INTERNATIONAL RENDERING SYMPOSIUM

International Production and Processing Expo, Atlanta Georgia

“The use of Animal Proteins and Fats in Aquaculture”

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Actual Challenges

with an increasing demand of feed ingredients

Shortage of feed ingredients


Foto: Mendoza Rojas, 2019

-Post pandemic (COVID-19)

-Fewer industries

-Lower supply of raw materials

-Reduction in availability of ingredients (Russia-Ukraine and Israel-Hamas War)

-Increased competition for human food Job losses Inflation

Fishmeal

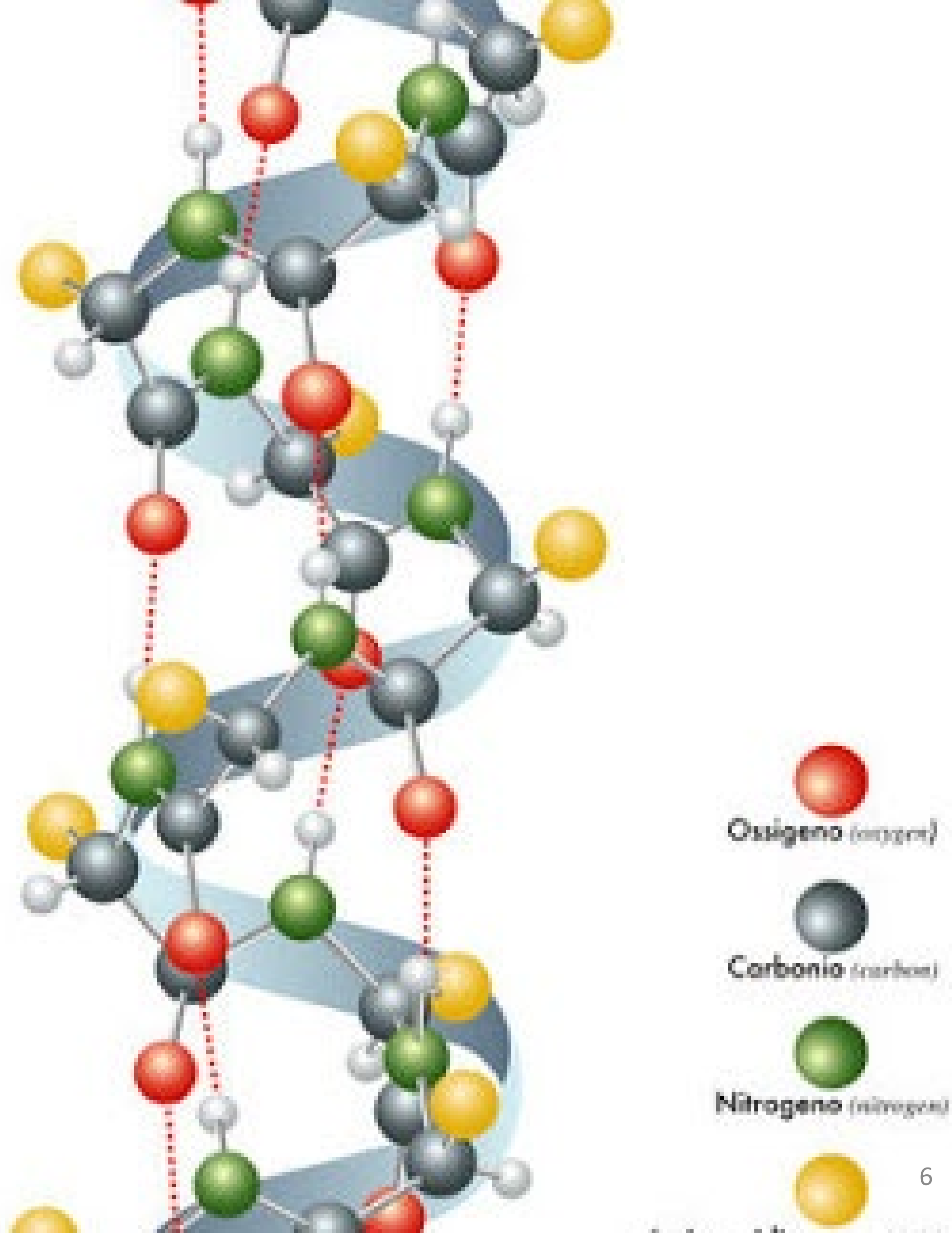
Aquafeed.com

Fishmeal properties

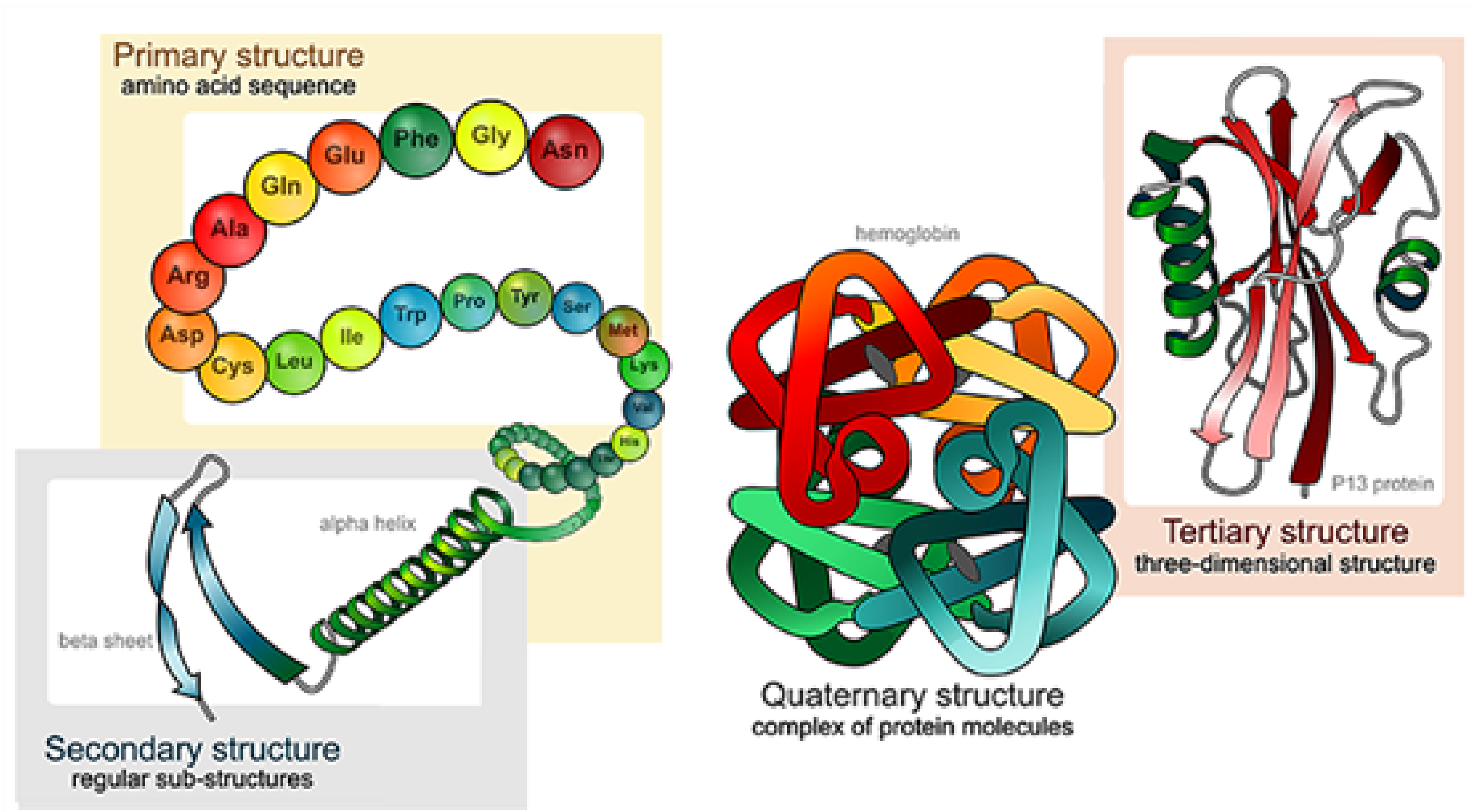
- High digestibility
- Nutrient characteristics:

Ideal: Amino acid and fatty acid profiles, cholesterol, palatability, growth promoters

