

Growth and Resistance to *Streptococcus iniae* of Nile Tilapia Fed Diets Containing Cottonseed Meal Supplemented with Essential Amino Acids

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OBJECTIVES

To evaluate the effect of replacement of soybean meal (SBM) by cottonseed meal (CSM) supplemented with lysine or lysine, methionine and isoleucine on:

- Growth performance
- Body composition
- Hematology
- Immune response
- and resistance of Nile tilapia to *Streptococcus iniae* challenge

METHODS

EXPERIMENTAL FISH

- **Species:** Nile tilapia
(*Oreochromis niloticus*)
- **Av. initial weight:** 6.42 ± 0.16 g
- **Stocking rate:** 35 fish/aquarium
- **No. of replicate:** 3 rep./treatment
- **No. of treatment:** 7
- **Experimental design:** CRD

CULTURE FACILITIES AND WATER

- **Tank:** 57-L aquarium equipped with flow-through and aeration
- **Water source:** Heated, Dechlorinated water
- **Water flow rate:** 0.5-1.0 L/min
- **Water temp.:** 25.2 ± 0.1 °C
- **D.O.:** 6.17 ± 0.08 mg/L
- **Tank cleaning:** Once weekly

Percentage composition of experimental diets

Ingredient	Percent (%) in diet						
	1	2	3	4	5	6	7
Menhaden fish meal	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Soybean meal	45.0	30.0	15.0	----	30.0	15.0	----
Cottonseed meal	----	16.9	33.7	50.6	16.9	33.7	50.6
Corn meal	31.8	31.8	31.8	31.8	31.8	31.8	31.8
Soybean oil	3.6	3.5	3.4	3.3	3.5	3.4	3.3
CMC	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin Mix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine (80%)	----	0.27	0.53	0.80	0.27	0.53	0.80
DL-methionine (99%)	----	----	----	----	0.03	0.06	0.08
Isoleucine (98%)	----	----	----	----	0.14	0.28	0.42
Corn starch	6.6	4.53	2.57	0.5	4.36	2.23	----

FEED AND FEEDING

- **Feed preparation:** Hobart meat grinder
- **Type of pellet:** 3-mm diameter pellet
- **Drying:** At room temp.
- **Feed storage:** Plastic bag kept at -20 °C
- **Feeding schedule:** 2 times daily to apparent satiation
- **Feeding duration:** 10 weeks

SAMPLING

- **Weight gain** : At 2-week intervals.
- **Body composition** : 4 pooled whole fish that has been bled/tank, 2 det./sample.
- **Hematology** : Blood of 4 fish/tank, 2 det./fish.
- **Immune responses** : Serum of 4 fish/tank, 2 det./fish.
- **Bacterial challenge** : 20 fish/tank by IP. injection 5×10^4 cfu/fish of *S. iniae* (wk 10).
- **Antibody titer** : 4 fish/tank prior to and 15 days after challenge.

STATISTICAL ANALYSES

- All data were analyzed by one-way analysis of variance using the General Linear Model (GLM) of SAS.
- Duncan's multiple range test was used to compare treatment means.
- Differences were considered significant at the 0.05 probability level.

RESULTS

Growth performance, feed utilization efficiency and survival of Nile tilapia fed various experimental diets

Diet	Weight gain (g)	Feed intake (g DM/fish)	FER	Survival
1	50.5 ^a	57.9 ^a	0.84 ^a	100
2	42.7 ^b	53.7 ^b	0.80 ^b	100
3	35.8 ^c	48.6 ^c	0.74 ^c	100
4	26.4 ^e	43.0 ^d	0.61 ^d	92.4
5	47.0 ^a	56.9 ^{ab}	0.82 ^b	99.0
6	31.9 ^{cd}	44.7 ^d	0.71 ^c	99.0
7	29.0 ^{de}	44.3 ^d	0.65 ^d	95.2
Pooled SEM	1.4	1.2	0.02	2.2

