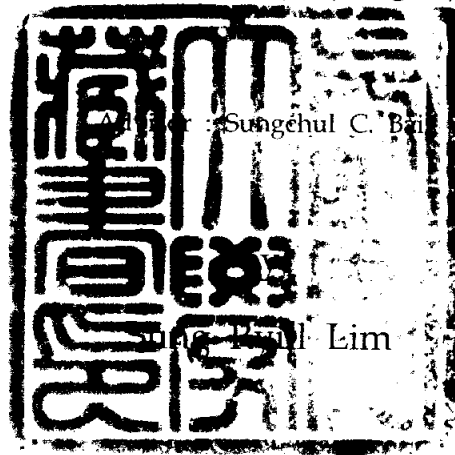


Evaluation of dehulled soybean meal as a fish
meal replacer in Korean rockfish (*Sebastes
schlegeli*) and olive flounder (*Paralichthys olivaceus*)

조피볼락 및 넙치 사료내 어분대체원으로서
탈피대두박의 평가



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주 심 이 학 박 사 김 동 수



위 원 농 학 박 사 김 창 훈



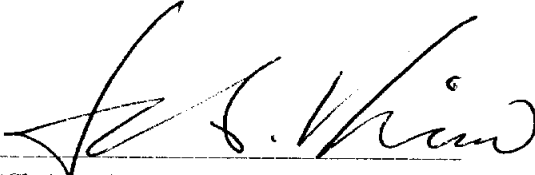
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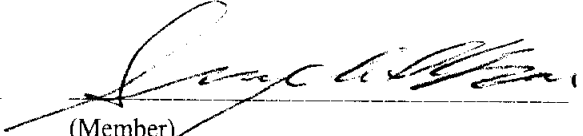
Evaluation of dehulled soybean meal as a fish meal replacer in
Korean rockfish (*Sebastes schlegeli*) and olive flounder
(*Paralichthys olivaceus*)

A Dissertation
by
Sung Ryul Lim

Approved as to style and content by :


(Chairman)


(Member)


(Member)

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요 약 문

두 실험은 조피볼락과 넙치에 있어서 사료내 어분대체원으로서 탈피대두박의 평가에 관한 연구를 하기 위하여 수행되었다. 조피볼락과 넙치의 어분대체 수준은 각각 아미노산 미첨가시 어분단백질의 20%, 아미노산 첨가시 어분단백질의 30%까지로 나타났다.

실험 1 : 조피볼락 사료내 어분대체원으로서 탈피대두박의 평가

(1) 치어기 조피볼락 사료내 어분대체원으로서 탈피대두박의 평가

본 실험은 조피볼락 치어에 있어서 사료내 어분대체원으로서 탈피대두박 (Dehulled soybean meal, DHSM)을 평가하고, 8가지 실험사료의 외견상 건물 및 단백질 소화율을 조사하기 위해 실시하였다. 실험사료는 주단백질원으로 북양어분 (white fish meal, FM), 탈피대두박, 콘글루텐밀 (corn gluten meal, CGM)을 사용하였으며, 조단백질 함량은 48%, 가용에너지는 16.0kJ/g으로 동일하게 맞추어 설계하였다. 2주간의 예비사육을 거친후 1차 사육실험은 8주간 실시하였으며 8가지 실험사료내 탈피대두박의 어분대체수준은 조단백질함량을 기준으로 다음과 같다; 100% FM (DHSM₀); 85% FM + 15% DHSM (DHSM₁₅); 80% FM + 20% DHSM (DHSM₂₀); 70% FM + 30% DHSM (DHSM₃₀); 55% FM + 45% DHSM (DHSM₄₅); 70%FM + 30% DHSM + metionine & lysine (Met & Lys)(DHSM_{30+AA}); 55%FM + 45% DHSM + Met & Lys (DHSM_{45+AA}); 40% FM + 60% DHSM + Met & Lys (DHSM_{60+AA}). 모든 사료구는 소화율 측정을 위해 지표 물질인 산화크롬 (Cr₂O₃)을 0.5%씩 첨가하였으며, L-lysine · HCl 첨가에 따르는 문제점들을 최소화하기 위해 수산화나트륨(NaOH)으로 중화 과정을 거쳤다. 2주간 예비사육 후, 실험어는 평균무게 2.5±0.04g(mean±SD)으로 300ℓ 원형 수조에 각

실험구 당 각각 40마리씩 3반복으로 무작위 배치하였고, 일일 사료공급량은 어체중의 4~5%로 1일 2회 공급하였다.

8주간 증체율과 사료효율에 있어서 DHSM₀, DHSM₁₅, DHSM₂₀ 및 DHSM_{30+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀, DHSM₁₅ 및 DHSM_{30+AA}구는 DHSM₃₀, DHSM₄₅, DHSM_{45+AA} 및 DHSM_{60+AA}구보다 유의적으로 높게 나타났다($P<0.05$). 일간성장률에 있어서 DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀ DHSM_{30+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀구는 DHSM₄₅, DHSM_{45+AA} 및 DHSM_{60+AA}구보다 유의적으로 높게 나타났다($P<0.05$). 단백질효율에 있어서 DHSM₀, DHSM₁₅ 및 DHSM_{30+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀구는 DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{45+AA} 및 DHSM_{60+AA}구보다 유의적으로 높게 나타났다($P<0.05$). 생존율에 있어서 DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM_{30+AA} 및 DHSM_{45+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀ 및 DHSM₂₀구는 DHSM₄₅ 및 DHSM_{60+AA}구보다 유의적으로 높게 나타났다($P<0.05$). 외견상 건물 소화율(AD)은 $75.9\pm 3.8\%$ (mean \pm SD)로 대체단백질원(탈피대두박)의 첨가비가 높아질수록 낮아지는 경향을 보였다. 외견상 건물 소화율에 있어서 DHSM₀, DHSM₁₅ 및 DHSM_{30+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀ 및 DHSM₁₅구는 DHSM₃₀, DHSM₄₅, DHSM_{45+AA} 및 DHSM_{60+AA}구보다 유의적으로 높게 나타났다($P<0.05$). 외견상 단백질 소화율(APD)은 $86.2\pm 3.5\%$ (mean \pm SD)로 나타났으며, 외견상 건물 소화율(AD)과 같은 경향을 보였다.

따라서, 상기의 결과는 치어기 조피볼락에 있어서 사료내 탈피대두박으로 어분 단백질의 20%까지, 탈피대두박내 결핍 아미노산(Met, Lys) 첨가시 어분단백질의 30%까지 대체할 수 있다는 것을 보여주었다.

(2) 육성기 조피볼락 사료내 어분대체원으로서 장기사육시 탈피대두박의 평가

본 실험은 육성기 조피볼락에 있어서 사료내 어분대체원으로서 탈피대두박(Dehulled soybean meal, DHSM)을 평가하기 위해 실시하였으며, 1차 사육실험의 연속되는 실험으로 1차 실험 종료시 그 중 4가지 사료구를 선택하고 2가지 사료구를 추가하여 장기 사육시 대체 효과를 알아보기 위해 12주간 실시하였다(총 20주간 사육실험). 실험사료는 조단백질원으로 북양어분(white fish meal, FM), 탈피대두박, 콘글루텐밀(corn gluten meal, CGM)을 사용하였으며, 조단백질 함량은 48%, 가용에너지는 16.0kJ/g으로 동일하게 맞추어 설계하였다. 2주간의 예비사육을 거친후 2차 사육실험은 12주간 실시하였으며 6가지 실험사료내 탈피대두박의 어분대체수준은 조단백질함량을 기준으로 다음과 같다.; 100% FM (DHSM₀); 90% FM + 10% DHSM (DHSM₁₀); 85% FM + 15% DHSM (DHSM₁₅); 80% FM + 20% DHSM (DHSM₂₀); 80%FM + 20% DHSM + Met & Lys (DHSM_{20+AA}); 70% FM + 30% DHSM+ Met & Lys (DHSM_{30+AA}). 실험어는 평균무게 21.5±0.05g (mean±SD)으로 300ℓ 원형 수조에 각 실험구 당 각각 20마리씩 3반복으로 재배치하였고, 일일 사료공급량은 1일 2회 어체중의 3~4로 1일 2회 공급하였다.

12주간 사육실험에 있어서 증체율, 일간성장률, 내장지방비율, 비만도, 생존율은 모든 실험구에서 유의적인 차이가 나타나지 않았다. 사료효율에 있어서 DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀ 및 DHSM_{20+AA}구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀는 M_{30+AA}구보다 유의적으로 높게 나타났다(P<0.05). 단백질효율에 있어서 DHSM₀, DHSM₁₀, DHSM₁₅ 및 DHSM₂₀구간에는 유의적으로 차이가 없었다, 하지만, DHSM₀구는 DHSM_{20+AA} 및 DHSM_{30+AA}구보다 유의적으로 높게 나타났다(P<0.05). 간중량지수에 있어서 DHSM₀, DHSM₁₀, DHSM₁₅, DHSM_{20+AA} 및 DHSM_{30+AA}구간에는 유의적으로 차이가 없었다. 하지만, DHSM₀, DHSM₁₅, 및 DHSM_{20+AA}구는 DHSM₂₀구보다 유의적으로 높게 나타났다(P<0.05).