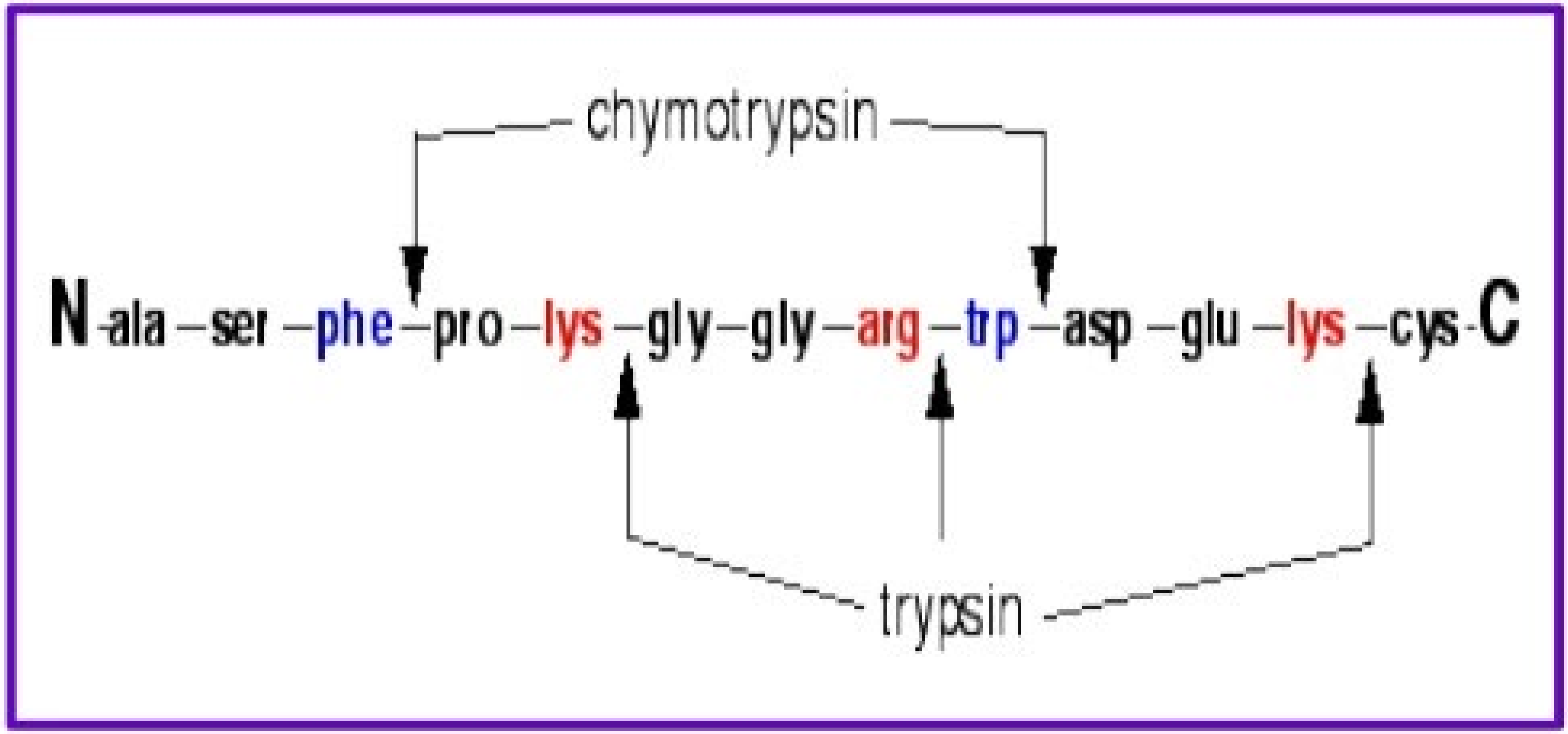
Biological value of proteins



Amino acids, structure and sequence



Broad specificity of Trypsin



In the Stomach:

parietal cells secrete HCl
chief cells secrete pepsinogen

endopeptidses

pepsinogen --(H^+)--> pepsin { general protease with preference for acidic & aromatic amino acids

Exocrine Pancreas secretion into the Small Intestine:

trypsinogen --(enteropeptidase)--> trypsin { arginine, lysine

chymotrypsinogen --(trypsin)--> chymotrypsin { tryptophane, phenylalanine, tyrosine, leucine

proelastase --(trypsin)--> elastase { alanine, glycine, serine

exopeptidases

procarboxypeptidases --(trypsin)--> carboxypeptidases

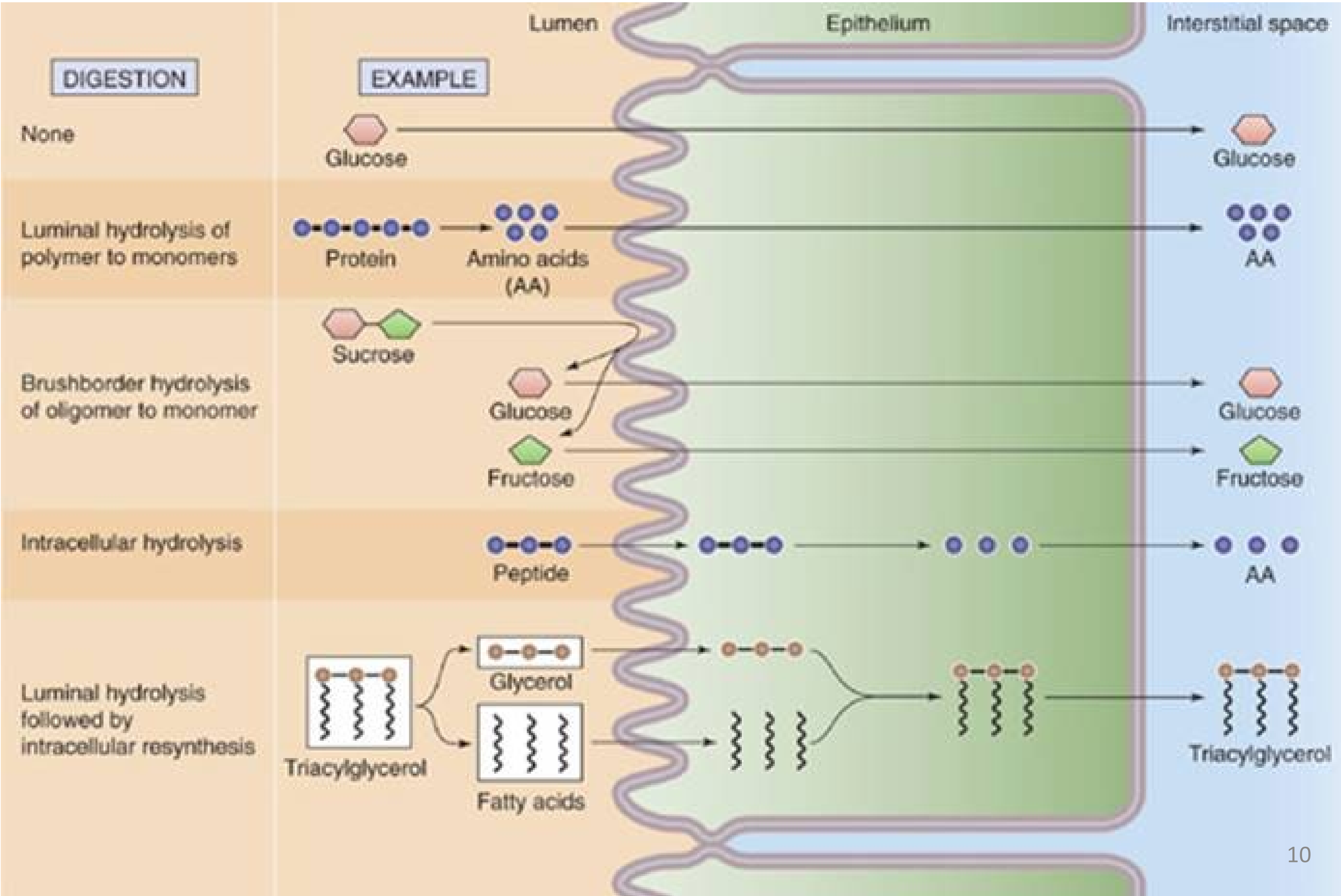
- A { hydrophobic amino acids
- B { basic amino acids: arginine, lysine

Secretion by the Brush Border of the Small Intestine:

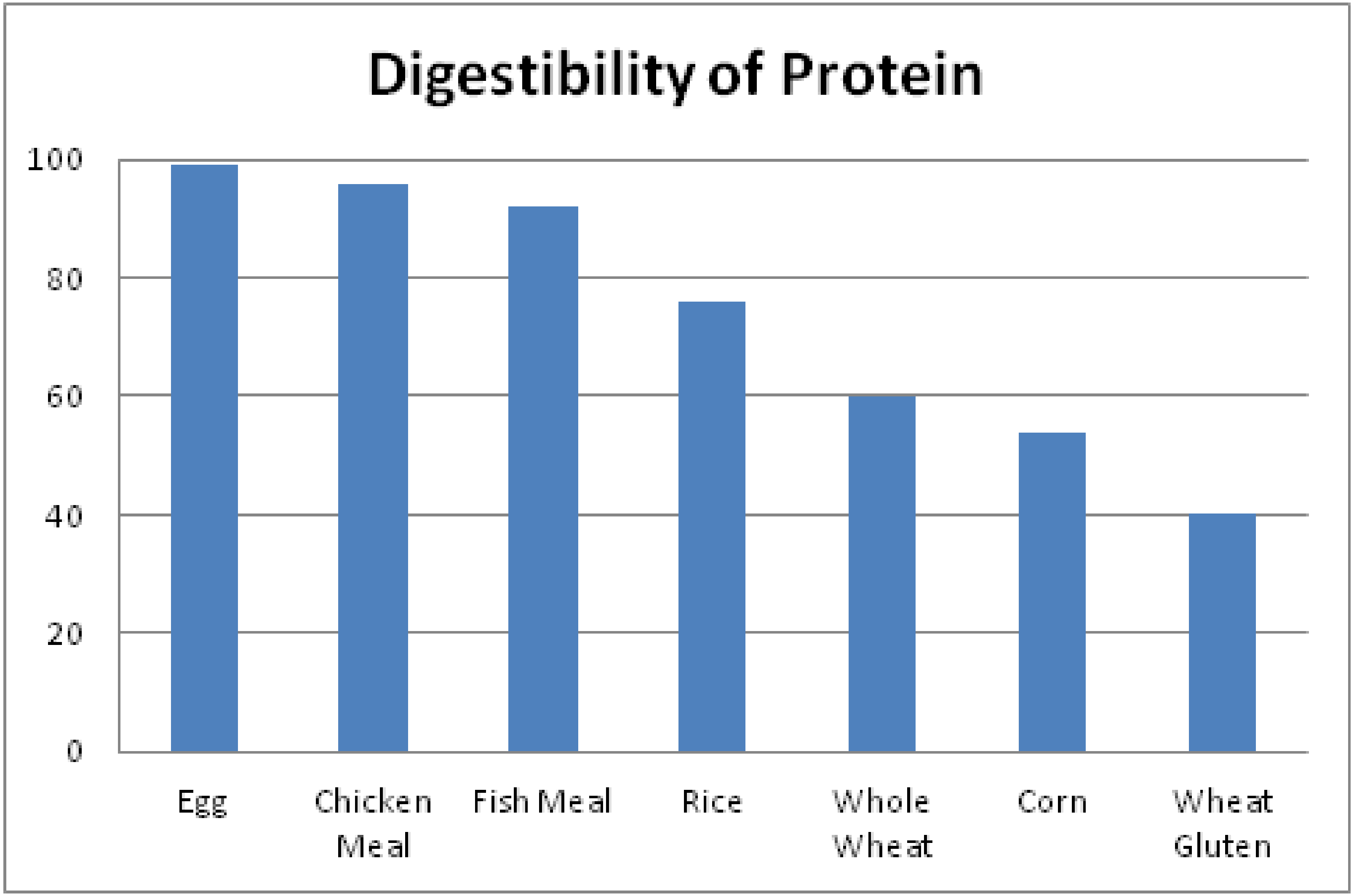
aminopeptidases { many

http://education.med.nyu.edu/mbm/aminoAcids/digestionAbsorption.shtml

Digestión y absorción



Why each type of protein has different digestibility?



