Innovative strategies on sparing dietary protein for tilapia

Yu-Hung Lin

Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan

- Proteins and amino acids are molecules critical because of the role they play in the structure and metabolism of all living organisms. Fish including tilapia cannot synthesize all amino acids and must acquire several in their diet, through the consumption of protein (NRC, 2011).
- Protein is expensive and is responsible for a large part of the cost of most prepared feeds.

Protein requirements of tilapia in freshwater

Species	Fish size (g)	Requirement (%)	Reference
O. mossambicus	1.0-2.5	29-38	Cruz and Laudencia (1977)
	Fry	50	Jauncey and Ross (1982)
	0.5-1.0	40	
	6-30	30-35	
	1.8	40	Jauncey (1982)
O. niloticus	1.5-7.5	36	Kubaryk (1980)
	3.2, 3.7	30	Wang et al. (1985)
	0.838	40	Siddiqui et al. (1988)
	40	30	
	24	27.5-35	Wee and Tuan (1988)
	0.012	45	El-Sayed and Teshima (1992)
O. aureus	0.3-0.5	36	Davis and Stickney (1978)
	0.16	40	Santiago and Laron (1991)
Tilapia zillii	1.7	35-40	Teshima et al. (1978)
	1.65	35	Mazid et al. (1979)
O. niloticus × *O. aureus*	0.6-1.1	32	Shiau and Peng (1993)
	21	28	Twibell and Brown (1998)

Protein requirements of tilapia at different salinities

Species	Fish size (g)	Salinity (ppt)	Requirement (%)	Reference
O. niloticus	0.024	0	30.4	De Silva and Perera (1985)
		5	30.4	
		10	28.0	
		15	28.0	
O. niloticus × *O. aureus*	2.88	32-34	24.0	Shiau and Huang (1989)
Florida red tilapia	10.6	37	20.0	Clark et al. (1990)

Facts:

- Protein source
- Protein to Energy (P/E) ratio

- Energy is not a nutrient but is a property of nutrients that are released during the metabolic oxidation of protein, carbohydrates and lipids.
- If insufficient non-protein energy is available, part of the protein is used as an energy source.

- Excess energy
  - produce fatty fish
  - reduce feed intake
  - inhibit proper utilization of other feedstuffs
- Therefore, it is critical to obtain the proper protein to energy (P/E) ratio in a diet for the most economical production of tilapia. Reduction of excess protein in the diet will also reduce the cost and amount of ammonia excreted by the tilapia.

Optimum protein-to-energy ration of tilapia

Species	Fish size (g)	Optimum P/E ratio	Reference
O. mossambicus	1.80	116.6 mg per kcal ME	Jauncey (1982)
	5.19	99.48 mg per kcal DE	El-Dahhar and Lovell (1995)
O. niloticus	0.012	110 mg per kcal GE	El-Sayed and Teshima (1992)
	1.7	120 mg per kcal DE	Kubaryk (1980)
O. aureus	2.5	123 mg per kcal DE	Winfree and Stickney (1981)
	7.5	108 mg per kcal DE	Winfree and Stickney (1981)
Tilapia zillii	1.65	95.3 mg per kcal DE	Mazid *et al.* (1979)
	50	103 mg per kcal DE	El-Sayed (1987)
O. niloticus × O. aureus	0.16	111 mg per kcal DE	and Laron (1991)

- Knowledge of the protein-sparing effects of non-protein nutrients such as lipids or carbohydrate is necessary and should be used to reduce diet costs and maximize nitrogen retention.