혈청내 총단백질 (3.5±0.3g/dL), 글루코스(48.4±15.1mg/dL)함량과 헤마토크리트(37.8±2.3%)는 모든 사료구에서 유의적인 차이가 없었다. 아울러 헤모

클로빈 함량은 $10.4 \pm 1.2\text{g}/100\text{ml}$ 으로 나타났으며 DHSM₀구는 DHSM₁₀, DHSM₁₅, DHSM₂₀ 및 DHSM_{30+AA}구와 비교하여 유의차가 없는 반면에 ($p>0.05$) DHSM_{20+AA}구는 유의적으로 높게 나타났다($P<0.05$). 혈청내 트리글리세라이드 함량은 $93.8 \pm 18.5\text{mg/dL}$ 으로 나타났으며 대조구인 DHSM₀구와 비교했을 때 모든구와 유의차를 나타내지 않았다. 하지만, DHSM_{30+AA}구는 DHSM_{20+AA}구와 비교했을 때 유의적으로 높게 나타났다($P<0.05$).

또, 등근육내 총 구성아미노산 함량은 $82.7 \pm 10.3\%$ 로 나타났으며 각 사료구들 사이의 총 구성아미노산 함량과 각 구성아미노산 사이에 유의차는 나타나지 않았다.

따라서, 상기의 결과는 육성기 조피볼락에 있어서 사료내 탈피대두박으로 어분 단백질의 20%까지, 탈피대두박내 결핍 아미노산(Met, Lys) 첨가시 어분단백질의 30%까지 대체할 수 있다는 것을 보여주었다.

실험 2 : 넙치 사료내 어분대체원으로서 탈피대두박의 효과

(1) 치어기 넙치 사료내 어분대체원으로서 탈피대두박의 평가

본 실험은 넙치 치어에 있어서 사료내 어분대체원으로서 탈피대두박(Dehulled soybean meal, DHSM)을 평가하고, 6가지 실험사료의 외견상 건물 및 단백질 소화율을 조사하기 위해 실시하였다. 실험사료는 주단백질원으로 북양어분(white fish meal, WFM), 탈피대두박, 콘글루텐밀(corn gluten meal, CGM)을 사용하였으며, 조단백질 함량은 50%, 가용에너지는 16.7kJ/g 으로 동일하게 맞추어 설계하였다. 1주간의 예비사육을 거친후 1차 사육실험은 8주간 실시하였으며 8가지 실험사료내 탈피대두박의 어분대체수준은 조단백질함량을 기준으로 다음과 같다; 100% FM (DHSM₀); 90% FM + 10% DHSM (DHSM₁₀); 80% FM + 20% DHSM (DHSM₂₀); 80% FM + 20% DHSM + Met & Lys (DHSM_{20+AA}); 70% FM + 30% DHSM + Met & Lys (DHSM_{30+AA}); 70% FM + 30% DHSM + Met & Lys + Attractant

(DHSM_{30+AA+Att}); 60% FM + 40% DHSM + Met & Lys (DHSM_{40+AA}); 60% FM + 40% DHSM + Met & Lys + Attractant (DHSM_{40+AA+Att}). 모든 사료구는 소화율 측정을 위해 지표 물질인 산화크롬(Cr₂O₃)을 0.5%씩 첨가하였으며, L-lysine · HCl 첨가에 따르는 문제점들을 최소화하기 위해 수산화나트륨(NaOH)으로 중화 과정을 거쳤다. 1주간 예비사육 후, 실험어는 평균무게 5.0±0.04g(mean±SD)으로 40 ℓ 사각 수조에 각 실험구 당 각각 25마리씩 3반복으로 무작위 배치하였고, 일일 사료공급량은 어체중의 4~5%로 1일 2회 공급하였다.

8주간 증체율과 사료효율에 있어서 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구는 DHSM_{40+AA}, DHSM_{40+AA+Att} 구보다 유의적으로 높게 나타났다(P<0.05). 일간성장률에 있어서 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구는 DHSM_{40+AA}, DHSM_{40+AA+Att} 구보다 유의적으로 높게 나타났다(P<0.05). 단백질효율에 있어서 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} 및 DHSM_{30+AA+Att} 구는 DHSM_{40+AA}, DHSM_{40+AA+Att} 구보다 유의적으로 높게 나타났다(P<0.05). 생존율에 있어서는 모든 구에서 유의적으로 차이가 없었다.

따라서, 상기의 결과는 치어기 넙치에 있어서 사료내 탈피대두박으로 어분 단백질의 20%까지, 탈피대두박내 결핍 아미노산(Met, Lys) 및 섭취촉진제 첨가시 어분단백질의 30%까지 대체할 수 있다는 것을 보여주었다.

(2) 육성기 넙치 사료내 어분대체원으로서 탈피대두박의 평가

본 실험은 육성기 넙치에 있어서 사료내 어분대체원으로서 탈피대두박

(Dehulled soybean meal, DHSM)을 평가하고, 6가지 실험사료의 외견상 건물 및 단백질 소화율을 조사하기 위해 실시하였다. 실험사료는 주단백질원으로 북양어분 (white fish meal, FM), 탈피대두박, 콘글루텐밀 (corn gluten meal, CGM)을 사용하였으며, 조단백질 함량은 50%, 가용에너지는 16.7kJ/g으로 동일하게 맞추어 설계하였다. 1주간의 예비사육을 거친후 1차 사육실험은 8주간 실시하였으며 6가지 실험사료내 탈피대두박의 어분대체수준은 조단백질함량을 기준으로 다음과 같다; 100% FM (DHSM₀); 90% FM + 10% DHSM (DHSM₁₀); 80% FM + 20% DHSM (DHSM₂₀); 70% FM + 30% DHSM (DHSM₃₀); 80% FM + 20% DHSM + Attractant (DHSM_{20+Att}); 70% FM + 30% DHSM + Attractant (DHSM_{30+Att}). 모든 사료구는 소화율 측정을 위해 지표 물질인 산화크롬(Cr₂O₃)을 0.5%씩 첨가하였으며, L-lysine · HCl 첨가에 따르는 문제점들을 최소화하기 위해 수산화나트륨(NaOH)으로 중화 과정을 거쳤다. 1주간 예비사육 후, 실험어는 평균무게 45.5±0.08g (mean±SD)으로 360ℓ 원형 수조에 각 실험구 당 각각 15마리씩 3반복으로 무작위 배치하였고, 일일 사료공급량은 어체중의 2~4%로 1일 2회 공급하였다.

10주간 증체율과 사료효율에 있어서 DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{30+Att}구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{30+Att}구는 DHSM₃₀구보다 유의적으로 높게 나타났고 DHSM_{20+Att}구보다는 유의적으로 낮게 나타났다(P<0.05). 일간성장률에 있어서 DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{30+Att}구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{30+Att}구는 DHSM₃₀구보다 유의적으로 높게 나타났고 DHSM_{20+Att}구보다는 유의적으로 낮게 나타났다(P<0.05). 단백질효율에 있어서 DHSM₀, DHSM₁₀ 및 DHSM₂₀구간에는 유의적으로 차이가 없었다. 하지만 DHSM₀, DHSM₁₀ 및 DHSM₂₀구는 DHSM₃₀구보다는 유의적으로 높게 나타났고 DHSM_{20+Att} 및 DHSM_{30+Att}구보다는 유의적으로 낮게 나타났다(P<0.05). 생존율에 있어서는 DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{20+Att} 및 DHSM_{30+Att}구간에는 유의적으로 차이가 없었다.

하지만, DHSM₀, DHSM₁₀, DHSM₂₀ 및 DHSM_{20+Att} 및 DHSM_{30+Att}구는 DHSM₃₀구보다는 유의적으로 높게 나타났다($P<0.05$). 외견상 단백질 소화율 (APD)은 $91.0\pm2.6\%$ (mean \pm SD)로 나타났으며, 외견상 건물 소화율(AD)과 같은 경향을 보였다.

따라서, 상기의 결과는 육성기 넙치에 있어서 사료내 탈피대두박으로 어분 단백질의 20%까지, 탈피대두박내 섬취촉진제 첨가시 어분단백질의 30%까지 대체할 수 있다는 것을 보여주었다.

Evaluation of dehulled soybean meal as a fish meal replacer in Korean rockfish, *Sebastes schlegeli* (Hilgendorf) and olive flounder *Paralichthys olivaceus* (Temminck et Schlegel)

Sung Ryul Lim

*Department of Fisheries Biology Graduate School
Pukyong National University*

Abstract

Two experiments were conducted to evaluate dehulled soybean meal (DHSM) as a fish meal (FM) replacer and to determine the proper inclusion level of dehulled soybean meal in Korean rockfish (*Sebastes schlegeli*) and olive flounder (*Paralichthys olivaceus*) diets. In the first experiment, those results indicated that DHSM could replace FM up to 20% without amino acids (Met, Lys) supplementation and 30% with amino acids supplementation for the maximum growth of Korean rockfish. In the second experiment, those results indicated that DHSM could replace FM up to 20% without amino acids (Met, Lys) supplementation and 30% with amino acids and/or attractant supplementation for the maximum growth of olive flounder.