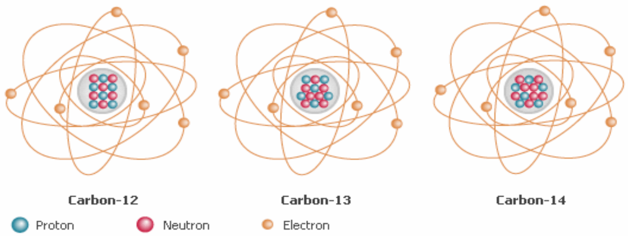
https://fourfriendspetfoods.co.uk/blog/article/digestibility-of-protein-in-high-quality-dog-food

HOWEVER.....

We need fishmeal substitution

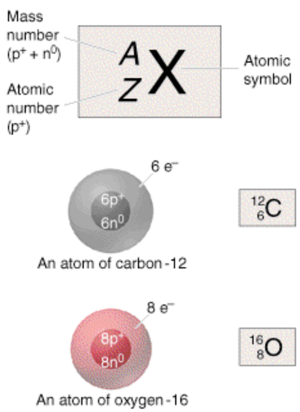
Substitution

How to measure protein assimilation?



Stable Isotopes

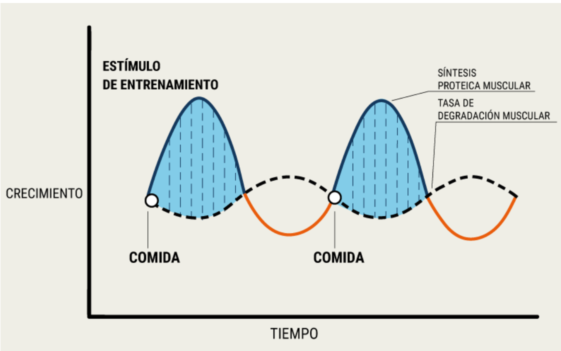
To study the protein assimilation



- ✓ **Atoms from the same element, but higher mass**
- ✓ **Organic mater C H O N S**

- ✓ **C : 6 protones y 6 neutrones = 12 IE 6 + 7 = 13**
- ✓ **N : 7 protones y 7 neutrones = 14 IE 7 + 8 = 15**

Protein turnover



https://www.foodspring.es/

Close to 70% of protein synthesis comes from recycled protein depending on age, organ, or species-specific

Muscular protein

Muscular synthesis

Muscle degradation

Amino acids

The protein that does not contain stable isotopes, that is, the light ones, is recycled with higher priority

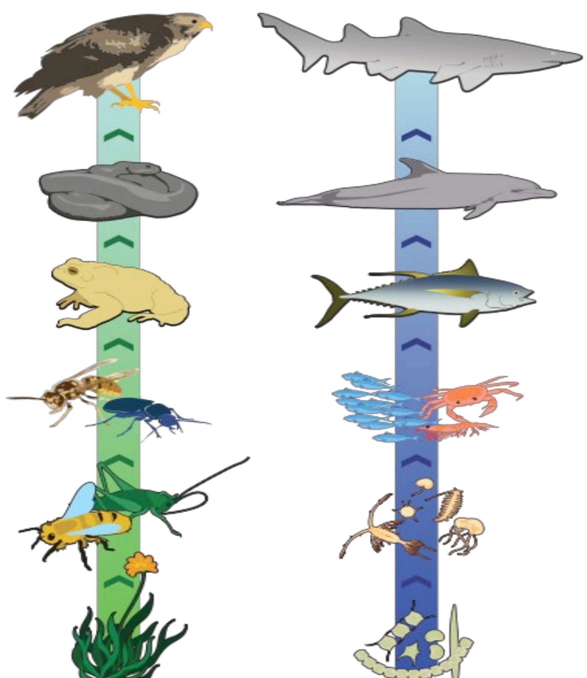
Choose protein sources with different isotopic concentration

First approach

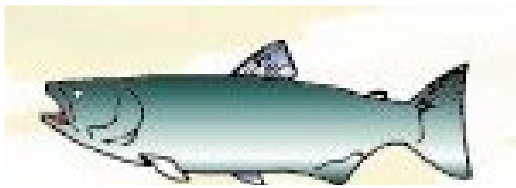
Formulated feeds for *Totoaba macdonaldi*, a carnivore fish

ISOTOPIC VALUE of ingredients:

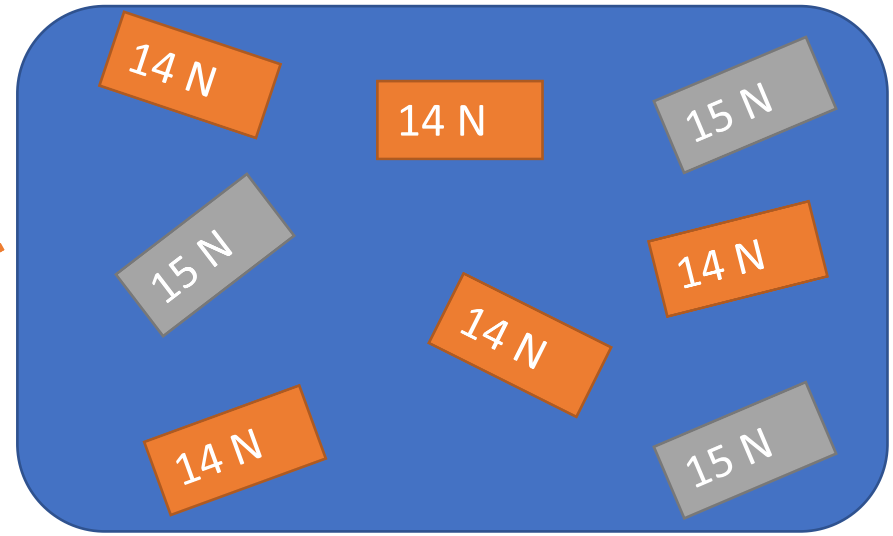
Fishmeal	$\delta^{15}N$ 14.1‰
Poultry by-product meal	$\delta^{15}N$ 3.4‰

