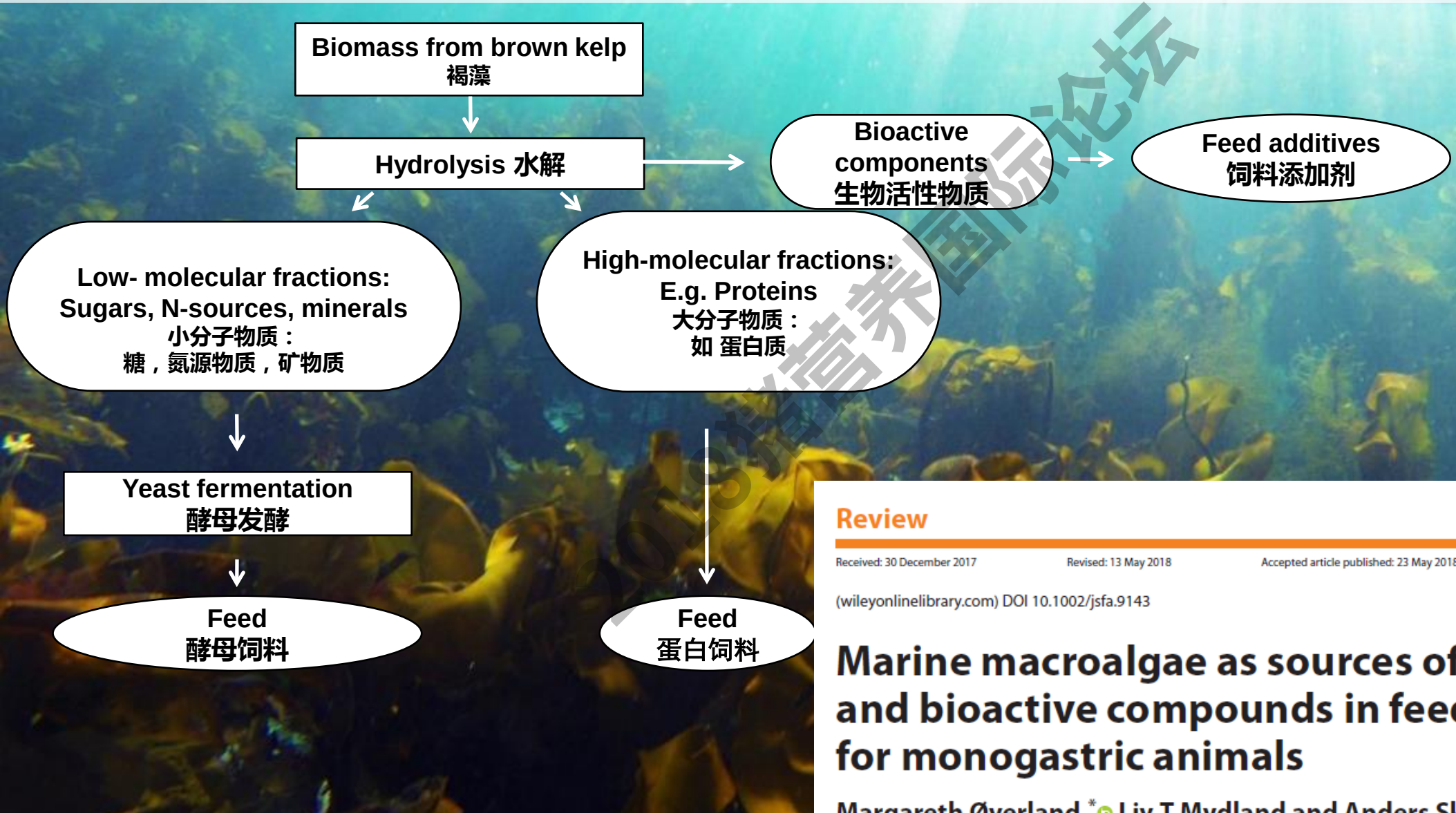
Advantages 优势：

- Large biomass production
  生物质产量大
- Don’t require any agricultural land, fertilizers, or fresh water
  不需要任何农业用地、肥料和淡水
- Can be cultivated in sea water
  可以海水培植
- Binds and recycles nutrients
  营养整合再利用



Source: Seaweed as a feed resource. Øverland et al., 2018., Review - J of the Sci. of Food & Agric.

Processing of seaweeds to feed
海藻饲料的生产流程



Review

SCI

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(wileyonlinelibrary.com) DOI 10.1002/jsfa.9143

Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals

Margareth Øverland,* Liv T Mydland and Anders Skrede

Trees and seaweeds in an integrated biorefinery process to produce feed

木质素和海藻综合使用加工成饲料

Biomass
生物质

Biorefinery processing
生物精炼加工

Yeast 酵母

Source: Foods of Norway: S. Sharma, S., et al., 2018, J. of Agricultura and Food Chemistry, in press

Conclusion 结论

Blue & green biomass
可再生生物质

Advanced technology to develop novel feed resources from natural gas and non-food biomass such as trees and seaweeds will be important to help meet the global food challenge.
开发利用天然气和非粮食生物质如树木和海藻等，生产新型饲料资源的先进技术，对于应对全球粮食紧缺的问题具有重要意义。

Technology
技术工艺

Microbial feed resources have an advantage in that they can be produced independently of arable land and climate, and they relieve pressure on food resources for direct human food production.
微生物饲料资源的优势在于，不依赖可用耕地和气候条件，减轻人类食品资源的压力。

Novel feed resources
新型饲料原料

Continued research and development in production of microbial ingredients will make an important contribution to securing the sustainability and economic viability of future feed market.
继续研究和开发微生物原料，将对未来饲料市场的可持续性和经济可行性做出重要贡献。

Value creation
创造价值