Experiment 1 : Evaluation of dehulled soybean meal as a fish meal replacer in Korean rockfish (*Sebastes schlegeli*)

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile Korean rockfish

This experiment was conducted to evaluate dehulled soybean meal (DHSM) as a fish meal (FM) replacer in juvenile Korean rockfish *Sebastes schlegeli* diets. Fish meal, a major animal protein source in the DHSM₀ diet, was replaced by DHSM on the protein equivalent base. Fish averaging 2.5 ± 0.04 g (Mean \pm SD) were distributed to each aquarium as a group of 40 fish reared in flow through system. Fish of triplicate groups were fed one of eight diets containing 48% crude protein and 16.0kJ available energy/g diet. Eight diets were formulated to replace fish meal with DHSM at 0, 10, 20, 30 & 45% without amino acid supplementation and 30, 45 & 60% with amino acid supplementation (DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{30+AA}, DHSM_{45+AA}, DHSM_{60+AA}). After 8 weeks of feeding trial, there was no significant differences in weight gain (WG) among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. However, WG of fish fed DHSM₀, DHSM₁₅ and DHSM_{30+AA} diets were significantly higher than that of fish fed DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets. There was no significant differences in feed efficiency (FE) among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. However, FE of fish fed DHSM₀ and DHSM₁₅ diets were significantly higher than that of fish fed

DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$). Condition factor (CF), hematocrit (PCV), hemoglobin (Hb) showed the same trend as hepatosomatic index (HSI). Survival of fish fed DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM_{30+AA}, DHSM_{45+AA} diets were not significantly different from those of fish fed the DHSM₀ diet ($P<0.05$). There was no significant differences in average survival among fish fed DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM_{30+AA} and DHSM_{45+AA} diets. Apparent protein digestibility (APD) showed the same trend as apparent dry matter digestibility (AD). There was no significant differences in APD among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and, DHSM_{30+AA} diets. However, APD of fish fed DHSM₀ and DHSM₁₅ diets were significantly higher than that of fish fed DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets. ($P<0.05$). Therefore, these results indicated that DHSM could replace FM up to 20% without amino acids supplementation and 30% with amino acids (Met, Lys) supplementation.

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing Korean rockfish

This experiment was conducted to get the information on the long term fish meal replacement effects of dehulled soybean meal (DHSM) and to determine the proper inclusion level of dehulled soybean meal in growing Korean rockfish *Sebastes schlegeli* diets for 12 weeks. Fish meal (FM), a major animal protein source in the control diet (DHSM₀) was replaced by DHSM on crude protein equivalent base. Fish averaging 21.5 ± 0.05 g (Mean \pm SD) were distributed to each aquarium as a group of

20 fish reared in the flow through system. Fish of triplicate groups were fed one of six diets containing 48% crude protein and 16.0kJ available energy/g diet. Six diets were formulated to replace fish meal with dehulled soybean meal at 0, 10, 15 & 20% without amino acid supplementation and 20 & 30% with amino acid supplementation (DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA}).

After 12 weeks of feeding trial, there was no significant difference in weight gain (WG), feed efficiency (FE) and specific growth rate (SGR, %) among fish fed six experimental diets. There was no significant differences in protein efficiency ratio (PER) among fish fed DHSM₀, DHSM₁₀, DHSM₁₅ and DHSM₂₀ diets. However, PER of fish fed DHSM₀ diets was significantly higher than that of fish fed DHSM_{20+AA} and DHSM_{30+AA} diets. There was no significant differences in hepatosomatic index (HSI) among fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM_{20+AA} and DHSM_{30+AA} diets. However, HSI of fish fed DHSM₀, DHSM₁₅ and DHSM_{20+AA} diets were significantly higher than that of fish fed DHSM₂₀ diets. There was no significant difference in intraperitoneal fat ratio (IPF ratio), condition factor (CF) and survival among fish fed six experimental diets. There was no significant differences in hemoglobin (Hb) content among fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM_{20+AA} and DHSM_{30+AA} diets. However, Hb content of fish fed DHSM_{20+AA} diets was significantly higher than that of fish fed DHSM₀, DHSM₁₀, DHSM₁₅ and DHSM₂₀ diets ($P<0.05$). There was no significant differences in triglyceride (TG) content among fish fed all of diets. However, triglyceride (TG) content of fish fed DHSM_{30+AA} diets was significantly higher than that of fish fed DHSM_{20+AA} diets ($P<0.05$). There was no significant difference in hematocrit (PCV),

total protein (TP), glucose contents and whole body proximate composition among fish fed six experimental diets.

Therefore, these results indicated that DHSM could replace FM up to 20% without amino acids supplementation and 30% with amino acids (Met, Lys) supplementation for the maximum growth of growing Korean rockfish.

Experiment 2: Evaluation of dehulled soybean meal as a fish meal replacer in olive flounder *Paralichthys olivaceus*

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile olive flounder

This experiment was conducted to evaluate dehulled soybean meal (DHSM) as a fish meal (FM) replacer and to determine the proper inclusion level of dehulled soybean meal in juvenile olive flounder *Paralichthys olivaceus* diets for 8 weeks. Fish meal, a major animal protein source in the control diet (DHSM₀), was replaced by dehulled soybean meal (DHSM) on crude protein equivalent base. Prior to the feeding trial, fish were fed control diet for 2 weeks to adjust to the experimental diets and conditions. Fish averaging $5.0 \pm 0.04\text{g}$ (Mean \pm SD) were distributed to each aquarium as a group of 25 fish reared in the flow through system. Fish of triplicate groups were fed one of eight diets containing 50% crude protein and 16.7kJ available energy/g diet. Eight diets were formulated to replace fish meal with dehulled soybean meal at 0, 10 & 20% without amino acid supplementation and 20, 30 & 40% with amino acid supplementation and 30% & 40% with amino acid & attractant supplementation (DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA}, DHSM_{30+AA+Att}, DHSM_{40+AA}, DHSM_{40+AA+Att}).

There was no significant differences in weight gain (WG) of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, average WG of fish fed DHSM₀, DHSM₁₀, DHSM₁₅,

DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets. There was no significant differences in feed efficiency (FE) of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, average FE of fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets. There was no significant differences in average specific growth rate (SGR) of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, average FE of fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets. There was no significant differences in protein efficiency ratio (PER) of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, PER of fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets. There was no significant differences in average survival of fish fed all of diets.

Therefore, these results indicated that DHSM could replace FM up to 20% without amino acids supplementation and 30% with amino acids (Met, Lys) supplementation for the maximum growth of juvenile olive flounder.

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing olive flounder

This experiment was conducted to evaluate dehulled soybean meal (DHSM) as a fish meal (FM) replacer and to determine the proper inclusion level of dehulled soybean meal in growing olive flounder *Paralichthys olivaceus* diets for 10 weeks. Fish meal, a major animal protein source in the control diet (DHSM₀), was replaced by dehulled soybean meal (DHSM) on crude protein equivalent base. Prior to the feeding trial, fish were fed control diet for 2 weeks to adjust to the experimental diets and conditions. Fish averaging $45.5 \pm 0.08\text{g}$ (Mean \pm SD) were distributed to each aquarium as a group of 15 fish reared in the flow through system. Fish of triplicate groups were fed one of six diets containing 50% crude protein and 16.7kJ available energy/g diet. Six diets were formulated to replace fish meal with dehulled soybean meal at 0, 10, 20 & 30% without amino acid supplementation and 20 & 30% with attractant supplementation (DHSM₀, DHSM₁₀, DHSM₂₀, DHSM₃₀, DHSM_{20+Att}, DHSM_{30+Att}).

There was no significant difference in weight gain (WG) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets. However, WG of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than those of fish fed DHSM₃₀ ($P < 0.05$). While WG of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in feed efficiency (FE) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, FE of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher than

those of fish fed DHSM₃₀ ($P<0.05$). While FE of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in specific growth rate (SGR) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets. However, SGR of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While SGR of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in protein efficiency ratio (PER) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, PER of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While PER of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in survival among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+Att} and DHSM_{30+Att} diets.

Therefore, these results indicated that DHSM could replace FM up to 20% without attractant supplementation and 30% with attractant supplementation for the maximum growth of growing olive flounder.

I. Introduction

The success of aquaculture industry most likely will depend on the reduction of fish meal use in fish feeds. Fish meal has traditionally been a major ingredient in fish diets because of its high protein quality and palatability, but is one of the most expensive ingredients in formulated fish diets. Also, It has been the major protein source in formulated olive flounder and Korean rockfish diets. The total domestic utilization of fish meal is approximately 100,000mt per year in 2001. It is obvious that the domestic supply of fish meal levelled off or decreased, but the demand of fish meal continuously increased. However, Lee and Bai (1997a) noted that the world supply of fish meal increased only about 27% during the past 20 years and fish meal output by the major fish meal producing countries actually declined. Because of limiting supply of fish meal around the world, the cost of producing fish would be expected to increase. For this reason, many studies have been conducted to replace or reduce its inclusion in fish diets by various less expensive alternative protein sources. Recently, fish nutritionists conducted many researches by using the stable supply ingredients such as plant protein sources (soybean meal, cottonseed meal, corn gluten meal etc) and animal protein sources (meat & bone meal, blood meal, feather meal, poultry by-product meal etc) to replace partial or all of the expensive fish meal in fish feed (Bai et al., 1997, 1998; Carter & Hauler, 2000; Lee & Bai, 1997a,b; Refstie et al., 2001). Soybean meal (SM) has been tested to some degree in this capacity because of its generally favorable protein content and amino acid profile (Mcgoogan & Gatlin III, 1997). There have been numerous studies on the

utilization of SM in the diet of various species of fish such as carp, rainbow trout, yellowtail, Atlantic salmon etc (Dabrowski et al., 1989; Jo et al., 1998; Shimeno et al., 1993; Refstie et al., 2001).