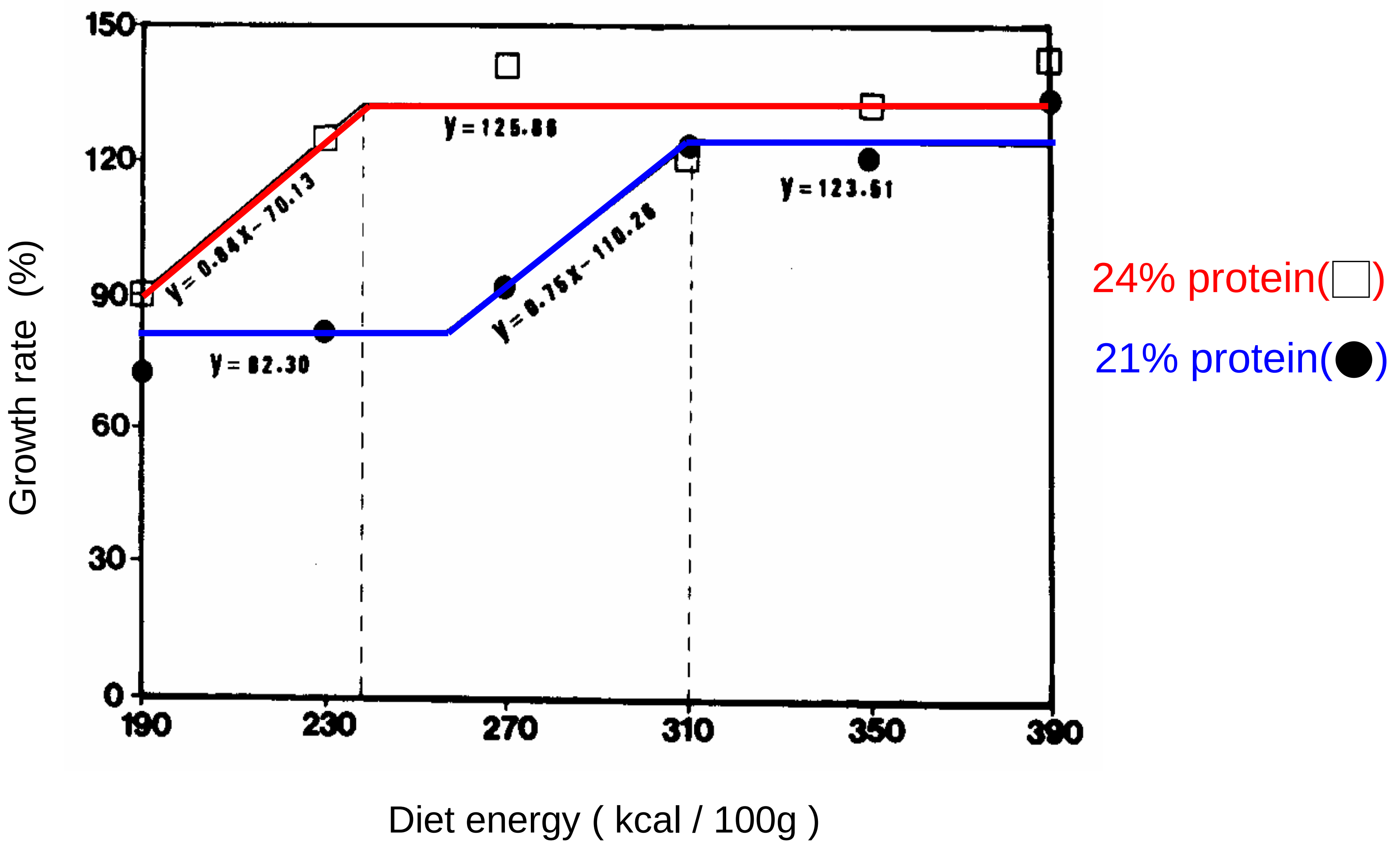
- Tilapia (O. *niloticus* × O. *aureus*) fed a 24% protein diet produced maximum growth when reared in a seawater. (Shiau and Huang, 1989)