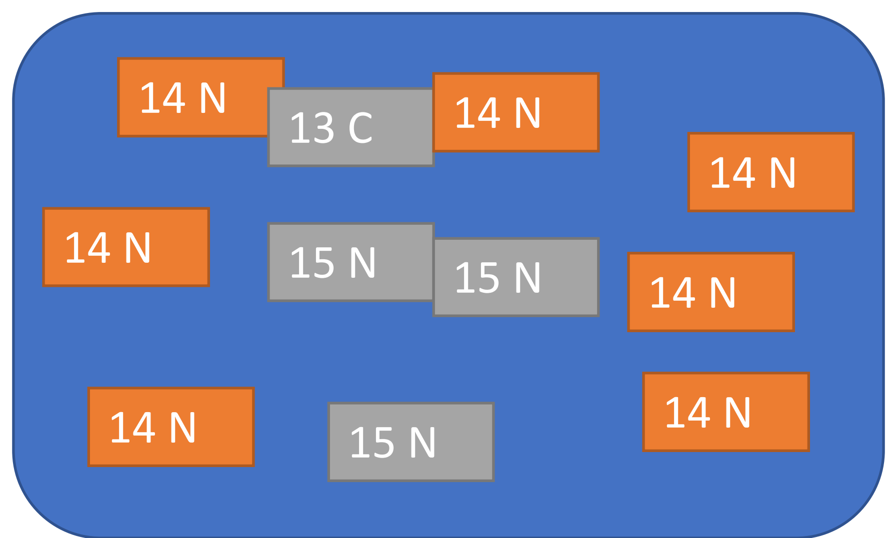


The relative abundance of an animal's stable isotopes reflects that of the diet

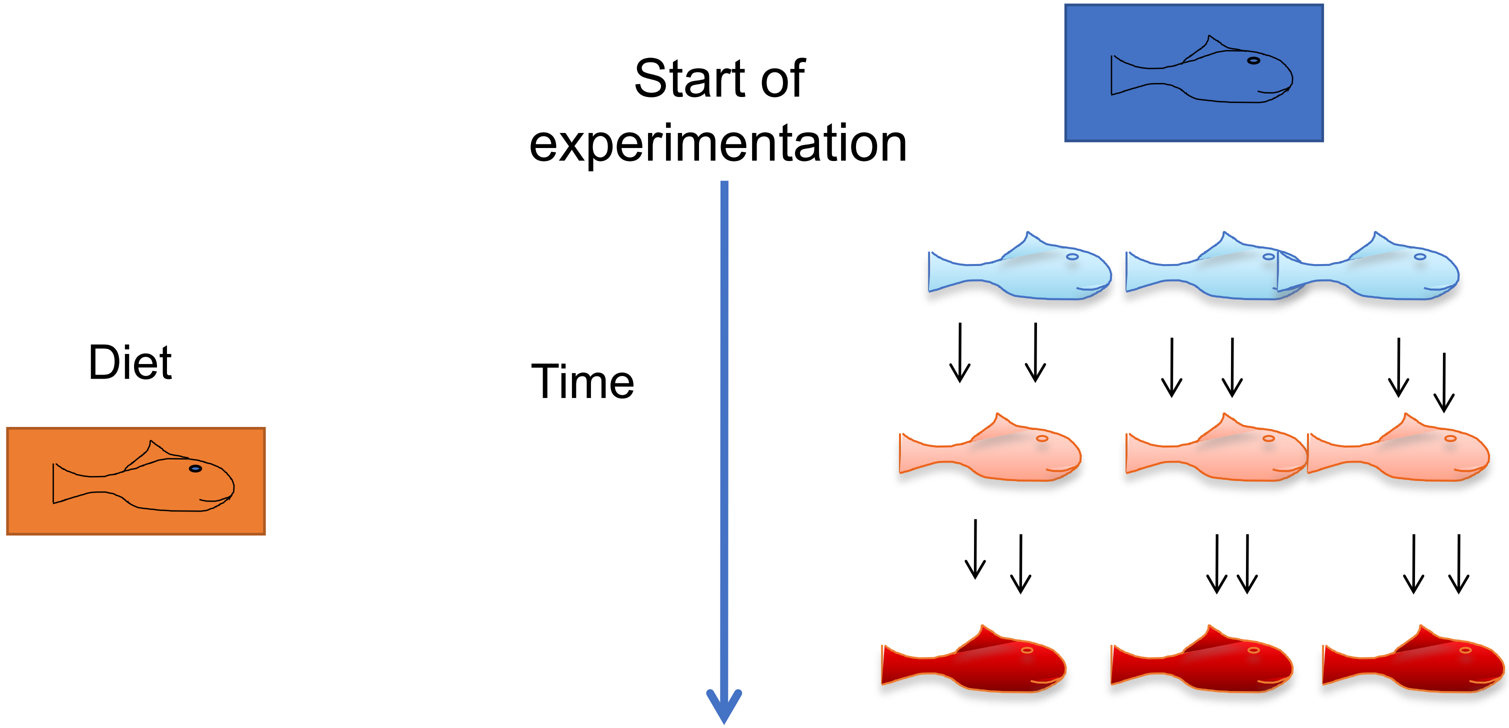


Diet





N	abundance
N14	99.66 %
N15	0.336 %



Isotopic turnover in tissues depends on the level of metabolic activity

From fastest to lowest: Blood, liver, muscle, skin, brain, collagen, bones,

Experiment with trout *Oncorhynchus mykiss*

Parés-Sierra et al., 2012

Ingredients (g per Kg), proximate composition on a dry weight basis (%) and $\delta^{15}N$ values of four experimental diets containing different levels of substitution of fishmeal with poultry by-products meal (PBM; 0, 33, 67 and 100% replacement). The four diets were fed to juvenile rainbow trout (*Oncorhynchus mykiss*) for 80 d. Fractional N contribution is expressed inside parentheses ( )

	TREATMENTS			
Ingredients	0PBM	33PBM	67PBM	100PBM
Poultry by-product meal (PBM)[1]	0.0 (0.0)	235 (0.3)	440 (0.6)	590 (0.9)
Fishmeal[2]	660 (0.9)	400 (0.5)	175 (0.2)	0 (0.0)
Corn meal	55 (0.01)	55 (0.01)	55 (0.01)	55 (0.01)
Poultry oil[3]	0.0	5.0	17	35
Fish oil[4]	72	52	28	0
Corn starch	91	131	166	201
Gelatin	60 (0.1)	60 (0.1)	60 (0.1)	60 (0.1)
Rovimix for carnivores fish[5]	30	30	30	30
Stay c[5]	4	4	4	4
Sodium Benzoate	2.3	2.3	2.3	2.3
Choline chloride	0.9	0.9	0.9	0.9
Tocopherol	0.1	0.1	0.1	0.1
Cellulose	25	25	25	25
TOTAL	1000	1000	1000	1000
Proximate composition (% of dry matter)				
Crude protein (%)	43.1	43.4	43.7	43.6
Crude fat (%)	12.5	12.5	12.5	12.3
Ash (%)	19.8	15.1	12.1	8.8
NFE[6]	24.5	29.0	31.7	35.3
Diet isotopic composition				
$\delta^{15}N$ (‰)	12.7	9.0	6.1	4.2

Parés-Sierra et al., 2012

Biological indices, for juvenile rainbow trout (*Oncorhynchus mykiss*) fed four experimental diets formulated to contain similar protein and lipid levels. Fishmeal and fish oil were incrementally substituted for poultry by-product meal (PBM; 0, 33, 67 and 100%) and poultry oil. Measurements were made after 80 d. Values within the same row with different superscripts letters were significantly different ($P<0.05$).

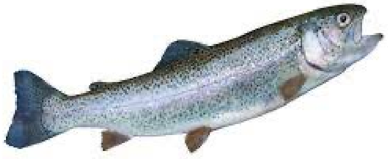
	Experimental Treatments			
	0PBM	33PBM	67PBM	100PBM
Biological indices				
Final weight (g)	21.8±1.5^{ab}	21.9±1.1^{ab}	23.5±1.6^{a}	20.9±0.5^{b}
TGC[1]	1.48±0.05	1.48±0.01	1.54±0.05	1.47±0.02
Weight gain (% of initial)	1480±39	1495±32	1587±32	1495±103
PER[2]	2.85±0.1	2.72±0.3	2.78±0.2	2.77±0.1

Parés-Sierra et al., 2012

Percentage of nitrogen retention by rainbow trout (*Oncorhynchus mykiss)* fed formulated diets with two levels (33 and 67%) replacement of fishmeal (FM) with poultry by-product meal (PBM). Nitrogen retention was estimated using a simple two-source mixing model considering the isotopic composition of the fishmeal and poultry by-product meal itself

		Treatments	
		33PBM	67PBM
Mixture model			
	Fishmeal	58.4 (30.8; 49.5)	24.8 (35.3; 15.9)
	PBM	41.6 (69.2; 50.5)	75.5 (64.5; 84.1)

	$\delta^{15}N$	**$\delta^{13}C$**
HP	14.0	-18.2
HA	3.4	-16.5



Parés-Sierra et al., 2012

Totoaba macdonaldi carnivore fish

Badillo et al., 2016

Ingredients (%) diets with different levels of replacement of FM for PBM in *Totoaba macdonaldi*

	Dietary treatments			
INGREDIENTS	**0PBM***	**33PBM***	**67PBM***	**100PBM***
PBM	0.0	23.5	44.0	59.0
FM	66.0	40.0	17.5	0.0
Poultry fat	0.0	0.5	1.7	3.5
Fish oil	7.2	5.2	2.8	0.0
Starch	9.1	13.1	16.6	20.1
Corn meal	5.5	5.5	5.5	5.5
Gelatin	6.0	6.0	6.0	6.0
Rovimix DSM	3.0	3.0	3.0	3.0
Stay C	0.4	0.4	0.4	0.4
Sodium benzoate	0.2	0.2	0.2	0.2
Tocopherol	0.01	0.01	0.01	0.01
Cellulose	2.5	2.5	2.5	2.5
Total	**100**	**100**	**100**	**100**

*PBM = % added into the diet

Badillo et al., 2016

Proximate composition of the diets and isotopic value δ^{13}C and δ^{15}N use to feed juvenile Totoabas fish (*Totoaba macdonaldi*) for 86 days.

Proximate composition	Tratamientos experimentales 0PBM*	33PBM*	67PBM*	100PBM*
Protein (%)	51.1	51.6	51.6	51.1
Lípids (%)	7.9	8.0	8.1	8.0
Ash (%)	15.5	14.1	11.5	11.4
NFE **	27.1	28.7	30.3	31.6
δ^{15}N (‰)	15.3	11.5	7.7	4.7
δ^{13}C (‰)	-15.5	-16.1	-15.2	-14.6

* PBM = Nivel de Inclusión de harina de subproducto de ave
** NFE = 100 - (% proteína cruda + % grasa cruda + % cenizas)

Badillo et al., 2016

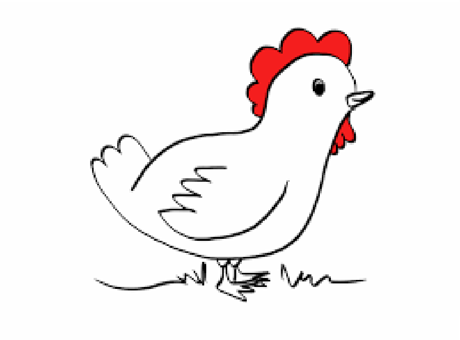
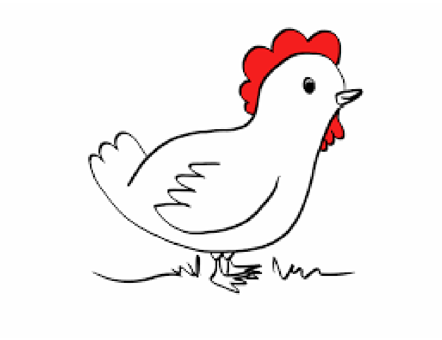
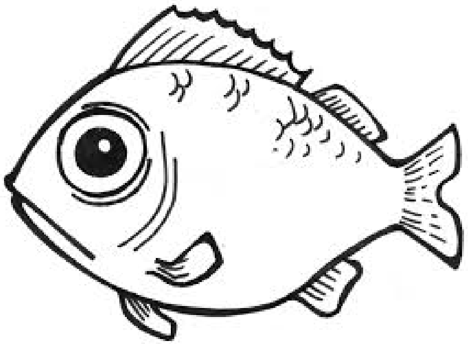
Biological index for totoaba (*Totoaba macdonaldi*) juveniles fed with four experimental diets with the same level of protein and lipids. Poultry by-product meal increased when replacing fish meal (HA; 0, 33, 67 and 100%). Values with different super index indicate statistical differences at P<0.05.

		Dietary treatments			
Biological index	Inicial	0PBM*	33PBM*	67PBM*	100PBM*[1]
SGR ** (% dia^{-1})		2.6±0.1[b]	2.9±0.2[b]	3.5±0.0[a]	1.6±0.5
TGC * **		0.70±0.0[b]	0.82±0.1[b]	1.06±0.2[a]	0.52±0.1
Survival rate (%)		76.0±10.6[ab]	78.7±10.1[a]	89.3±2.4[a]	52.4±3.8
Proximate composition of muscle (% dry matter)					
Crude proteína (%)	82.7±0.6	81.1±1.3	82.6±0.7	81.3±1.1	82.2±0.2
Crude fat (%)	1.3±0.2	2.2±0.4	2.2±0.9	2.1±0.1	2.3±0.7
Ash (%)	8.9±0.4	4.8±0.1[b]	5.6±0.2[a]	6.1±0.1[a]	5.9±0.3
ELN** (%)**	7.0	11.9	9.6	10.5	9.6

*PBM = % added into the diet
**SGR = Specific growth rate: 100*[(ln final weight- ln initial weight) / days]
***TGC =Thermal growth coeficient
****NFE = 100 - (% crude protein+ % crude fat + % ash)

Badillo et al., 2016

Based on the model 2 to 1

Ingredient composition of diets g Kg-1 (51% CP and 12% GC) to feed *Totoaba macdonaldi* with different levels of fish oil replacement. Using tallow and microalgae concentrate.