Olive flounder (*Paralichthys olivaceus*) is one of the commercially important fish species in Korea, its production is the top among the Korean mariculture finfish species. Culture of olive flounder has rapidly increase in the last 20 years with annual production of 3mt in 1981 to 39,628mt in 2001. Additionally, the production of formulate feeds of olive flounder also increased to 23,993 MT in 2001. And Korean rockfish (*Sebastes schlegeli*) is one of the most important commercial aquaculture fish species in Korea. This species has desirable characteristics for culture including the high tolerance to water temperature changes, the easiness of seeding production because of a ovoviviparous reproductive style and the ability to withstand high stocking density. Commercial culture systems of this species have been rapidly developed since 1987, and its production reached at 39,321 mt among the total finfish mariculture production, which was approximately 100,000 mt in Korea (The Ministry of Maritime Affairs and Fisheries, 1999). However, there are only a few studies for evaluation of dehulled soybean meal (DHSM) in Korean rockfish and olive flounder. Additionally, more information is needed for the practical use of SM in Korean rockfish and olive flounder diets.

Therefore, the purpose of this study is to evaluate DHSM as a fish meal replacer in Korean rockfish and olive flounder.

II. Materials and Methods

Experiment 1: Evaluation of dehulled soybean meal as a fish meal replacer in Korean rockfish (*Sebastes schlegeli*)

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile Korean rockfish

Experimental design and diets

An eight week feeding trial was conducted to determine the inclusion level of dehulled soybean meal (DHSM) that could replace fish meal (FM) in juvenile Korean rockfish diets. Eight experimental diets were formulated to contain 48% CP and 16.0 kJ available energy g⁻¹ by varying lipid content (Table 1). Eight diets were formulated to replace fish meal with dehulled soybean meal at 0, 10, 20, 30 & 45% without amino acid supplementation and 30, 45 & 60% with amino acid supplementation (DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{30+AA}, DHSM_{45+AA}, DHSM_{60+AA}). Estimate available energy (Lee & Putnam, 1973; Garling & Wilson, 1976) of experimental diets were adjusted to have 16.0 kJ g⁻¹ (16.7, 16.7 and 37.7 kJ g⁻¹ for protein, carbohydrate and lipid, respectively). The approximate and essential amino acids (EAA) composition of FM and DHSM are shown in table 2. Two EAA (methionine and lysine) were selected to study the effect of adding EAA to Korean rockfish diets. The EAA composition of the experimental diets

is shown in table 3. White fish meal, dehulled soybean meal, and corn gluten meal served as the major protein sources. Squid liver oil and wheat meal were used as lipid and carbohydrate sources, respectively. Cellulose were also included in the diets to match CP and energy levels. Dehulled soybean meal were obtained from ASA (American Soybean Association / Korea). Dehulled soybean meal was composed of 53.5% crude protein, 1.9% crude fat and 7.9% ash on a dry-matter basis. Procedures for diet preparation and storage were as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, squid liver oil together with 30% filtered tap water, experimental diets were pelleted by using a laboratory pelleting machine.

Experimental fish and feeding trial

Juvenile Korean rockfish (*Sebastes schlegeli*) were obtained from Tong-young, Korea and the feeding trials were conducted at the Institute of Fisheries Sciences (Pukyong National University, Busan, Korea). Prior to the feeding trial, the fish were fed DHSM₀ diet for 1 weeks to allow them to adjust to the experimental diets and conditions. Feeding trials were conducted in 300 ℓ aquaria each having a water flow rate of 1.0 ℓ /min. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5 ± 0.5 ppm. The water temperature was maintained at 23 ± 2 °C (mean \pm SD) the salinity was maintained at 31 ± 1 g/L (mean \pm SD), and the pH was maintained at 7.5 ± 0.3 (mean \pm SD) and photoperiod of 12h light : 12h dark (06:00 to 18:00) was used throughout the experimental periods. In the first 8-week experimental period, three groups of 40 fish averaging 2.5 ± 0.04 g (mean \pm SD) were randomly

assigned to each diet treatment. Each diet were fed to triplicate groups to apparent satiation 2 times per day at a rate of 2.5% dry matter basis diet of wet body weight. Total fish weight in each aquarium was determined every 2 weeks, and the amount of diet fed to fish was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing.

Fecal collections and digestibility determination

Fecal samples were collected each day by careful pipetting from each tank in the morning and 2h after feeding. Feces were immediately frozen and stored at -20°C pending analysis. Prior to analysis individual collections from each tank were pooled to a composite sample per treatment. Apparent dry matter digestibility (AD) and apparent protein digestibility (APD) was estimated by the equation :

$$AD = 100 - 100 \times \left(\frac{\% \text{ Cr}_2\text{O}_3 \text{ in feed}}{\% \text{ Cr}_2\text{O}_3 \text{ in feces}} \right)$$

$$APD = 100 - \left(\frac{100\% \text{ Cr}_2\text{O}_3 \text{ in feed}}{\% \text{ Cr}_2\text{O}_3 \text{ in feces}} \times \frac{\% \text{ Protein in feces}}{\% \text{ Protein in feed}} \right)$$

Eight diets and feces were dried in an oven at 60°C for 24 h and ground with a mortar and pestle, then triplicate samples of each diet and feces were subjected to chemical analysis. Crude protein content (N × 6.25) was determined by the microKjeldhal method. The apparent digestibility of dry matter, protein of eight diets were determined by the

chromic oxide (Cr_2O_3) method (Hanley, 1987). Cr_2O_3 concentrations were determined by flame atomic absorption spectrophotometer following combustion of sample in muffle furnace, before and after digestion in nitric acid (AOAC, 1995). Apparent digestibility (AD) values were calculated as previously described by Cho & Slinger (1979) and Sugiura et al. (1998).

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with MS-222 (100ppm), and then weighed, and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER). Three fish from each aquarium were randomly selected to determine hepatosomatic index (HSI) and condition factor (CF). Blood samples were obtained from the caudal vein with a syringe. Hematocrit (PCV) was determined on three fish randomly selected per aquarium by the microhematocrit method (Brown, 1980), and hemoglobin (Hb) was measured with the same fish by the cyan-methemoglobin procedure using Drabkins solution. Hb standard prepared from human blood (Sigma Chemical, St. Louis, Missouri) was used. The muscle structural amino acids were quantified by amino acid analyzer S433 (Sykno, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4mm \times 150mm, Absorbance; 570nm 440nm, Reagent flow rate; 0.25ml/min, Buffer flow rate; 0.45ml/min, Reactor temperature; 120, Reactor Size; 15m. Analyses of crude protein, moisture and ash were performed by the standard procedure of AOAC (1995). Crude fat was determined using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying

samples for 12hours.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

Table 1. Composition and proximate analysis of the eight experimental diets (% of dry matter basis)¹

Ingredients	Diets ¹²							
	DHSM ₀	DHSM ₁₅	DHSM ₂₀	DHSM ₃₀	DHSM ₄₅	DHSM 30+AA	DHSM 45+AA	DHSM 60+AA
White fish meal ²	60	51	48	42	33	42	33	24
Corn gluten meal ³	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Soybean meal ⁴	0	11.5	15.3	22.9	34.4	22.9	34.4	45.8
Wheat meal ⁵	6.0	6.0	6.0	6.0	6.0	6.0	6.0	5.0
Dextrin ⁶	12.0	8.2	7.0	4.5	0.9	5.1	2.1	0
Methionine	0	0	0	0	0	0.5	0.5	0.5
L-Lysine · HCl ⁷	0	0	0	0	0	0.52	0.78	1.04
Squid oil ⁸	7.0	7.6	7.8	8.2	8.7	8.0	8.26	8.60
Vitamin premix ⁹	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix ¹⁰	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CMC ¹¹	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	0	0.71	0.94	1.42	2.0	0	0	0.04

Proximate analysis

Moisture	26.5	27.6	28.2	27.3	28.0	28.1	28.7	29.5
Crude protein	48.0	47.9	47.7	47.6	47.8	48.9	49.1	49.3
Crude lipid	12.5	12.5	12.5	12.5	12.5	12.3	12.0	11.8
Crude ash	13.2	12.2	11.8	11.2	10.2	11.2	10.2	9.2

¹ Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

^{2,3} Suhyup Co. Busan, Korea

⁴ American Soybean Association/ Korea

⁵ Young Nam Flourmills Co., Pusan, Korea

^{6,11} United States Biochemical, Cleveland, Ohio 44122

⁷ 0.5% L-lysine · HCl (0.4% lysine)

⁸ E-Wha oil Co., Ltd., Pusan, Korea

⁹ Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ; Choline bitartrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl- α -Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B₁₂, 0.06

¹⁰ Contains (as mg/kg in diets) : NaCl, 437.4; MgSO₄ · 7H₂O, 1379.8; NaH₂P₄ · 2H₂O, 877.8; Ca(H₂PO₄)₂ · 2H₂O, 1366.7; KH₂PO₄, 2414; ZnSO₄ · 7H₂O, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; MnSO₄, 0.016; FeSO₄, 0.0378; CuSO₄, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; NaSeO₃, 0.00025

¹¹ Carboxymethylcellulose

¹² DHSM₀, 100% fish meal (FM) ; DHSM₁₅, 85% FM + 15% dehulled soybean meal (DHSM); DHSM₂₀, 80% FM + 20% DHSM; DHSM₃₀, 70% FM + 30% DHSM ; DHSM₄₅, 55% FM + 45% DHSM ; DHSM_{30+AA}, 70% FM + 30% DHSM + Met & Lys ; DHSM_{45+AA}, 55% FM + 45% DHSM + Met & Lys; DHSM_{60+AA}, 40% FM + 60% DHSM + Met & Lys

Table 2. Proximate analysis and amino acid composition of white fish meal and dehulled soybean meal (% of dry matter basis)¹

	White fish meal	Dehulled soybean meal
Crude protein	68.1	53.5
Crude lipid	9.0	1.9
Crude ash	21.4	7.9
Arginine	4.21	3.86
Histidine	1.34	1.51
Lysine	4.53	3.16
Leucine	4.52	3.63
Isoleucine	2.67	2.20
Methionine	1.68	0.63
Phenylalanine	2.34	2.61
Threonine	2.57	1.96
Tryptophan	0.60	0.52
Valine	3.02	2.10

¹ Values of amino acid were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University.