Whole-body proximate composition of Nile tilapia fed various experimental diets

Diets	Moisture	Percent wet weight basis		
	(%)	Protein	Lipid	Ash
1	72.61	15.23 ^{cb}	7.41 ^a	3.63
2	72.53	15.92 ^a	7.82 ^a	3.60
3	72.32	15.81 ^{ab}	7.89 ^a	3.45
4	74.15	15.13 ^c	6.00 ^b	3.59
5	72.16	15.70 ^{ab}	8.19 ^a	3.51
6	72.23	15.79 ^{ab}	7.25 ^a	3.74
7	72.29	15.77 ^{ab}	7.16 ^a	3.59
Pooled SEM	0.54	0.18	0.34	0.16

Hematological parameters of Nile tilapia fed various experimental diets

Diets	Red blood cell x10 ⁶ /uL	White blood cell x10 ⁵ /uL	Hemoglobin (g/dL)	Hematocrit (%)
1	2.20 ^{ab}	3.20	9.37 ^a	32.17 ^b
2	2.58 ^a	3.52	9.41 ^a	36.58 ^a
3	1.77 ^{bc}	4.40	6.27 ^c	27.25 ^c
4	1.47 ^c	4.83	5.31 ^c	21.89 ^d
5	2.28 ^{ab}	3.36	9.31 ^a	34.97 ^{ab}
6	1.81 ^{bc}	3.88	7.49 ^b	28.25 ^c
7	1.56 ^c	3.99	6.18 ^c	26.29 ^c
Pooled SEM	0.17	0.40	0.36	1.12

Selected innate immune responses of Nile tilapia fed various experimental diets

Diets	Serum protein (mg/mL)	Total Immunoglobulin (mg/mL)	Lysozyme activity ($\mu\text{g/mL}$)	Alternative complement (Units/mL)
1	24.52	1.74	70.57	109.42
2	25.19	1.97	54.24	85.66
3	24.72	1.54	63.59	97.40
4	24.31	1.40	70.00	87.05
5	23.79	1.04	79.26	86.24
6	24.15	1.71	62.61	94.37
7	23.77	1.90	55.63	69.80
Pooled SEM	0.91	0.37	6.90	11.20

Cumulative mortality of juvenile tilapia to *S. iniae* challenge and antibody production against *S. iniae* 15 day post-challenge

Diets	Cumulative Mortality (%)	Antibody titer (Log ₁₀)
1	81.7	1.76
2	80.0	1.51
3	71.7	1.56
4	70.0	1.61
5	78.3	1.79
6	65.0	1.45
7	75.0	1.51
Pooled SEM	6.27	0.13

SUMMARY

- Except for the WG and FI of fish fed the 16.5% CSM supplemented with lysine + methionine + isoleucine, WG, FI and FE decreased at each incremental level of CSM.
- Survival was not affected by dietary treatments.
- Total replacement of SBM by CSM + lysine resulted in decreased body fat content.

SUMMARY

- Increasing dietary CSM levels to 33.7 and 50.6% with either lysine or lysine + methionine + isoleucine supplementation (as replacements of 2/3 or 100% SBM) significantly reduced RBC, Hb and Ht.
- Serum protein, total Ig, lysozyme activity, alternative complement and Ab titer were not affected by dietary CSM levels and/or EAA supplementation.
- Dietary level of CSM with EAA supplementation also has no effect on the resistance of Nile tilapia to *S. iniae* infection challenge.

CONCLUSION

- Relative to channel catfish, Nile tilapia utilize CSM very poorly.
- The poor performance of diets containing CSM may be due to deficiency of essential dietary nutrients (EAA) and/or the presence of antinutritional factors other than gossypol, such as cyclopropene fatty acids (sterculic and malvalic acids).
- Supplementation of lysine alone did not improve the nutritional value of CSM-containing diets.
- However, supplementation of lysine + methionine + isoleucine, significantly improve the performance of the 16.5%-CSM diet.
- Immune responses and resistance of fish to *S. iniae* challenge were not affected by dietary levels of CSM and EAA supplementation.