Tilapia case#1

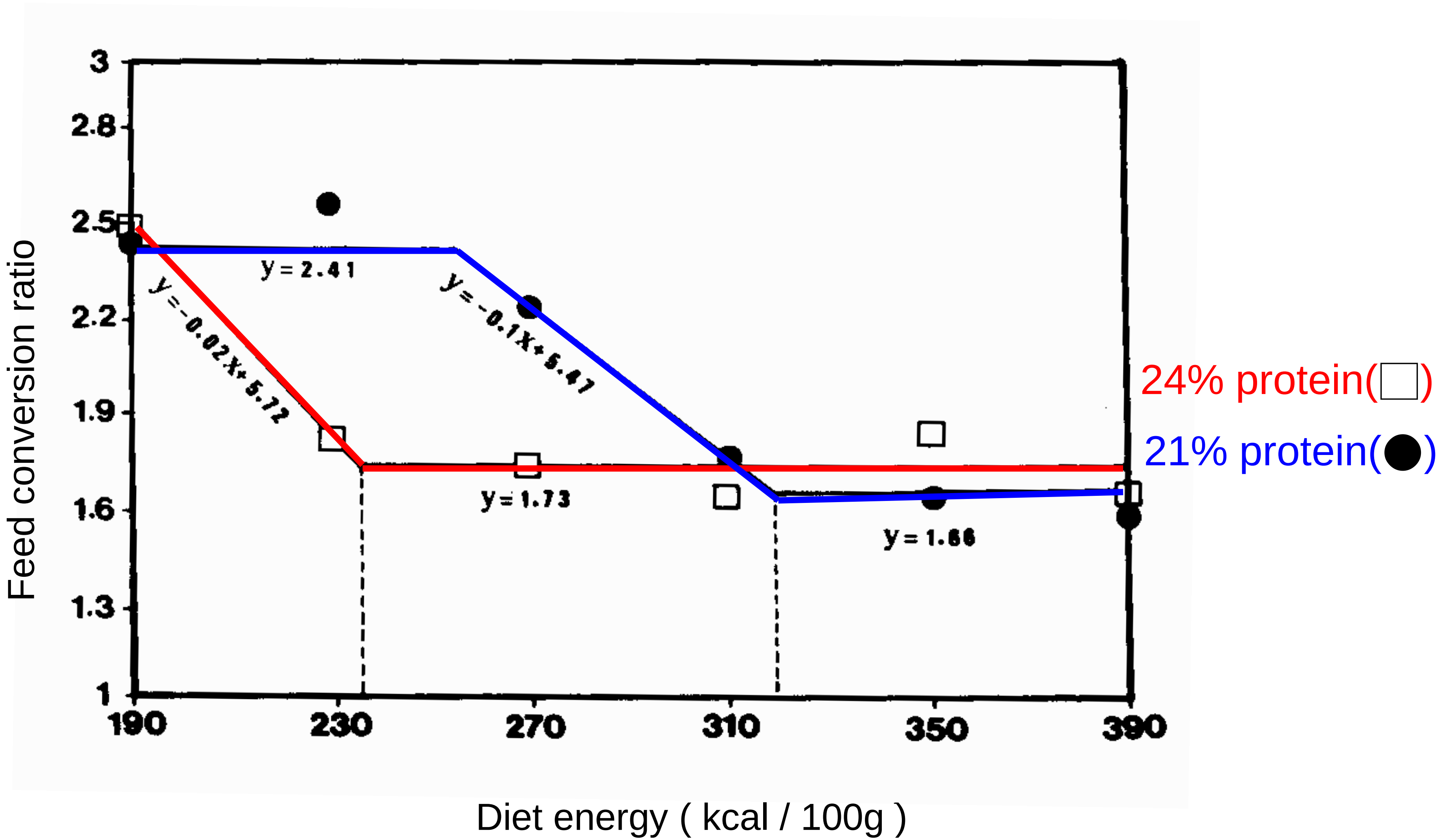
- Two dietary protein levels (21% and 24%) were used along with six energy level at each protein level (190, 230, 270, 310,350 and 390 kcal/100 g diet).

Basal diet (%)

	21% protein	24% protein
White fishmeal	31	36
Corn oil	3	3
Corn starch	2.5	1.5
Dextrin	10	6
CMC	3	3
Vitamin mixture	1	1
Mineral mixture	2	2
α-Cellulose	47	47



150
120
90
60
30
0
190
230
270
310
350
390
Growth rate (%)
Diet energy (kcal / 100g)
y = 0.84X - 70.13
y = 125.86
y = 0.75X - 110.26
y = 123.61
y = 82.30
24% protein(□)
21% protein(●)


Feed conversion ratio
3
2.8
2.5
2.2
1.9
1.6
1.3
1
190
230
270
310
350
390
y = 2.41
y = -0.02X+5.72
y = -0.1X+5.47
y = 1.73
y = 1.66
24% protein(□)
21% protein(●)
Diet energy (kcal / 100g)