Table 3. Calculated essential amino acid (EAA) composition of eight experimental diets (% of dry matter basis)¹

Ingredients	Diets ³							
	DHSM ₀	DHSM ₁₅	DHSM ₂₀	DHSM ₃₀	DHSM ₄₅	DHSM 30+AA	DHSM 45+AA	DHSM 60+AA
Arginine	3.71	3.65	3.64	3.52	3.44	3.56	3.47	3.38
Histidine	1.27	1.30	1.31	1.30	1.32	1.32	1.34	1.36
Isoleucine	2.51	2.44	2.42	2.32	2.23	2.34	2.25	2.15
Leucine	4.94	5.09	5.16	5.15	5.29	5.21	5.34	5.47
Lysine	3.86	3.64	3.57	3.34	3.10	4.08	4.22	4.34
Met + Cys ²	2.13	2.01	1.98	1.86	1.73	2.57	2.44	2.31
Phe + Tyr ²	4.17	4.26	4.30	4.26	4.32	4.30	4.36	4.42
Threonine	2.36	2.33	2.33	2.26	2.22	2.29	2.25	2.20
Trptophan	0.54	0.56	0.56	0.56	0.57	0.56	0.57	0.58
Valine	2.86	2.83	2.83	2.75	2.70	2.78	2.73	2.68

¹ Values are calculated from the ingredients used in this study

² Dispensable amino acids, but cystine spares methionine and tyrosine spares phenylalanine

³ Refer to the table 1

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing Korean rockfish

Experimental design and diets

Consecutive twelve-week feeding trial was conducted to determine the inclusion level of dehulled soybean meal (DHSM) that could replace fish meal (FM) in growing Korean rockfish diets. Six experimental diets were formulated to contain 48% CP and 16.0 KJ available energy g^{-1} by varying lipid content (Table 4). Six diets were formulated to replace fish meal with dehulled soybean meal at 0, 10, 15 & 20% without amino acid supplementation and 20 & 30% with amino acid supplementation (DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₃₀, DHSM_{20+AA}, DHSM_{30+AA}). Estimate available energy (Lee & Putnam, 1973; Garling & Wilson, 1976) of experimental diets were adjusted to have 16.0 KJ g^{-1} (16.7, 16.7 and 37.7 KJ g^{-1} for protein, carbohydrate and lipid, respectively). Two EAA (methionine and lysine) were selected to study the effect of adding EAA to Korean rockfish diets. The EAA composition of the experimental diets is shown in table 5. White fish meal, dehulled soybean meal, and corn gluten meal served as the major protein sources. Squid liver oil and wheat meal were used as lipid and carbohydrate sources, respectively. Cellulose were also included in the diets to match CP and energy levels. Dehulled soybean meal were obtained from ASA (American Soybean Association/Korea). Dehulled soybean meal was composed of 53.5% crude protein, 1.9% crude fat and 7.9% ash on a dry-matter basis. Procedures for diet preparation and storage were as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, squid liver oil

together with 30% filtered tap water, experimental diets were pelleted by using a laboratory pelleting machine.

Fish and feeding trials

Growing Korean rockfish (*Sebastes schlegeli*) were obtained from Tong-young, Korea and the feeding trials were conducted at the Institute of Fisheries Sciences (Pukyong National University, Busan, Korea). Prior to the feeding trial, the fish were fed DHSM₀ diet for 1 weeks to allow them to adjust to the experimental diets and conditions. Feeding trials were conducted in 300 L aquaria each having a water flow rate of 1.0 L/min by the flow through system. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5 ± 0.5 ppm. The water temperature was maintained at 18 ± 1 °C (mean ± SD) the salinity was maintained at 31 ± 1g/L (mean ± SD), and the pH was maintained at 7.5 ± 0.3 (mean ± SD) and photoperiod of 12h light : 12h dark (06:00 to 18:00) was used throughout the experimental periods. In the second 12-week experimental period, three groups of 20 fish averaging 21.5 ± 0.05 g (mean ± SD) were randomly assigned to each diet treatment. Each diet were fed to triplicate groups to apparent satiation 2 times per day. Total fish weight in each aquarium was determined every 4 weeks, and the amount of diet fed to fish was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing. Feeding trial was carried out at the Institute of Fisheries Sciences.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with MS-222

(100ppm), and then weighed, and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER). Three fish from each aquarium were randomly selected to determine hepatosomatic index (HSI) and condition factor (CF). Blood samples were obtained from the caudal vein with a syringe. Haematocrit (PCV) was determined on three fish randomly selected per aquarium by the microhematocrit method (Brown, 1980), and hemoglobin (Hb) was measured with the same fish by the cyan-methemoglobin procedure using Drabkins solution. Hb standard prepared from human blood (Sigma Chemical, St. Louis, Missouri) was used. The muscle structural amino acids were quantified by amino acid analyzer S433 (Sykna, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4mm × 150mm, Absorbance; 570nm 440nm, Reagent flow rate; 0.25ml/min, Buffer flow rate; 0.45ml/min, Reactor temperature; 120, Reactor Size; 15m. Analyses of crude protein, moisture and ash were performed by the standard procedure of AOAC (1995). Crude fat is determined using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying samples for 12hours.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means.

Table 4. Composition and proximate analysis of six experimental diets (% of dry matter basis)¹

Ingredients	Diets ¹²					
	DHSM ₀	DHSM ₁₀	DHSM ₁₅	DHSM ₂₀	DHSM 20+AA	DHSM 30+AA
White fish meal ²	60	54	51	48	48	42
Corn gluten meal ³	8.5	8.5	8.5	8.5	8.5	8.5
Soybean meal ⁴	0	7.6	11.5	15.3	15.3	22.9
Wheat meal ⁵	6.0	6.0	6.0	6.0	6.0	6.0
Dextrin ⁶	12.0	9.7	8.2	7.0	6.9	5.1
Methionine	0	0	0	0	0.5	0.5
L-Lysine · HCl ⁷	0	0	0	0	0.5	0.5
Squid oil ⁸	7.0	7.3	7.6	7.8	7.8	8.0
Vitamin premix ⁹	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix ¹⁰	3.0	3.0	3.0	3.0	3.0	3.0
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5	0.5
CMC ¹¹	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	0	0.37	0.71	0.94	0	0
<i>Proximate analysis</i>						
Moisture	19.2	20.7	19.8	20.5	18.8	17.8
Crude protein	48.1	48.0	47.9	47.7	48.7	48.9
Crude lipid	12.4	12.5	12.6	12.6	12.3	12.2
Crude ash	13.2	12.5	12.2	11.9	11.5	11.3

¹ Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

^{2,3} Suhyup Co. Busan, Korea

⁴ American Soybean Association/ Korea

⁵ Young Nam Flourmills Co., Busan, Korea

^{6,11} United States Biochemical, Cleveland, Ohio 44122

⁷ 0.5% L-lysine · HCl(0.4% lysine)

⁸ E-Wha oil Co., Ltd., Busan, Korea

⁹ Contains(as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate,150; Choline bitartrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl- α -Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B₁₂, 0.06

¹⁰ Contains (as mg/kg in diets) : NaCl, 437.4; MgSO₄ · 7H₂O, 1379.8; NaH₂P₄ · 2H₂O, 877.8; Ca(H₂PO₄)₂ · 2H₂O, 1366.7; KH₂PO₄, 2414; ZnSO₄ · 7H₂O, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; MnSO₄, 0.016; FeSO₄, 0.0378; CuSO₄, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; NaSeO₃, 0.00025

¹¹ Carboxymethylcellulose

¹² Refer to the table 1.

Table 5. Calculated essential amino acid (EAA) composition of the six experimental diets (% of dry matter basis)¹

Amino acid	Diets ³					
	DHSM ₀	DHSM ₁₀	DHSM ₁₅	DHSM ₂₀	DHSM _{20+AA}	DHSM _{30+AA}
Arginine	3.37	3.36	3.29	3.29	3.22	3.18
Histidine	1.15	1.18	1.17	1.18	1.16	1.14
Isoleucine	2.28	2.26	2.20	2.19	2.14	2.11
Leucine	4.49	4.62	4.60	4.66	4.56	4.51
Lysine	3.51	3.51	3.28	3.23	3.78	3.73
Met + Cys ²	1.94	1.89	1.82	1.79	2.37	2.34
Phe + Tyr ²	3.80	3.88	3.84	3.88	2.80	3.75
Threonine	2.15	2.15	2.11	2.11	2.06	2.04
Tryptophan	0.49	0.51	0.50	0.51	0.50	0.49
Valine	2.61	2.61	2.56	2.56	2.51	2.48

¹ Values are calculated from the ingredients used in this study.

² Dispensable amino acids, but cystine spares methionine and tyrosine spares phenylalanine

³ Refer to the table 1.