	Tratamientos			
Ingredientes g Kg^{-1}	**PBM/FM**	**PBM/FM+Tallow**	**PBM/FM+Tallow 50% DHA**	**PBM/FM+Tallow 100% DHA**
Fish meal	150.0	150.0	150.0	150.0
Poultry byproduct meal	480.4	480.4	480.4	480.4
starch	221.2	221.0	219.0	215.0
Gelatin	80.0	80.0	80.0	80.0
Fish oil	25.0	0	0	0
Beef tallow	**0**	**25.0**	**25.0**	**25.0**
DHA/EPA concentrate*	0	0	2.0	6.0
Otros	43.4	43.6	43.6	43.6

PBM: Poultry by-producto meal
FM: Fishmeal
* From microalgae

Mata et al., 2018

Fatty acids composition (mg g-1 lipid) of fish oil, beef tallow and the diets fed to *Totoaba macdonaldi* containing beef tallow and two levels of DHA compared to fish oil.

		Complementary lipid sources		Dietary treatments			
	Fatty Acids	Fish oil	Tallow	PBM+FO	PBM+ TALLOW	PBM+TALLOW + 50% DHA	PBM+TALLOW + 100% DHA
	14:0	103.55	31.92	21.32	14.10	16.61	20.68
	16:0	279.20	267.99	242.42	238.66	250.93	231.55
	18:0	60.41	175.24	76.10	105.75	83.05	93.87
	Other SFAs[a]	30.49	15.32	10.35	6.69	7.30	7.06
	Σ SFAs[b]	473.65	490.47	350.19	365.19	357.89	353.17
	16:1n-7	108.1	28.03	66.25	61.22	58.43	55.17
	18:1n-7	33.08	26.37	11.65	9.31	15.10	10.72
	18:1n-9	142.03	350.69	268.89	311.04	317.50	320.08
	20:1n-9	ND	5.85	3.77	3.46	4.08	5.57
	Other MUFAs[c]	3.71	ND	3.60	1.13	2.00	1.91
	Σ MUFAs[d]	286.92	410.94	354.16	386.16	397.10	393.44
	18:2n-6	34.51	21.67	133.32	132.55	127.54	128.64
	20:4n-6	3.95	ND	1.55	0.56	0.48	0.99
	Other n-6[e]	6.17	ND	4.71	4.20	4.92	4.91
	Σ n-6[f]	44.63	21.67	139.58	137.32	132.95	134.55
	18:3n-3	13.08	0.66	11.23	10.83	9.00	9.40
	20:5n-3	55.79	ND	30.14	5.07	6.31	5.97
	22:5n-3	6.13	ND	3.58	2.14	1.93	2.19
DHA	**22:6n-3**	47.91	ND	29.99	9.25	12.70	17.68

Biological indices of totoabas fed with different levels of fish oil replacement

Biological index	Tratamientos			
	PBM/FM	**PBM/FM+Tallow**	**PBM/FM+Tallow 50% DHA**	**PBM/FM+Tallow 100% DHA**
Survival	96±4	100	92±8	92±4
SGR	2.63±0.07	2.58±0.05	2.64±0.04	2.63±0.11
FCR	1.39±0.2[ab]	1.59±0.03[ab]	1.18±0.3[ab]	1.09±0.08[a]

SGR= Specific growth rate: 100*[(ln final weight- ln initial weight) / days]
FCA= Conversion efficiency: feed ingestion/growth

Mata et al., 2018

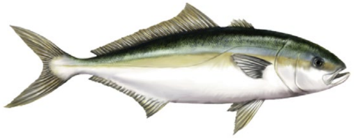
Sustitución parcial a total de harina y aceite de pescado en dietas para *Seriola dorsalis*

Manriquez-Patiño et al., 2021

Table 1. Ingredient composition and proximate analysis, and amino acid content of diets formulated to contain 45% crude protein (CP) and 12% crude fat (CF), used for experimental diets to replace fishmeal and fish oil replaced by poultry by-product meal and bovine tallow with an algae extract (DHA source). Diets were fed to juvenile yellowtail, *Seriola dorsalis*, for 48 days. Amino acids were calculated from the ingredient content.

(Manriquez-Patiño et al., 2021)

	TREATMENTS			
Ingredients	**Control**	**T-Low**	**T-Med**	**T-Total**
Fish meal [a]	21	14	7	0
Poultry by product meal [b]	22	29.3	36.6	44
Yeast by-product (77% CP) [c]	5	5	5	5
Soybean meal 42% [d]	4	4	4	4
Yeast by-product (48% CP) [e]	4	4	4	4
Gelatin [f]	6	6	6	6
Corn gluten [g]	4	4	4	4
Rovimix [h]	2	2	2	2
Stay C [i]	0.1	0.1	0.1	0.1
Taurine [j]	2	2	2	2
Beef tallow [k]	0	2.7	2.8	2.9
Starch [l]	21.6	21	20.9	20.7
Lysine [m]	1	1	1	1
Methionine [n]	0.4	0.4	0.4	0.4
DHA-Nature™ (24% DHA) [o]	1	2.80	3.40	4
Fish oil [p]	6	1.8	0.9	0
Sodium benzoate [q]	0.2	0.2	0.2	0.2
Choline chloride [q]	0.09	0.09	0.09	0.09
Cholesterol [r]	0	0.2	0.25	0.3



Proximate composition and amino acid content in diets

Ingredients	TREATMENTS Control	T-Low	T-Med	T-Total
Proximal composition (% dry matter)				
Crude protein (%)	45.37	45.32	45.31	45.30
Crude fat (%)	12.08	12.10	12.09	12.11
Ash (%)	2.5	2.6	2.7	2.7
NFE (by difference)				
Aminoacid content (%)				
Methionine	0.94	0.91	0.88	0.84
Methionine+Cysteine	0.70	0.68	0.67	0.66
Cysteine	0.21	0.22	0.22	0.22
Lysine	2.55	2.58	2.59	2.61
Taurine	1.91	1.89	1.87	1.84
Threonine	1.06	1.04	1.02	1.00
Valine	1.28	1.25	1.21	1.17
Arginine	1.75	1.77	1.80	1.82
Tryptophan	0.19	0.17	0.15	0.13
Isoleucine	1.03	1.00	0.96	0.93
Leucine	1.74	1.71	1.68	1.65
Phenylalanine	0.94	0.92	0.90	0.88
Tyrosine	0.47	0.43	0.38	0.32

(Manriquez-Patiño et al., 2021)

Table 2. Fatty acid content of four different diets formulated to contain from partial to total substitution of fish meal and fish oil fed to juvenile yellowtail (*Seriola dorsalis*) for 48 days.

Fatty acids	TREATMENTS			
	Control	**T-Low**	**T-Med**	**T-Total**
14:0	6.96	7.42	8.19	8.41
16:0	22.60	24.15	24.53	24.62
16:1n-7	6.72	4.30	4.69	4.47
18:0	5.20	8.52	8.58	8.52
18:1n-9	18.94	28.41	25.53	26.39
18:1n-7	2.61	1.88	2.10	1.88
18:2n-6	10.35	12.38	11.26	11.52
18:3n-3	1.41	1.08	0.93	0.67
18:4n-3	0.91	0.17	0.25	0.10
20:0	0.83	0.50	0.44	0.29
20:2n-6	0.40	0.34	0.36	0.32
20:4n-6	1.52	0.88	0.94	0.89
20:5n-3	6.23	0.78	0.99	0.23
22:5n-3	1.12	0.37	1.03	0.24
22:6n-3	7.19	4.43	5.05	5.75
24:1n-9	0.48	0.23	0.39	0.26
Others	6.48	4.19	4.61	5.56
Total	**100**	**100**	**100**	**100**

(Manriquez-Patiño et al., 2021)

Table 3. Biological indices of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=3) are given. Values in the same row with a different superscript were significantly different (P <0.05).

Biological indices		**TREATMENTS**			
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
Initial weight (g)	14.51±0.02	14.30±0.14	14.77±0.26	14.55±0.18	0.122
Final weight (g)	43.48±1.41	37.90±2.17	42.88±9.95	39±1.82	0.065
Weight gain (%)	200.94±9.39	163.2±16.79	191.4±25.28	168.8±15.2	0.087
Total initial length (cm)	10.98±0.09	10.82±0.15	10.99±0.10	10.92±0.05	0.285
Total final length (cm)	15.42±0.154[a]	14.42±0.174[b]	14.99±0.516[ab]	14.83±0.30	0.031
SGR (%/day)	2.28±0.06	2.02±0.13	2.21±0.22	2.05±0.11	0.170
TCG	1.01±0.05	0.82±0.08	0.98±0.14	0.85±0.07	0.096
FI (% day $^{-1}$)	2.95±0.11	3.09±0.02	2.99±0.10	2.95±0.29	0.723
FCR	1.39±0.06	1.61±0.08	1.45±0.16	1.52±0.10	0.177
PER	1.59±0.07	1.37±0.07	1.53±0.18	1.46±0.10	0.203
HIS	2.23±0.24	1.87±0.44	1.89±0.23	2.34±0.72	0.510
VIS	17.10±0.80	15.17±2.01	15.73±0.76	17.33±3.79	0.596
Survival (%)	100	100	100	100	
CF					

SGR = (ln final weight –ln initial weight)*100/t
TGC = [(final weight ⅓ - initial weight ⅓) / (T°C x days)] x 1000.
FCR = total feed consumed / wet weight gained.
Protein Efficiency Ratio (PER) = weight gain / protein intake.
FI = 100 x (total amount of the feed consumed / ((initial body weight + final body weight) / 2) / days).
HSI = (hepatopancreas weight / body weight) x 100.
VSI = (viscera weight / body weight) x 100.
Condition factor (CF) (g cm^3-1)= total fish weight (g) / $length^3$ (cm)

(Manriquez-Patiño et al., 2021)

Table 2. Fatty acid content of four different diets formulated to contain from partial to total substitution of fish meal and fish oil fed to juvenile yellowtail (*Seriola dorsalis*) for 48 days.