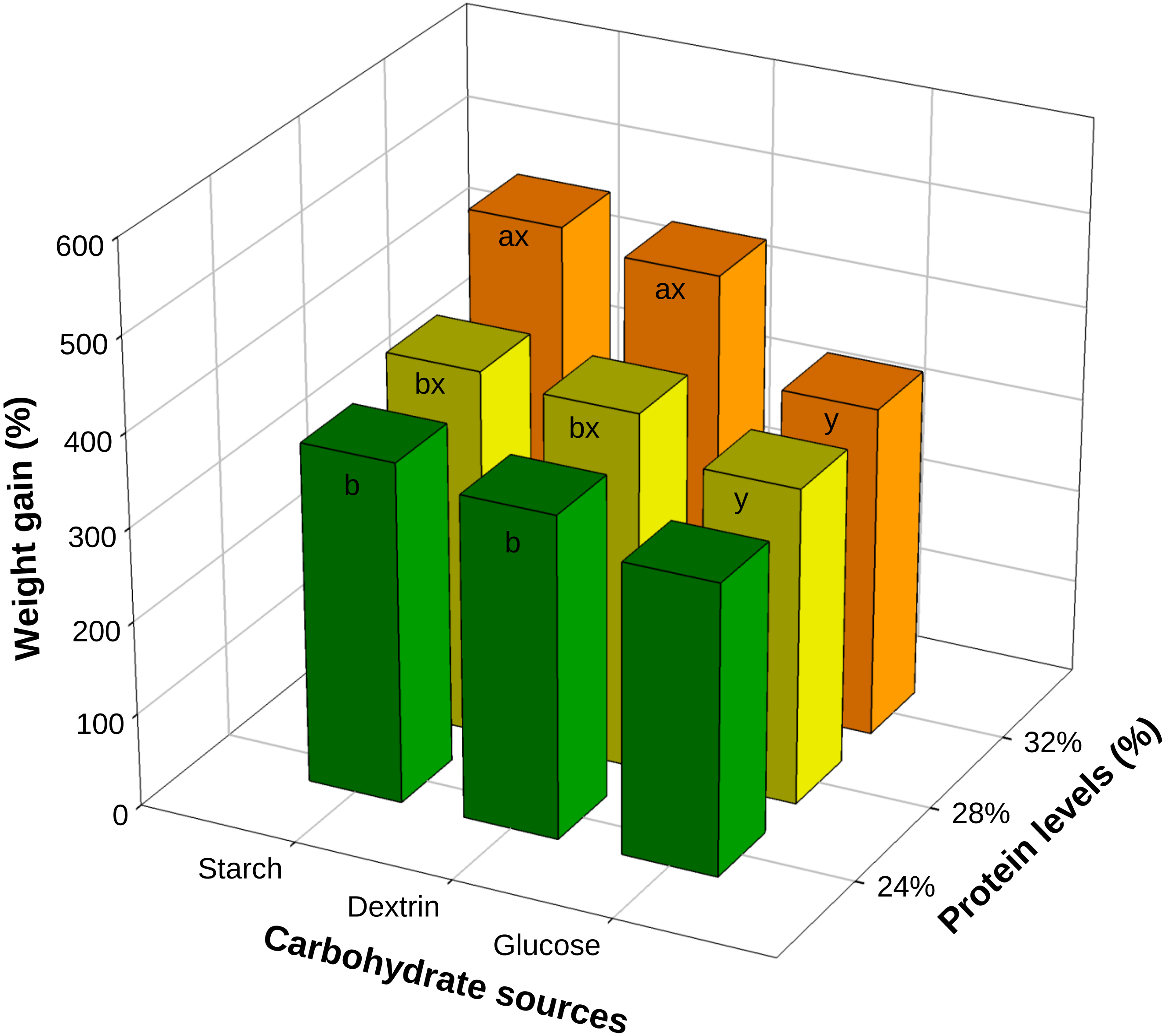
- These data suggest that when the energy level of the diet is 310 kcal/100 g, the dietary protein level for hybrid tilapia reared in seawater can be lowered from 24% to 21%.

Shiau and Huang, Aquaculture 91, 143-153 (1989)

- Carbohydrates are the cheapest dietary energy sources for domestic animals and their utilization by different species of cultured fish is of interest to fish nutritionists and feed producers.
- Protein sparing by carbohydrate

Tilapia case#2

- Shiau and Peng (1993) conducted a study to evaluate the possible protein-sparing effects of carbohydrates in tilapia.
- Three dietary protein level (32, 28, 24%) were achieved by substitution with three levels (33, 37, 41%) and three sources (glucose, dextrin, starch) of dietary carbohydrates.


Weight gain (%)
600
500
400
300
200
100
0
ax
ax
bx
bx
y
y
b
b
Starch
Dextrin
Glucose
Carbohydrate sources
24%
28%
32%
Protein levels (%)

- This study suggests that starch or dextrin could spare some protein in tilapia diet when the dietary protein is low.

Shiau and Peng, Aquaculture 117: 327-334 (1993)

Facts#1

- Fish in general utilize dietary carbohydrate poorly. In addition, common carbohydrate sources also show low digestibility due to the high fiber content.