Experiment 2: Evaluation of dehulled soybean meal as a fish meal replacer in olive flounder (*Paralichthys olivaceus*)

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile olive flounder

Experimental design and diets

An eight week feeding trial was conducted to determine the inclusion level of dehulled soybean meal (DHSM) that could replace fish meal (FM) in juvenile olive flounder diets. Eight experimental diets were formulated to contain 50% CP and 16.7 kJ available energy g^{-1} by varying lipid content (Table 6). Eight diets were formulated to replace fish meal with dehulled soybean meal at 0, 10 & 20% without amino acid supplementation and 20, 30 & 40% with amino acid supplementation and 30% & 40% with amino acid & attractant supplementation (DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA}, DHSM_{30+AA+Att}, DHSM_{40+AA}, DHSM_{40+AA+Att}). Estimate available energy (Lee & Putnam, 1973; Garling & Wilson, 1976) of experimental diets were adjusted to have 16.7 kJ g^{-1} (16.7, 16.7 and 37.7 kJ g^{-1} for protein, carbohydrate and lipid, respectively). The approximate and essential amino acids (EAA) composition of FM and DHSM are shown in Table 7. Two EAA (methionine and lysine) were selected to study the effect of adding EAA to olive flounder diets. The EAA composition of the experimental diets is shown in Table 8. White fish meal, dehulled soybean meal, and corn gluten meal served as the major protein sources. Squid liver oil and wheat

flour were used as lipid and carbohydrate sources, respectively. Cellulose were also included in the diets to match CP and energy levels. Dehulled soybean meal were obtained from ASA (American Soybean Association / Korea). Dehulled soybean meal was composed of 50.4% crude protein, 2.1% crude fat and 7.1% ash on a dry-matter basis. Procedures for diet preparation and storage were as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, squid liver oil together with 30% well water, experimental diets were pelleted by using a laboratory pelleting machine.

Experimental fish and feeding trials

Juvenile olive flounder (*Paralichthys olivaceus*) were obtained from Tong-young and the feeding trials were conducted at the Institute of Fisheries Sciences (Pukyong National University, Pusan, Korea). Prior to the feeding trial, the fish were fed DHSM₀ diet for 1 weeks to allow them to adjust to the experimental diets and conditions. Feeding trials were conducted in 40 ℓ aquaria each having a water flow rate of 0.8 ℓ /min. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5 ± 0.5 ppm. The water temperature was maintained at 23 ± 2 °C (mean ± SD) the salinity was maintained at 31 ± 1g/L (mean ± SD), and the pH was maintained at 7.5 ± 0.3 (mean ± SD) and photoperiod of 12h light : 12h dark (06:00 to 18:00) was used throughout the experimental periods. In the 8-week experimental period, three groups of 25 fish averaging 5.0 ± 0.04 g (mean ± SD) were randomly assigned to each diet treatment. Each diet were fed to triplicate groups twice per day at a rate of 4-5% dry matter basis of wet body weight. Total fish

weight in each aquarium was determined every 2 weeks, and the amount of diet fed to fish was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing. Feeding trial was carried out at the Institute of Fisheries Sciences.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with MS-222 (100ppm), and then weighed, and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER). Three fish from each aquarium were randomly selected to determine hepatosomatic index (HSI) and condition factor (CF). Blood samples were obtained from the caudal vein with a syringe. Haematocrit (PCV) was determined on three fish randomly selected per aquarium by the microhematocrit method (Brown, 1980), and hemoglobin (Hb) was measured with the same fish by the cyan-methemoglobin procedure using Drabkins solution. Hb standard prepared from human blood (Sigma Chemical, St. Louis, Missouri) was used. The muscle structural amino acids were quantified by amino acid analyzer S433 (Sykam, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4mm × 150mm, Absorbance; 570nm 440nm, Reagent flow rate; 0.25ml/min, Buffer flow rate; 0.45ml/min, Reactor temperature; 120, Reactor Size; 15m. Analyses of crude protein, moisture and ash were performed by the standard procedure of AOAC (1995). Crude fat was determined using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying samples for 12hours.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

Table 6. Composition and proximate analysis of the eight experimental diets (% of dry matter basis)¹

Ingredients	Diets ¹²							
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM	DHSM	DHSM	DHSM	DHSM
				20+AA	30+AA	30+AA+Att	40+AA	40+AA+Att
White fish meal ²	60	54	48	48	42	42	36	36
Corn gluten meal ³	6.9	6.9	6.9	5.5	5.4	5.0	5.4	4.9
Soybean meal ⁴	0	8.84	17.7	17.7	26.5	26.5	35.4	35.4
Wheat meal ⁵	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Dextrin ⁶	11.9	8.6	5.4	5.7	2.5	2.6	0	0.30
Methionine	0	0	0	0.5	0.5	0.5	0.5	0.5
L-Lysine · HCl ⁷	0	0	0	0.54	0.56	0.56	0.57	0.57
Squid oil ⁸	7.5	7.7	7.9	7.95	8.2	8.2	8.06	8.00
Vitamin premix ⁹	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix ¹⁰	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CMC ¹¹	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	0.03	0.20	0.42	0.42	0.67	0.52	0.42	0.21
Attractant	0	0	0	0	0	0.5	0	0.5
<i>Proximate analysis</i>								
Moisture	18.2	18.4	18.9	19.0	18.6	18.6	18.5	18.7
Crude protein	50.4	50.3	50.3	50.0	49.8	50.0	49.9	50.0
Crude lipid	11.7	11.7	11.8	11.8	11.9	11.8	11.4	11.3
Crude ash	11.9	11.3	10.8	10.8	10.2	10.2	9.7	9.7

¹ Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

^{2,3} Suhyup Co. Busan, Korea

- ⁴ American Soybean Association/Korea
- ⁵ Young Nam Flourmills Co., Busan, Korea
- ^{6,11} United States Biochemical, Cleveland, Ohio 44122
- ⁷ 0.5% L-lysine · HCl (0.4% lysine)
- ⁸ E-Wha oil Co., Ltd., Busan. Korea
- ⁹ Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ;Choline bitartrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150;Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl- α -Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B₁₂, 0.06
- ¹⁰ Contains (as mg/kg in diets) : NaCl, 437.4; MgSO₄ · 7H₂O, 1379.8; NaH₂P₄ 2H₂O, 877.8; Ca(H₂PO₄)₂ · 2H₂O, 1366.7; KH₂PO₄, 2414; ZnSO₄ · 7H₂O, 226.4; Fe-Citrate, 299 ; Ca-lactate, 3004; MnSO₄, 0.016; FeSO₄, 0.0378; CuSO₄, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; NaSeO₃, 0.00025
- ¹¹ Carboxymethylcellulose
- ¹² DHSM₀, 100% fish meal (FM) ; DHSM₁₀, 90% FM + 10% dehulled soybean meal (DHSM); DHSM₂₀, 80% FM + 20% DHSM; DHSM_{20+AA}, 80% FM + 20% DHSM + Met & Lys ; DHSM_{30+AA}, 70%FM + 30% DHSM + Met & Lys ; DHSM_{30+AA+Att}, 70%FM + 30% DHSM + Met & Lys + Attractant ; DHSM_{40+AA}, 60%FM + 40% DHSM + Met & Lys; DHSM_{40+AA+Att}, 60% FM + 40% DHSM + Met & Lys + Attractant.

Table 7. Proximate analysis and amino acid composition of white fish meal and dehulled soybean meal (% of dry matter basis)¹

	White fish meal	Dehulled soybean meal
Crude protein	72.2	50.4
Crude lipid	6.8	2.1
Crude ash	19.4	7.1
Arginine	4.02	3.84
Histidine	1.41	1.36
Lysine	4.92	3.22
Leucine	4.91	3.85
Isoleucine	2.72	2.50
Methionine	1.83	0.80
Phenylalanine	2.62	2.57
Threonine	2.52	1.96
Tryptophan	0.79	0.56
Valine	3.23	2.33

¹ Values of amino acid were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University.

Table 8. Calculated essential amino acid (EAA) composition of eight experimental diets (% of dry matter basis)¹

Ingredients	Diets ³							
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM 20+AA	DHSM 30+AA	DHSM 30+AA+Att	DHSM 40+AA	DHSM 40+AA+Att
Arginine	3.16	3.29	3.43	3.40	3.50	3.49	3.62	3.62
Histidine	1.17	1.22	1.27	1.25	1.28	1.28	1.33	1.32
Isoleucine	2.26	2.33	2.42	2.38	2.43	2.42	2.50	2.49
Leucine	4.54	4.61	4.69	4.52	4.55	4.49	4.60	4.55
Lysine	3.74	3.74	3.75	3.74	3.71	3.70	3.69	3.69
Met + Cys ²	2.21	2.16	2.11	2.07	1.99	1.98	1.93	1.92
Phe + Tyr ²	3.83	3.97	4.12	4.00	4.10	4.06	4.22	4.19
Threonine	2.05	2.08	2.12	2.09	2.10	2.09	2.13	2.12
Tryptophan	0.62	0.62	0.63	0.62	0.62	0.62	0.63	0.62
Valine	2.68	2.70	2.73	2.68	2.68	2.67	2.69	2.68

¹ Values are calculated from the ingredients used in this study.

² Dispensable amino acids, but cystine spares methionine and tyrosine spares phenylalanine.