Fatty acids	TREATMENTS			
	Control	**T-Low**	**T-Med**	**T-Total**
14:0	6.96	7.42	8.19	8.41
16:0	22.60	24.15	24.53	24.62
16:1n-7	6.72	4.30	4.69	4.47
18:0	5.20	8.52	8.58	8.52
18:1n-9	18.94	28.41	25.53	26.39
18:1n-7	2.61	1.88	2.10	1.88
18:2n-6	10.35	12.38	11.26	11.52
18:3n-3	1.41	1.08	0.93	0.67
18:4n-3	0.91	0.17	0.25	0.10
20:0	0.83	0.50	0.44	0.29
20:2n-6	0.40	0.34	0.36	0.32
20:4n-6	1.52	0.88	0.94	0.89
20:5n-3	6.23	0.78	0.99	0.23
22:5n-3	1.12	0.37	1.03	0.24
22:6n-3	7.19	4.43	5.05	5.75
24:1n-9	0.48	0.23	0.39	0.26
Others	6.48	4.19	4.61	5.56
Total	**100**	**100**	**100**	**100**

Table 6. Fatty acid content in the muscle tissue of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=3) are given.

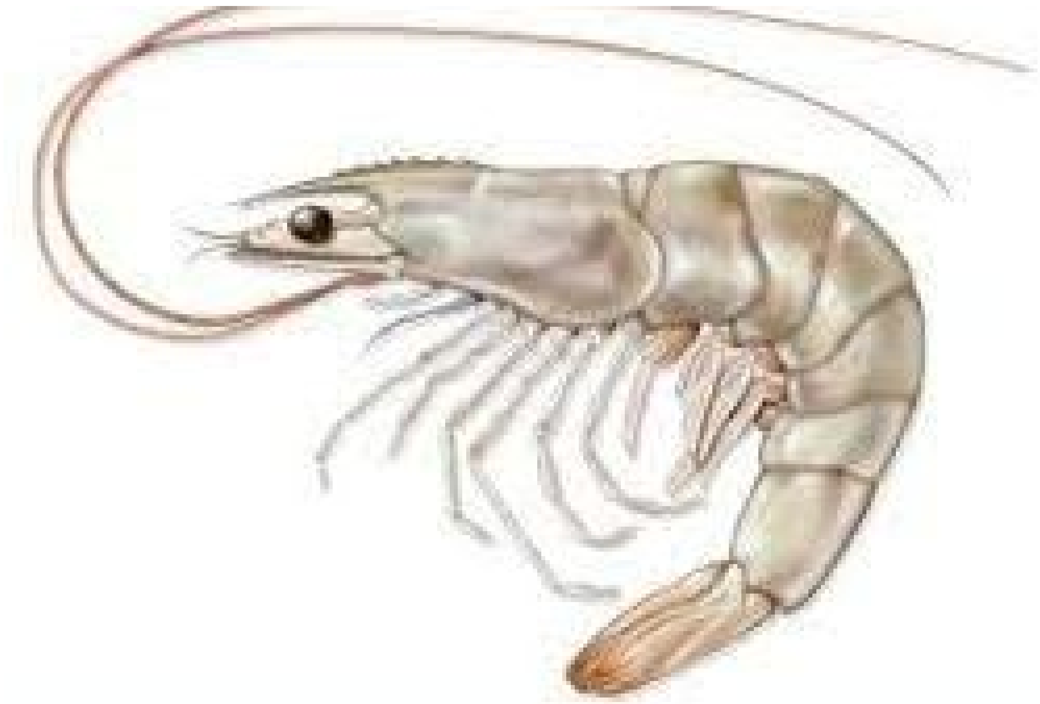
Fatty acids	TREATMENTS				
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
14:0	3.38 ± 0.87^{b}	2.29 ± 0.10^{a}	1.98 ± 0.59^{a}	2.10 ± 0.51^{a}	0.01
16:0	17.20 ± 0.00^{a}	16.64 ± 0.23^{a}	15.33 ± 0.23^{b}	15.16 ± 1.12^{b}	0.007
16:1n-7	3.58 ± 0.21^{c}	2.46 ± 0.12^{ab}	2.26 ± 0.18^{a}	2.84 ± 0.51^{ab}	0.001
18:0	8.84 ± 0.03	9.08 ± 0.31	8.49 ± 0.17	9.32 ± 1.58	0.380
18:1n-9	11.61 ± 0.27^{b}	18.63 ± 0.78^{a}	17.65 ± 0.41^{a}	20.10 ± 4.08^{a}	0.001
18:1n-7	3.72 ± 0.19^{c}	3.07 ± 0.22^{b}	2.63 ± 0.23^{b}	1.53 ± 0.51^{a}	0.001
18:2n-6	7.51 ± 0.28^{c}	14.77 ± 1.07^{a}	13.18 ± 0.35^{b}	14.70 ± 1.14^{a}	0.001
18:3n-3	1.41 ± 0.05^{a}	1.05 ± 0.03^{bc}	0.93 ± 0.35^{ab}	0.49 ± 0.38^{c}	0.001
18:4n-3	1.05 ± 0.10^{b}	0.34 ± 0.07^{a}	0.28 ± 0.08^{a}	0.40 ± 0.27^{a}	0.001
20:0	1.53 ± 0.20^{a}	1.04 ± 0.05^{b}	0.84 ± 0.04^{ab}	0.76 ± 0.09^{c}	0.001
20:2n-6	0.78 ± 0.09^{a}	0.51 ± 0.16^{b}	0.64 ± 0.03^{ab}	0.63 ± 0.13^{ab}	0.250
20:4n-6	3.20 ± 0.18^{a}	2.12 ± 0.27^{c}	2.65 ± 0.20^{b}	2.04 ± 0.10^{c}	0.001
20:5n-3	6.53 ± 0.30^{a}	2.96 ± 0.28^{b}	3.02 ± 0.37^{b}	1.40 ± 0.63^{c}	0.001
22:5n-3	2.26 ± 0.11^{a}	2.48 ± 0.17^{b}	4.20 ± 0.04^{c}	4.72 ± 0.03^{d}	0.001
22:6n-3	20.90 ± 0.48^{ab}	18.29 ± 0.69^{b}	21.81 ± 1.43^{a}	20.38 ± 3.24^{ab}	0.050
Others	6.49^{a}	4.26^{c}	4.12^{bc}	3.26^{c}	0.001
Total	**100**	**100**	**100**	**100**	

Values in the same row with a different superscript were significantly different ($P < 0.05$).

(Manriquez-Patiño et al., 2021)

¿What about shrimp?

- **Studies have shown that fishmeal can be replaced**
- **However, we must continue looking for alternatives as raw material prices continue to rise**



Litopenaeus vannamei

Effect of soybean meal as substitute of fishmeal or PBM in *Litopenaeus vannamei*

Ingredients and proximate composition of diets

INGREDIENTS	Fishmeal	PBM	PBM+SBM-L	PBM+SBM-H
Soybeam meal			35	47
poultry by-product meal		63	32.5	18
Fish meal	61			
Corn gluten			8	15
Soybean oil	1.5		2.7	3
Fish oil	2	1	2.5	4

Paramter	FM	PBM	PBM+SBM-L	PBM+SBM-H
Protein	45.8±0.1	48.3±1.6	43.5±0.9	42.6±0.3
Lipid	11.21±0.1	12.82±0.3	14.24±0.5	13.57±0.2
Ash	14.42±0.0	9.65±0.0	7.75±0.1	7.24±0.1
NFE	28.57	29.23	34.71	36.59

Unpublished results

Effect of soybean meal as substitute of fishmeal or PBM in *Litopenaeus vannamei*

Biological indices of Litopenaeus vannamei fed with different protein sources and mixture

	Experimental diets			
	FM	PBM	PBM+SBM-L	PBM+SBM-H
Growth performance				
Initial weight (g)	0.177 ± 0.02	0.165 ± 0.02	0.147 ± 0.03	0.173 ± 0.01
Final weight (g)	1.13 ± 0.07	1.02 ± 0.15	1.03 ± 0.11	0.81 ± 0.09
SGR (% $día^{-1}$)	4.23 ± 0.58[a]	4.12 ± 0.58[a]	4.5 ± 1.09[a]	3.46 ± 0.6[b]
WGR (%)	538.8	521.5	598.8	367.6
Survival (%)	96.00 ± 4.00	97.33 ± 2.31	96.00 ± 6.93	90.67 ± 8.33

Unpublished results

The substitution effect of fish meal and poultry by-products by Soybean meal (60 days)

Ingredient formulation and proximate composition (g kg^{-1} on a dry matter basis, DM) of diets containing different levels of soybean meal (SBM) used to fed *Litopenaeus vannamei* juveniles for 60 days.

50/50 75/25 100

Ingredients, g kg^{-1} DM	Experimental diets			
	SBM Free	SBM L	SBM M	SBM H
Fish meal	180.0	90.0	45.0	0.0
Poultry byproduct meal	190.0	95.0	47.5	0.0
Soybean meal	0.0	296.8	445.4	593.6
White wheat meal	120.0	120.0	74.5	0.0

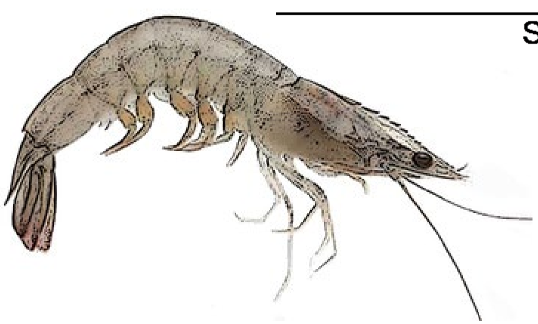
Parameters	SBM Free	SBM L	SBM M	SBM H	P Val
Total protein (g dL^{-1})	9.20 ± 0.39[a]	8.78 ± 0.10[a]	7.91 ± 0.35[b]	8.56 ± 0.25[a]	0.0
Glucose (mg dL^{-1})	52.26 ± 2.95[a]	30.92 ± 4.02[b]	36.67 ± 8.60[a]	104.77 ± 41.09[a]	0.0
Cholesterol (mg dL^{-1})	29.35 ± 3.1[a]	35.74 ± 11.99[a]	34.28 ± 1.78[a]	30.33 ± 2.18[a]	0.3
Triglycerides (mg dL^{-1})	70.68 ± 13.35[a,b]	50.06 ± 10.18[a]	100.87 ± 13.76[b]	123.11 ± 14.79[b,c]	0.0

The substitution effect of fish meal and poultry by-products by Soybean meal (60 days)

Growth performance, hepatosomatic index and muscle tissue proximate composition of *Litopenaeus vannamei* juveniles fed diets containing different levels of soybean meal (SBM).