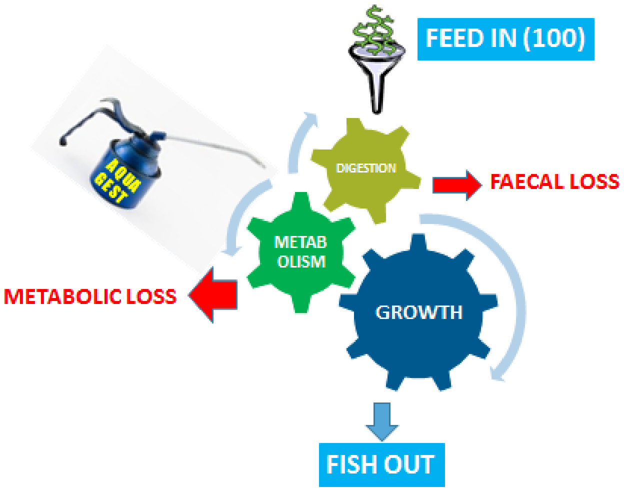
Facts#2

- High dietary fat levels cause fatty fish.

Innovative strategy of protein sparing for tilapia – lipid-enhancing feed additive

Rationale

- digestive/metabolic enhancement
  - metabolic/faecal losses ↓
  - protein efficiency ↑
  - dietary protein requirement ↓
- Conditions for reducing protein level without affecting performance
  - Maintain protein quality (essential amino acid levels)
  - Maintain feed intake



Experimental design

- Experimental design : reduce 2% protein in a standard growout feed for tilapia:
  - replacing 4.35% soybean meal with filler material (oyster shell)
  - Compensating with a digestive/metabolic enhancer (Aquagest OMF, Nutriad International NV, Belgium)

Determine effect

- fish performance
- filleting yield and carcass composition
- condition factor = W (g)/L(cm)^3
- a range of physiological parameters (haematological parameters, gut histology
- metabolic enzyme - Glucose-6-phosphate dehydrogenase (G6PDH)

Experimental feeds

	CONTROL 28/7	LOPRO 26/7+AG
local fish meal	10.9	10.9
meat and bone meal	2.2	2.2
soybean meal	13.0	8.7
rapeseed meal	8.7	8.7
wheat meal	10.9	10.9
wheat flour (japan)	10.9	10.9
coconut meal	8.7	8.7
whole fat rice bran	10.9	10.9
corn DDGS	19.6	19.6
salt	0.7	0.7
local fish oil	2.6	2.6
DCP	0.4	0.4
premix	0.4	0.4
choline	0.0	0.0
oyster shell powder	-	4.3
AQUAGEST OMF	**-**	**0.3**
TOTAL	100	100
Proximate composition		
Moisture	8.7	8.3
Crude protein	27.8	26.1
Crude fat	7.4	7.4
Ash	8.2	10.4

Experimental diets were designed by NPUST based on a common feed formulation for tilapia (Hanaqua Tech Inc., Taiwan) Aquagest OMF were provided by Nutriad International NV, Belgium

Feed preparation

- produced by Tungkang Biotech Research Center
- Treatments:
  1. CONTROL 28/7 CP/CF
  2. LOPRO 26/7 CP/CF + 3 kg/MT Aquagest OMF

Extruder in Tungkang Biotech Research Center, Pingtung, Taiwan

Trial conditions

- male hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), initial weight 175 g (Tainan, Taiwan)
- trial performed at a farm in Changjhih, Pingtung with monitoring and sampling by National Pingtung University of Science and Technology, Taiwan
- fish were acclimated to farm conditions for 2 month fed commercial tilapia diet (Hanaqua Tech Inc., Taiwan)
- trial units: cement tanks 5 m × 5 m (0.75 m depth); each tank was divided into two units using a nylon net

Trial conditions (cont's)

- flow-through system with underground freshwater (80% water change every three weeks)
- 45 fish per unit, 3 replicate units per treatment
- culture period : 18 wks (July 28 - Nov 30, 2013)