³ Refer to the table 1

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing olive flounder

Experimental design and diets

An twelve week feeding trial was conducted to determine the inclusion level of dehulled soybean meal (DHSM) that could replace fish meal (FM) in growing olive flounder diets. Six experimental diets were formulated to contain 50% CP and 16.7 kJ available energy g⁻¹ by varying lipid content (Table 9). Six diets were formulated to replace fish meal with dehulled soybean meal at 0, 10, 20 & 30% without amino acid supplementation and 20 & 30% with amino acid supplementation (DHSM₀, DHSM₁₀, DHSM₂₀, DHSM₃₀, DHSM_{20+Att}, DHSM_{30+Att}). Estimate available energy (Lee & Putnam, 1973; Garling & Wilson, 1976) of experimental diets were adjusted to have 16.7 kJ g⁻¹ (16.7, 16.7 and 37.7 kJ g⁻¹ for protein, carbohydrate and lipid, respectively). White fish meal, dehulled soybean meal, and corn gluten meal served as the major protein sources. Squid liver oil and wheat meal were used as lipid and carbohydrate sources, respectively. Cellulose were also included in the diets to match CP and energy levels. Dehulled soybean meal were obtained from ASA (American Soybean Association / Korea). Dehulled soybean meal was composed of 50.4% crude protein, 2.1% crude fat and 7.1% ash on a dry-matter basis. Procedures for diet preparation and storage were as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, squid liver oil together with 30% filtered tap water, experimental diets were pelleted by using a laboratory pelleting machine.

Experimental fish and feeding trials

Growing olive flounder (*Paralichthys olivaceus*) were obtained from Tong-young, Korea and the feeding trials were conducted at the Institute of Fisheries Sciences (Pukyong National University, Busan, Korea). Prior to the feeding trial, the fish were fed DHSM₀ diet for 1 weeks to allow them to adjust to the experimental diets and conditions. Feeding trials were conducted in 300 ℓ aquaria each having a water flow rate of 1 ℓ /min. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5 ±0.5 p.p.m. The water temperature was maintained at 17 ± 1 °C (mean ± SD) the salinity was maintained at 31 ± 1g/L (mean ± SD), and the pH was maintained at 7.5 ± 0.3 (mean ± SD) and photoperiod of 12h light : 12h dark (06:00 to 18:00) was used throughout the experimental periods. In the 12-week experimental period, three groups of 15 fish averaging 45.5 ± 0.08 g (mean ± SD) were randomly assigned to each diet treatment. Each diet were fed to triplicate groups twice per day at a rate of 2-4% dry matter basis of wet body weight. Total fish weight in each aquarium was determined every 4 weeks, and the amount of diet fed to fish was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing. Feeding trial was carried out at the Institute of Fisheries Sciences.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with MS-222 (100ppm), and then weighed, and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER). Three fish from each aquarium were randomly selected to

determine hepatosomatic index (HSI) and condition factor (CF). Blood samples were obtained from the caudal vein with a syringe. Hematocrit (PCV) was determined on three fish randomly selected per aquarium by the microhematocrit method (Brown, 1980), and hemoglobin (Hb) was measured with the same fish by the cyan-methemoglobin procedure using Drabkins solution. Hb standard prepared from human blood (Sigma Chemical, St. Louis, Missouri) was used. The muscle structural amino acids were quantified by amino acid analyzer S433 (Sykam, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4mm × 150mm, Absorbance; 570nm 440nm, Reagent flow rate; 0.25 ml/min, Buffer flow rate; 0.45ml/min, Reactor temperature; 120, Reactor Size; 15m. Analyses of crude protein, moisture and ash were performed by the standard procedure of AOAC (1995). Crude fat was determined using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying samples for 12hours.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

Table 9. Composition and proximate analysis of six experimental diets (% of dry matter basis)¹

Ingredients	Diets ¹²					
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM ₃₀	DHSM 20+Att.	DHSM 30+Att.
White fish meal ²	60	54	48	42	48	42
Corn gluten meal ³	6.9	6.9	6.9	6.9	6.9	6.9
Soybean meal ⁴	0	8.8	17.7	26.5	17.7	26.5
Wheat meal ⁵	8.2	8.2	8.2	8.2	8.2	8.2
Dextrin ⁶	11.9	8.6	5.4	2.0	5.3	2.0
Squid oil ⁷	7.5	7.7	7.9	8.2	8.0	8.2
Vitamin premix ⁸	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix ⁹	3.0	3.0	3.0	3.0	3.0	3.0
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5	0.5
CMC ¹⁰	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	0.03	0.2	0.42	0.7	0	0.23
Attractant	0	0	0	0	0.5	0.5
<i>Proximate analysis</i>						
Moisture	19.8	20.2	19.8	20.0	19.1	19.3
Crude protein	50.0	50.2	50.1	50.0	50.3	50.3
Crude lipid	11.8	11.7	11.7	11.8	11.6	11.5
Crude ash	11.9	11.3	10.8	10.3	10.7	10.4

¹ Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

^{2,3} Suhyup Co. Busan, Korea

⁴ American Soybean Association / Korea

⁵ Young Nam Flourmills Co., Busan, Korea

^{6,11} United States Biochemical, Cleveland, Ohio 44122

⁷ E-Wha oil Co., Ltd., Busan. Korea

⁸ Contains(as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate,150; Choline bitartrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B₁₂, 0.06

⁹ Contains (as mg/kg in diets) : NaCl, 437.4; MgSO₄ · 7H₂O, 1379.8; NaH₂P₄ · 2H₂O, 877.8; Ca(H₂PO₄)₂ · 2H₂O, 1366.7; KH₂PO₄, 2414; ZnSO₄ · 7H₂O, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; MnSO₄, 0.016; FeSO₄, 0.0378; CuSO₄, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; NaSeO₃, 0.00025

¹⁰ Carboxymethylcellulose

¹² Refer to the table 1.

Table 10. Calculated essential amino acid (EAA) composition of the six experimental diets (% of dry matter basis)¹

Amino Acid	Diets ³					
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM ₃₀	DHSM _{20+Att}	DHSM _{30+Att}
Arginine	3.23	3.36	3.47	3.60	3.44	3.57
Histidine	1.19	1.24	1.28	1.33	1.27	1.32
Isoleucine	2.30	2.39	2.45	2.52	2.43	2.50
Leucine	4.63	4.71	4.75	4.82	4.71	4.77
Lysine	3.82	3.82	3.79	3.79	3.76	3.75
Met + Cys ²	2.26	2.21	2.13	2.07	2.11	2.06
Phe + Tyr ²	3.91	4.06	4.17	4.31	4.13	4.27
Threonine	2.09	2.13	2.15	2.18	2.13	2.16
Tryptophan	0.63	0.64	0.64	0.64	0.63	0.64
Valine	2.73	2.76	2.76	2.79	2.74	2.76

¹ Values are calculated from the ingredients used in this study.

² Dispensable amino acids, but cystine spares methionine and tyrosine spares phenylalanine

³ Refer to the table 1.

III. Results

Experiment 1: Evaluation of dehulled soybean meal as a fish meal replacer in Korean rockfish

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile Korean rockfish

Weight gain (WG, %), feed efficiency (FE, %), specific growth rate (SGR, %), protein efficiency ratio (PER, %), hepatosomatic index (HSI, %), condition factor (CF), hematocrit (PCV, %), hemoglobin (Hb, g/100ml) and survival (%) during the first 8-week experiment are shown in Table 11. WG of fish fed DHSM₀, DHSM₁₅ and DHSM_{30+AA} diets were significantly higher than those of fish fed DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$). However, there was no significant difference in WG among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. FE of fish fed DHSM₀ and DHSM₁₅ diets were significantly higher than those of fish fed DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$). However, there was no significant difference in FE among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. SGR of fish fed DHSM₀ diet was significantly higher than those of fish fed DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$). However, there was no significant difference in SGR among fish fed DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀ and DHSM_{30+AA} diets. PER of fish fed DHSM₀ diet was significantly higher than those of fish fed DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$).

However, there was no significant difference in PER among fish fed DHSM₀, DHSM₁₅ and DHSM_{30+AA} diets. HSI of fish fed DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM_{30+AA} and DHSM_{45+AA} diets were significantly higher than those of fish fed DHSM_{60+AA} diet ($P<0.05$). However, there was no significant different in HSI among fish fed DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{30+AA} and DHSM_{45+AA} diets. CF, PCV, Hb showed the same trend as HSI. survival (%) of fish fed DHSM₀ and DHSM₂₀ diets were significantly higher than those of fish fed DHSM₄₅ and DHSM_{60+AA} diets ($P<0.05$). However, there was no significant difference in survival among fish fed DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM_{30+AA} and DHSM_{45+AA} diets. Crude protein of wholebody in fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets were not significantly different from each other (Table 12). Average apparent digestibility (AD) and apparent protein digestibility (APD) are shown in Table 13. APD showed the same trend as AD. APD ranged from $86.2\pm3.5\%$ in this study. APD of fish fed DHSM₀ and DHSM₁₅ diets were significantly higher than those of fish fed DHSM₃₀, DHSM₄₅, DHSM_{45+AA} and DHSM_{60+AA} diets ($P<0.05$). However, there was no significant difference in APD among fish fed DHSM₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. The APD in DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{30+AA}, DHSM_{45+AA} and DHSM_{60+AA} diets was 91.3%, 89.2%, 87.4%, 86.2%, 81.3%, 88.3%, 84.2% and 81.9%, respectively.