Initial weight 5.5 g	Experimental diets			
	SBM Free	SBM L	SBM M	SBM H
Growth performance				
Final weight (g)	16.17 ± 0.79^{a}	16.93 ± 0.74^{a}	15.39 ± 2.06^{a,b}	13.96 ± 0.78^{b}
SGR (% día^{-1})	1.76 ± 0.10^{a}	1.81 ± 0.04^{a}	1.63 ± 0.15^{a}	1.47 ± 0.09^{b}
WGR (g semana^{-1})	1.23 ± 0.10^{a}	1.31 ± 0.07^{a}	1.12 ± 0.21^{a,b}	0.96 ± 0.09^{b}
Biomass gain (kg)	246.76 ± 6.06^{a}	268.66 ± 10.88^{a}	225.29 ± 50.76^{a,b}	172.58 ± 34.9
Survival (%)	96.00 ± 4.00	97.33 ± 2.31	96.00 ± 6.93	90.67 ± 8.33
Hepatosomatic index	0.032 ± 0.003	0.033 ± 0.003	0.030 ± 0.001	0.033 ± 0.001
Muscle tissue proximate composition g kg^{-1} DM				
Moisture	732.41 ± 2.79	732.42 ± 6.26	730.58 ± 7.68	740.42 ± 4.3
Protein	877.01 ± 7.11	878.55 ± 4.67	880.63 ± 7.97	887.00 ± 7.91
Lipids	20.05 ± 0.70^{b}	23.39 ± 0.73^{a}	20.79 ± 1.96^{a,b}	20.33 ± 0.75^{b}
Ash	57.06 ± 1.25	55.11 ± 0.56	54.10 ± 3.44	56.13 ± 2.79

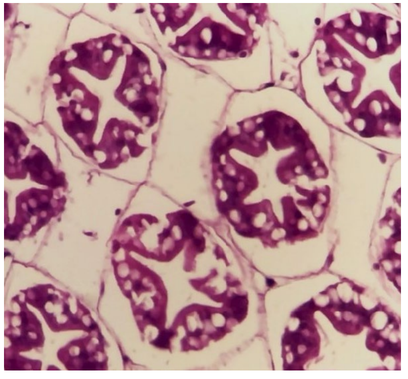
SGR: Specific growth rate; WGR: weekly growth rate; FCR: Feed conversion ratio.



The substitution effect of fish meal and poultry by-products by Soybean meal (60 days)

Effectof soybeanmeal on the hepatopancreas histology

0-SBM



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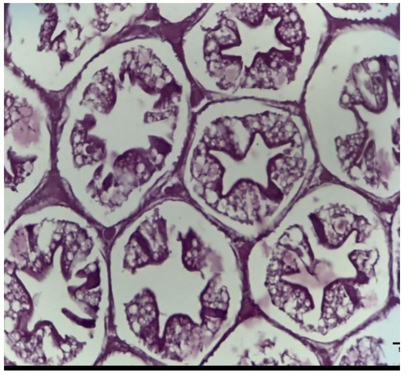

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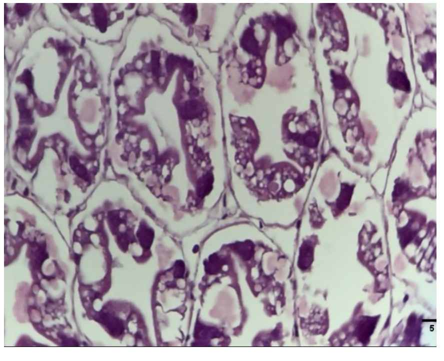

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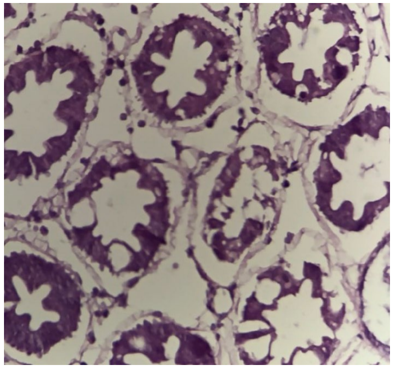
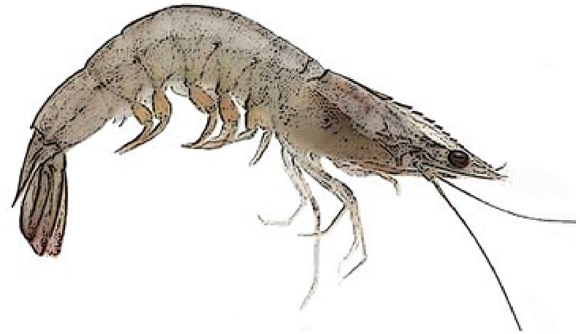


Bovine meat and bone meal?

(BBM)

López-Ortiz et al., 2023

Effect of substitution of poultry by-product meal by bovine by-product meal on overall performance for *Litopenaeus vannamei*

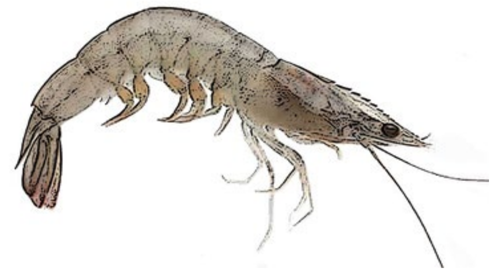


Ingredients and proximate composition (g Kg^{-1} on a dry matter basis, DM) of four isoproteic and isolipidic diets formulated to contain different bovine by-product meal (BBM) levels fed white-leg shrimp *Litopenaeus vannamei* for 50 days.

Ingredients, g Kg^{-1}, DM	Dietary bovine meal levels			
	Control	L-BBM	M-BBM	H-BBM
Bovine by-product meal[a]	0	135.9	271.6	406.9
Poultry by-product meal[a]	300	200	100	0
Soybean 65% ADM [b]	200	200	200	200
Wheat flour [c]	98.9	61.0	23.3	0
Gelatin[d]	80	80	80	80
Corn gluten[e]	40	40	40	40
Beef tallow[f]	0	2.0	6.0	8.0
Maizena™ [g] (corn starch)	170	170	168	154
Metionine[g]	10	10	10	10
Rovimix[h]	30	30	30	30
Stay C[h]	5.0	5.0	5.0	5.0
Phospholipids[i]	10	10	10	10
Cholesterol[j]	5.0	5.0	5.0	5.0
DHA-Nature™ 17%[k]	50	50	50	50
Sodium benzoate[l]	1.0	1.0	1.0	1.0
BHT[l]	0.1	0.1	0.1	0.1

López-Ortiz et al., 2023

Effect of substitution of poultry by-product meal by bovine by-product meal on overall performance for *Litopenaeus vannamei*

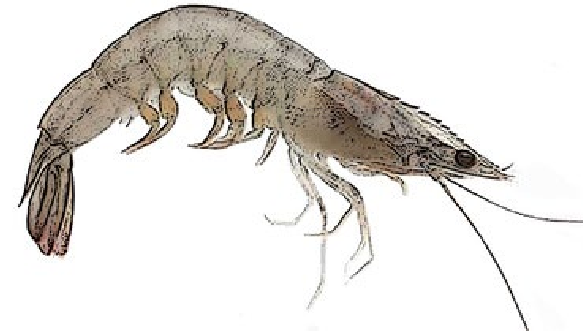


Overall performance of white leg juveniles of *Litopenaeus vannamei,* fed with four diets containing four different bovine by-product meal (BBM); 0, 13.6, 27.2, and 40.7% in replacement of poultry-byproduct meal (PBM) for 50 days.

	Dietary treatments				
	Control	**L-BBM**	**M-BBM**	**H-BBM**	**P value**
IBW (g)	0.47 ± 0.006	0.47 ± 0.002	0.47 ± 0.004	0.478± 0.001	0.237
FBW (g)	1.70 ± 0.04[c]	2.27 ± 0.059[b]	1.73 ± 0.040[c]	2.66 ± 0.132[a]	0.001
RWG (%)	258.61 ± 6.62[c]	383.84 ± 11.44[b]	267.26 ± 7.36[c]	457.05 ± 26.97[a]	0.001
SGR (%)	2.55 ± 0.03[c]	3.15 ± 0.04[b]	2.60 ± 0.04[c]	3.43 ± 0.09[a]	0.001
FCR	2.76 ± 0.12[a]	1.93 ± 0.07[b]	2.65 ± 0.09[a]	1.51 ± 0.12[c]	0.001
HI	6.32 ± 0.41	5.73 ± 0.22	8.06 ± 2.77	6.64 ± 1.25	0.748
TGC	0.303 ± 0.005[c]	0.39 ± 0.007[b]	0.30 ± 0.006[c]	0.44 ± 0.016[a]	0.001
Sv (%)	100 ± 0[a]	97.78 ± 3.50[ab]	96.67 ± 4.43[ab]	88.89 ± 4.6[b]	0.043

López-Ortiz et al., 2023

Dry matter digestibility of shrimp fed with BBM in substitution of PBM



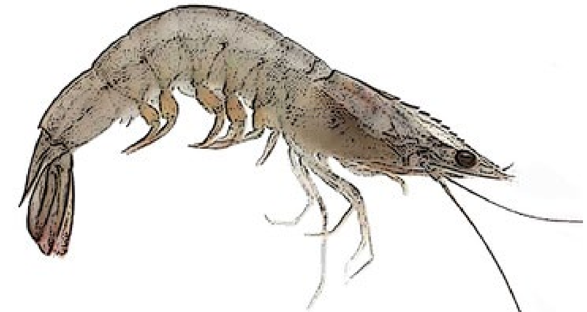
Apparent digestibility coefficient calculated using acid insoluble ash as internal marked in diets containing four different bovine by-product meal (BBM); 0, 13.6, 27.2, and 40.7% in replacement of poultry-byproduct meal (PBM) for 50 days, fed to *Litopenaeus vannamei* (n=3).