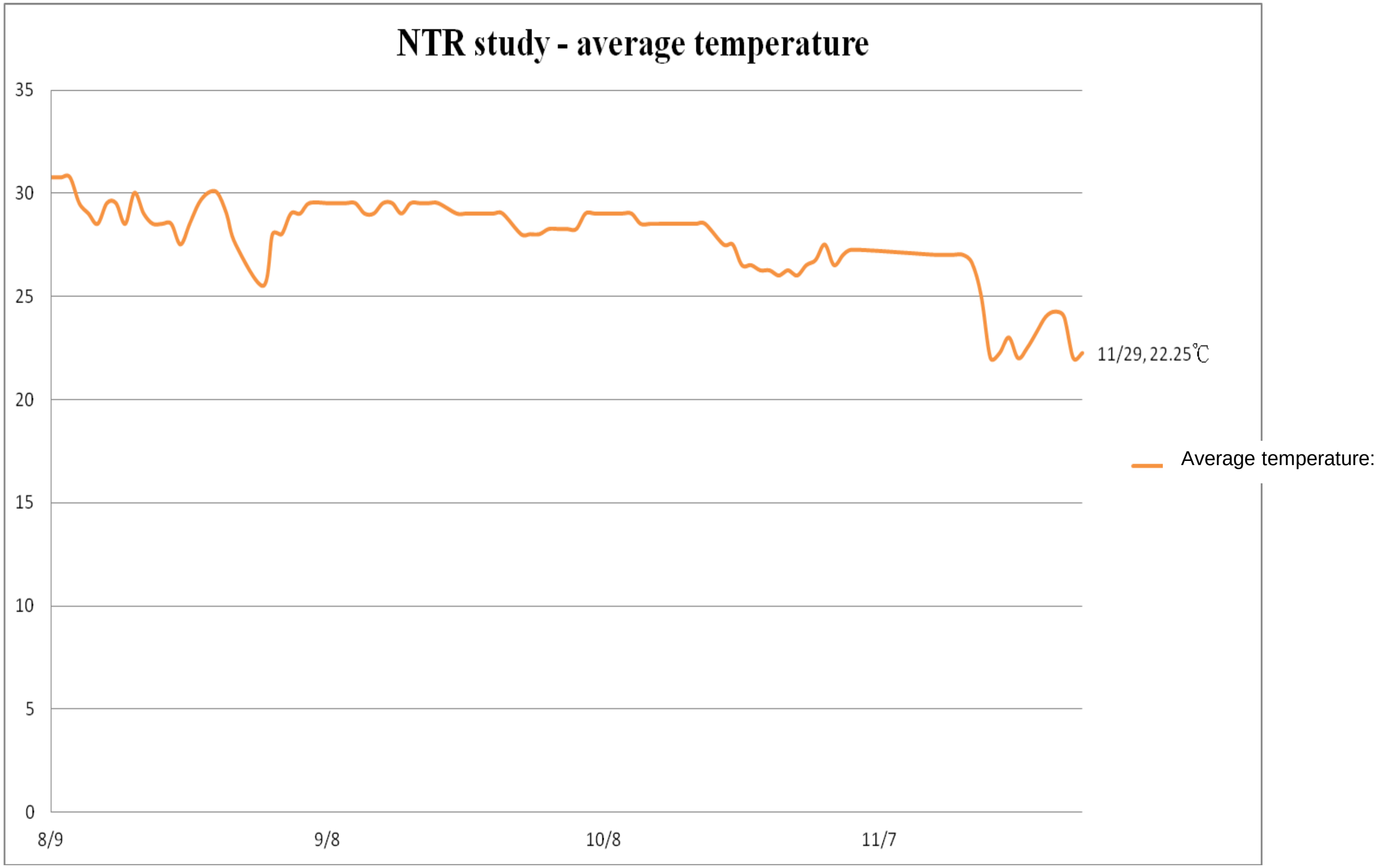
Trial conditions

- Fish were fed with 2-2.5% of their body weight per day (close to the maximal daily ration for tilapia according to feed consumption during the acclimation period of the study)
- The daily ration was divided into two equal meals (08:00 and 15:00 h) and hand-fed
- Water temperature was recorded daily
- Water quality parameters (ammonium and nitrite) weekly

Trial conditions (cont’s)

- Fish were weighed once every 3 weeks by National Pingtung University of Science and Technology, to monitor growth performance and adjust feeding rations

Temperature during the trial



Fish performance

	CONTROL 28/7	LOPRO 26/7+AG	% change vs control	statistics
Survival (%)	93.3 ± 2.2	96.3 ± 2.6	+3.2%	
Initial weight (g)	177.3 ± 1.8	174.7 ± 2.1	-1.5%	
Final weight (g)	469.6 ± 13.2	482.0 ± 12.1	+2.6%	
Daily weight gain (g/d)	2.32 ± 0.11	2.44 ± 0.08	+5.2%	
Feed intake (g/fish)	860.5 ± 21.6	840.3 ± 17.0	-2.4%	
Food Conversion Ratio (FCR)	2.95 ± 0.20	2.74 ± 0.04	-7.1%	P<0.05
Protein Efficiency Ratio (PER)	1.22 ± 2.2	1.40 ± 0.02	+14.8%	P<0.05
Protein retention (%)	27.70 ± 1.73	29.72 ± 0.04	+7.3%	P<0.05