Dietary treatment	Apparent digestibility (%)
Control	85.76 ± 0.55^{b}
L-BBM	90.20 ± 0.55^{a}
M-BBM	91.31 ± 0.88^{a}
H-BBM	91.51 ± 0.34^{a}
P value	0.001

L-BBM (low inclusion); M-BBM (medium inclusion); H-BBM (high inclusion). Mean ± SD values in each row with different superscripts were significantly different ($P < 0.05$, Tukey's test).

López-Ortiz et al., 2023

Hemolymph biochemistry parameters of white shrimp *Litopenaeus vanamei* fed on poultry meal balance with different percentages of bovine by-product meal inclusion (Mean ± SD). Three samples per experimental unit were analyzed



Hemolymph biochemistry parameters of white shrimp *Litopenaeus vanamei* fed on poultry meal balance with different percentages of bovine by-product meal inclusion (Mean ± SD). Three samples per experimental unit were analyzed.

Treatment	Parameters			
	Protein (gdL^{-1})	Glucose ($mgdL^{-1}$)	Cholesterol ($mgdL^{-1}$)	Triglycerides ($mgdL^{-1}$)
Control	7.03 ± 0.34^{a}	9.78 ± 0.33^{b}	7.71 ± 0.72^{b}	8.77 ± 0.23^{b}
L-BBM	7.16 ± 0.06^{a}	17.74 ± 2.67^{a}	9.39 ± 2.04^{b}	11.82 ± 0.52^{b}
M-BBM	4.18 ± 0.75^{b}	10.57 ± 1.88^{b}	19.91 ± 8.38^{b}	11.17 ± 2.04^{b}
H-BBM	5.98 ± 0.59^{ab}	14.55 ± 1.10^{ab}	40.80 ± 4.43^{a}	29.52 ± 1.88^{a}
P value	0.001	0.009	0.001	0.001

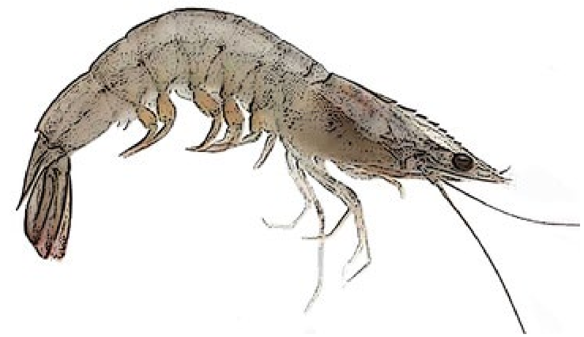
-BBM (low inclusion); M-BBM (medium inclusion); H-BBM (high inclusion). Mean ± SD valu
ı each row with different superscripts were significantly different ($P < 0.05$, Tukey's test).

López-Ortiz et al., 2023

Fatty acid composition of juveniles of *Litopenaeus vannamei* hepatopancreas fed with diets fed the different Bovine by-product meal in replacement of poultry by-product meal.

	Dietary treatments				
Fatty acid	Control	L-BBM	M-BBM	H-BBM	P value
C14	3.28 ± 0.39^{b}	3.27 ± 0.05^{b}	4.53 ± 0.05^{a}	4.40 ± 0.15^{a}	0.04
C16	18.54 ± 0.28^{c}	23.07 ± 0.51^{a}	19.72 ± 0.23b^{c}	20.86 ± 0.54^{b}	0.001
C18	8.55 ± 0.67^{ab}	6.85 ± 0.13^{c}	8.85 ± 0.32^{a}	7.65 ± 0.22^{ab}	0.027
C20	0.37 ±0.06	0.24 ± 0	0.53 ± 0.11	0.29 ± 0.05	0.083
∑SFA	30.76	33.44	33.65	33.21	-
C14:1	0.06 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.22 ± 0.06^{a}	0.19 ± 0.01^{ab}	0.033
C16:1	1.64 ± 0.12	2.05 ± 0.13	1.77 ± 0.03	2.09 ± 0.07	0.035
C18:1n9	26.53 ± 1.11^{b}	33.72 ± 0.49^{a}	28.90 ± 0.16^{b}	33.54 ± 0.33^{a}	0.001
C20:1n9	1.05 ± 0^{a}	0.77 ± 0.01^{b}	0.80 ± 0.06^{b}	0.79 ± 0.05^{b}	0.006
∑MUFA	29.31	36.66	31.7	36.62	-
C18:2n6	13.36 ± 0.22^{a}	11.11 ± 0.20^{b}	10.57 ± 0.06^{b}	8.33 ± 0.06^{c}	0.001
C20:4n6	5.30 ± 0.40^{a}	1.91 ± 0.17^{b}	4.29 ± 0.30^{a}	1.76 ± 0.27^{b}	0.001
∑PUFA n6	18.67	13.01	14.87	10.09	-
C18:3n3	0.65 ± 0.07	0.57 ± 0.07	0.55 ± 0.14	0.60 ± 0.02	0.887
C20:3n3	3.45 ± 0.42^{a}	1.22 ± 0.09^{bc}	2.25 ± 0.08^{b}	0.99 ± 0.18^{c}	0.001
C20:5n3	1.94 ± 0.10^{ab}	1.66 ± 0.05^{b}	2.14 ± 0.11^{a}	2.28 ± 0.71^{a}	0.005
C22:6n3	11.44 ± 0.44^{b}	10.04 ± 0.10^{c}	11.44 ± 0.25^{b}	12.67 ± 0.08^{a}	0.001
∑PUFA n3	17.49	13.5	16.39	16.56	-
∑PUFA´s	36.16	26.51	31.26	26.65	-
Others	3.75 ± 0.01	3.34 ± 0.09	3.37 ± 0.20	3.49 ± 0.17	0.232

Values represent means ± standard error. ∑SFA, ∑MUFA, ∑PUFA n6, ∑PUFA n3, ∑PUFA are the sum of saturated, monounsaturated, polyunsaturated, polyunsaturated n3 and polyunsaturated n6 respectively. ^{abc} Different letters mean statistical differences among experimental diets, according to Tukey´s test ($P < 0.05$)



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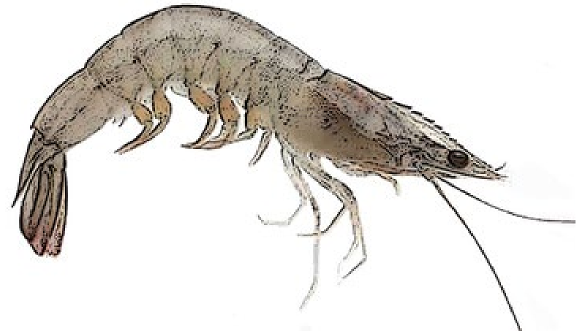
Pork meat and bone meal?

(PoBM)

Effect of substitution of poultry by-product meal by Pork by-product meal on overall performance for *Litopenaeus vannamei*



Ingredients and proximate composition (g Kg^{-1} on a dry matter basis, DM) of four isoproteic and isolipidic diets formulated to contain different pork by-product meal (PoBM) levels fed white-leg shrimp *Litopenaeus vannamei* for 61 days.

Ingredients, g Kg^{-1}, DM	Dietary bovine meal levels			
	Control	L-PoBM	M-PoBM	H-PoBM
Pork by-product meal (Po)[a]	0	176	250	330
Poultry by-product meal (P)[a]	290	135	67.5	0
Soybean 42% [b]	250	250	250	250
Wheat flour [c]	111.5	88.5	88.07	67.0
Gelatin[d]	80	80	80	80
Beef tallow[f]	8.0	10.0	11.0	12.0
Maizena™ [g] (corn starch)	167.4	167.4	167.4	167.4
Rovimix[h]	25	25	25	25
Stay C[h]	2.0	2.0	2.0	2.0
Phospholipids[i]	10	10	10	10
Cholesterol[j]	5.0	5.0	5.0	5.0
DHA-Nature™ 17%[k]	50	50	50	50
Sodium benzoate[l]	1.0	1.0	1.0	1.0
BHT[l]	0.1	0.1	0.1	0.1
Proximate composition, (%, DM)				
Crude Protein	36.92	37.30	37.38	37.73
Crude Lipid	9.92	9.93	9.93	9.99
Ash	5.73	5.73	5.73	5.73
NFE	47.44	47.05	46.96	46.56

Effect of substitution of poultry by-product meal by pork by-product meal on overall performance for *Litopenaeus vannamei*

Overall performance of white leg juveniles of *Litopenaeus vannamei,* fed with four diets containing four different pork by-product meal (PoBM); 0, 13.6, 27.2, and 40.7% in replacement of poultry-byproduct meal (PBM) for 61 days.

	Dietary treatments				
	Control	L-PoBM	M-PoBM	H-PoBM	P value
IBW (g)	2.66 ± 0.003	2.67 ± 0.003	2.66 ± 0.003	2.67 ± 0.02	0.761
FBW (g)	4.94 ± 0.30[b]	5.47 ± 0.30[ab]	6.24 ± 0.23[a]	6.08 ± 0.31[ab]	0.039
RWG (%)	85.5 ± 10.8	105.16 ± 10.8	134.36 ± 8.3	127.41 ± 9.82	0.057
SGR (%)	1.01 ± 0.09	1.17 ± 0.09	1.39 ± 0.06	1.46 ± 0.07	0.051
FCR	6.92 ± 1.022	5.84 ± 0.759	4.45 ± 0.695	5.46 ± 0.61	0.246
HI	3.62 ± 0.16	3.73 ± 0.32	3.62 ± 0.25	3.18 ± 0.06	0.143
Sv (%)	90 ± 2.854	88.33 ± 3.962	85 ± 2.348	81.66 ± 5.945	00.687

L-BBM (low inclusion); M-BBM (medium inclusion); H-BBM (high inclusion). Mean ± SD values in each row with different superscripts were significantly different ($P < 0.05$, Tukey's test). IBW= Initial body weight, FBW= final body weight, RWG= relative weight gain, SGR= specific growth rate, FCR= conversion ratio, HI= hepatosomatic index, TGR= thermal growth coefficient and Sv= survival.

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