Processing parameters

	CONTROL 28/7	LOPRO 26/7+AG	% change vs control
Hepatosomatic Index HSI (%)	3.30 ± 0.74	3.09 ± 0.26	-6.4%
Visceral fat (%)	6.31 ± 1.81	5.83 ± 1.62	-7.6%
Conditioning factor (g/cm3)	1.88 ± 0.14	2.02 ± 0.04	+7.4%
Filet yield (%) with skin	41.46 ± 0.17	41.91 ± 0.40	+1.1%
Filet yield (%) without skin	34.82 ± 0.14	35.20 ± 0.34	+1.1%

Processing the fish at Hung-Yi Frozen Food Factory, Pingtung

Visceral fat

Fish composition

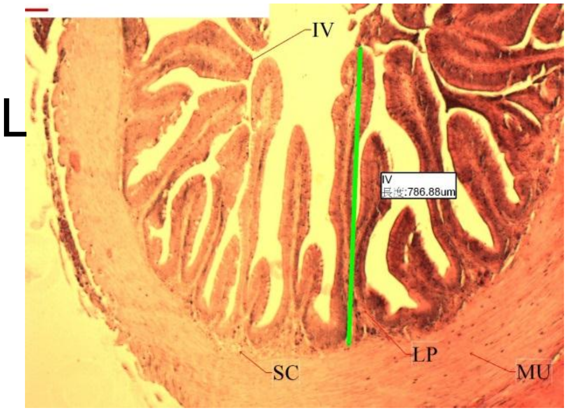
carcass composition	CONTROL 28/7	LOPRO 26/7+AG
moisture (%)	78.1 ± 0.4	77.6 ± 1.3
crude protein (%)	20.1 ± 1.3	19.3 ± 1.4
crude fat (%)	1.2 ± 0.7	1.7 ± 0.5
ash (%)	1.12 ± 0.10	1.07 ± 0.01

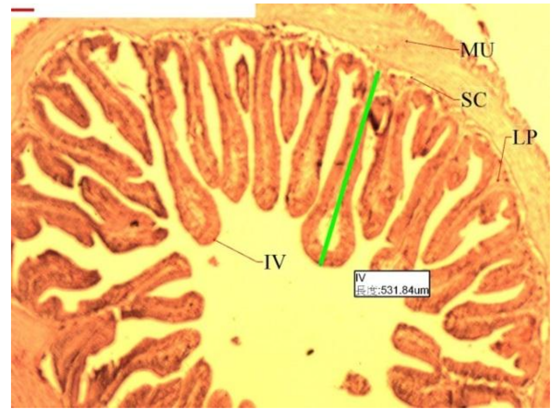
Haematological indices

haematological indices	CONTROL 28/7	LOPRO 26/7+AG	statistics
white blood cells	235 ± 11	236 ± 12	
red blood cells	2.55 ± 0.08	2.50 ± 0.24	
haemoglobin	10.12 ± 0.56	10.64 ± 0.82	
haematocrit	33.1 ± 2.3	31.6 ± 3.0	
plasma TG	15.73 ± 0.40	13.53 ± 0.80	$P<0.05$
G6PDH	163 ± 40	267 ± 29	$P<0.05$

Histology of the mid gut

CONTROL
28/7





LOPRO
26/7+AG

Images of the histomorphological changes (40X) with light filter of midgut in fish fed different diets for 18 weeks. IV, intestinal villus; LP, lamina propria, SC, stratum compactum; MU, muscularis.

gut histology	CONTROL 28/7	LOPRO 26/7	statistics
villi length (µm)	452 ± 42	341 ± 30	$P<0.05$

Fish fed the LOPRO diet supplemented with AQUAGEST OMF

- Better integrity of gut villi compared to fish fed control diet (less vacuolization)
- Gut villi showed a different morphology, resulting in shorter more robust villi

Summary

LOPRO 26/7 + AQUAGEST OMF 3 kg/MT versus CONTROL 28/7:

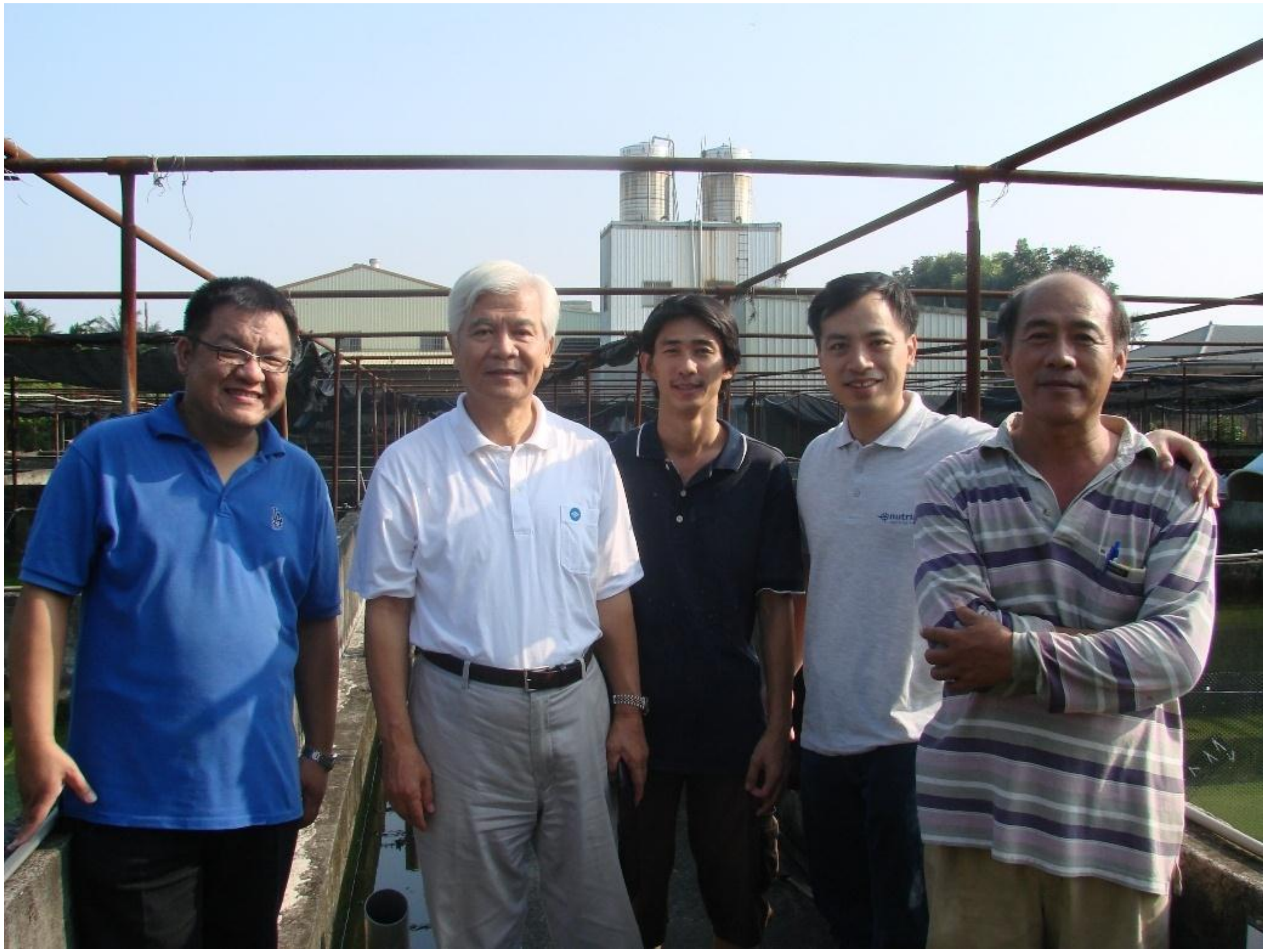
- Improved fish performance
  - Reduced FCR (-7.1%)
  - Improved growth (+5.2%)
  - Improved survival (+3.2%)
- More efficient conversion of feed protein into fish
  - Improved protein retention (+7.3%)
  - Improved protein efficiency ratio (+14.8%)
- Improved processing yield
  - Improved condition factor (+7.4%)
  - Improved fileting weight (+1.1%)
  - Reduced visceral fat (-7.6%)
- Carcass composition and hematology not affected
- Physiological indicators confirming mode of action:
  - Reduced plasma TG
  - Increased G6PDH
  - Improved integrity of gut villi in the midgut

Conclusion

Protein sparing effects for tilapia can be provided by

- lipid
- carbohydrate
- digestive/metabolic enhancing feed additives

Acknowledgement:



Thank you!