Table 11. Effects of the experimental diets for the first 8 weeks in juvenile Korean rockfish (*Sebastes schlegelii*)¹

	Diets ²								Pooled SEM ³
	DHSM ₀	DHSM ₁₅	DHSM ₂₀	DHSM ₃₀	DHSM ₄₅	DHSM _{30+AA}	DHSM _{45+AA}	DHSM _{60+AA}	
WG(%) ⁴	509 ^a	491 ^a	439 ^{ab}	334 ^b	188 ^d	465 ^a	325 ^{bc}	210 ^{cd}	25.1
FE(%) ⁵	87.6 ^a	85.1 ^a	76.0 ^{ab}	66.4 ^b	38.7 ^c	79.3 ^{ab}	63.2 ^b	40.2 ^c	3.80
SGR(%) ⁶	3.23 ^a	3.17 ^{ab}	3.01 ^{ab}	2.62 ^{ab}	1.88 ^d	3.09 ^{ab}	2.58 ^{bc}	1.97 ^{cd}	0.11
PER ⁷	1.82 ^a	1.77 ^{ab}	1.58 ^{bc}	1.38 ^{cd}	0.81 ^e	1.65 ^{ab}	1.32 ^d	0.82 ^e	0.08
HSI ⁸	2.85 ^a	3.05 ^a	3.00 ^a	2.97 ^a	2.68 ^{ab}	2.95 ^a	2.90 ^a	2.50 ^b	0.01
CF ⁹	1.80 ^a	1.76 ^a	1.74 ^a	1.70 ^a	1.65 ^{ab}	1.75 ^a	1.70 ^a	1.60 ^b	0.01
PCV ¹⁰	32.5 ^a	30.3 ^a	31.0 ^a	29.7 ^a	28.2 ^{ab}	30.6 ^a	29.5 ^a	26.1 ^b	0.02
Hb ¹¹	9.80 ^a	9.79 ^a	9.72 ^a	9.70 ^a	8.84 ^{ab}	9.75 ^a	9.00 ^a	8.50 ^b	0.03
Survival(%)	98.3 ^a	96.7 ^{ab}	99.2 ^a	87.5 ^{ab}	85.8 ^b	95.0 ^{ab}	96.7 ^{ab}	85.8 ^b	1.27

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/ \sqrt{n} .

⁴ WG(%) = Weight gain (%) : [(final wt. - initial wt.) / initial wt.] \times 100.

⁵ FE = Feed efficiency : (wet weight gain / dry feed intake) \times 100.

⁶ SGR = Specific growth rate : [(log_e final wt. - log_e initial wt.)/days] \times 100.

⁷ PER =Protein efficiency ratio : wet wt. gain / protein intake.

⁸ HSI = Hepatosomatic index : (liver weight \times 100/ body weight).

⁹ CF = Condition factor : (wet weight / total length³) \times 100.

¹⁰PCV = Hematocrit (%).

¹¹Hb = Hemoglobin (g/100ml).

Table 12. Whole body proximate composition (%) of juvenile Korean rockfish (*Sebastes schlegeli*) fed eight experimental diets for the first 8 weeks¹

Diet ²	Moisture	Crude protein	Crude lipid	Ash
DHSM ₀	70.8	17.9 ^a	8.9 ^a	4.3 ^c
DHSM ₁₅	69.5	17.9 ^a	8.8 ^a	4.4 ^b
DHSM ₂₀	69.9	17.4 ^{ab}	8.9 ^a	4.5 ^b
DHSM ₃₀	69.0	16.9 ^b	8.7 ^b	4.6 ^{ab}
DHSM ₄₅	68.7	16.0 ^c	8.6 ^b	4.7 ^a
DHSM _{30+AA}	69.6	17.5 ^{ab}	8.7 ^b	4.3 ^c
DHSM _{45+AA}	68.3	16.8 ^b	8.8 ^a	4.6 ^{ab}
DHSM _{60+AA}	68.4	16.1 ^c	8.6 ^b	4.7 ^a
Pooled SEM	0.17	0.11	0.03	0.02

¹ Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/ \sqrt{n} .

Table 13. Apparent dry matter digestibility (AD) and apparent protein digestibility (APD) in juvenile Korean rockfish (*Sebastes schlegeli*) diets¹

Diets ²	AD(%)	APD(%)
DHSM ₀	82.0 ^a	91.3 ^a
DHSM ₁₅	78.3 ^a	89.2 ^a
DHSM ₂₀	77.4 ^{ab}	87.4 ^{ab}
DHSM ₃₀	75.2 ^b	86.2 ^b
DHSM ₄₅	71.0 ^c	81.3 ^c
DHSM _{30+AA}	78.1 ^{ab}	88.3 ^{ab}
DHSM _{45+AA}	74.2 ^b	84.2 ^b
DHSM _{60+AA}	71.0 ^c	81.9 ^c
Pooled SEM ³	0.78	0.72

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

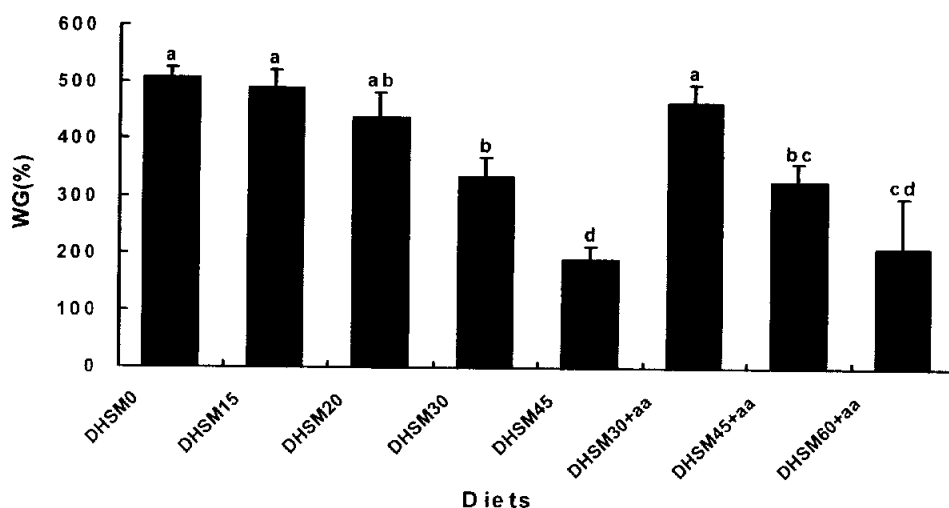


Fig.1. Average weight gain (WG, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$)

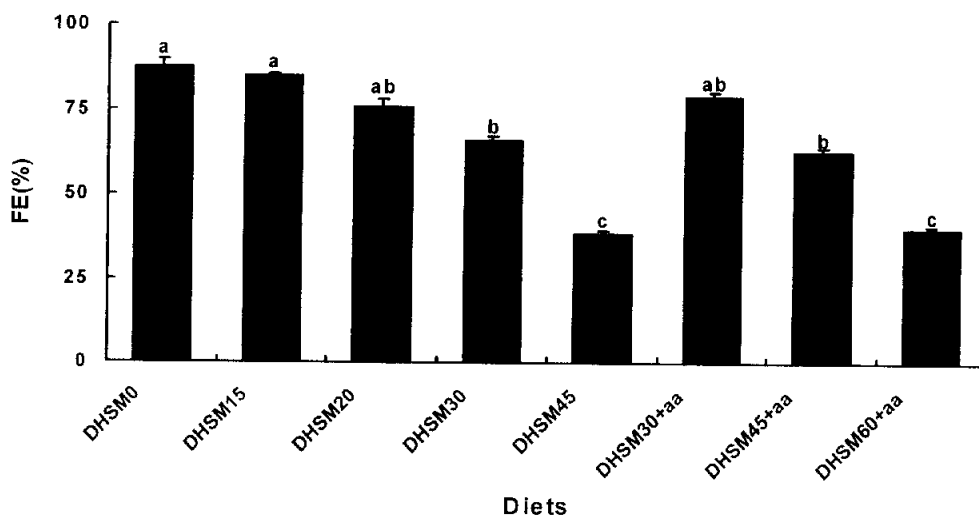


Fig.2. Average feed efficiency (FE, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$)

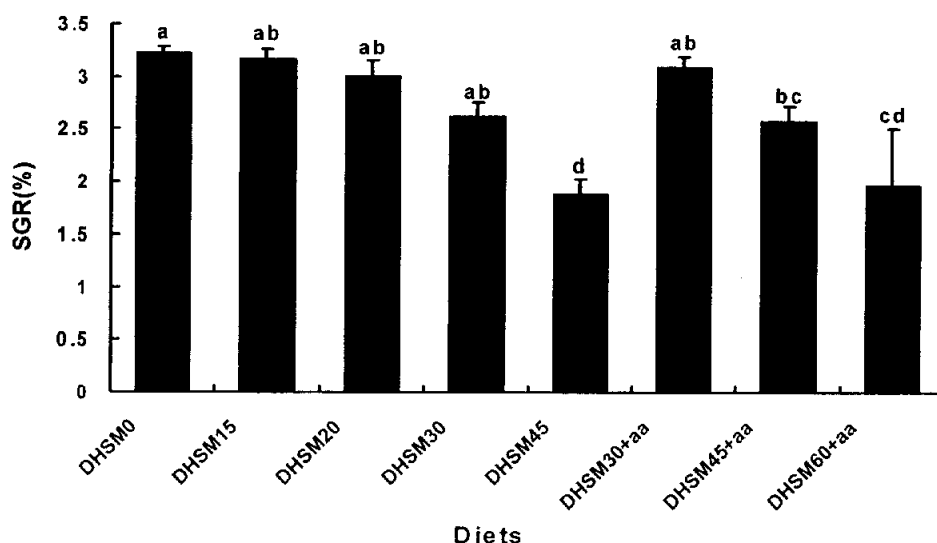


Fig.3. Average specific growth rate (SGR, %) from fish fed 8 diet for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

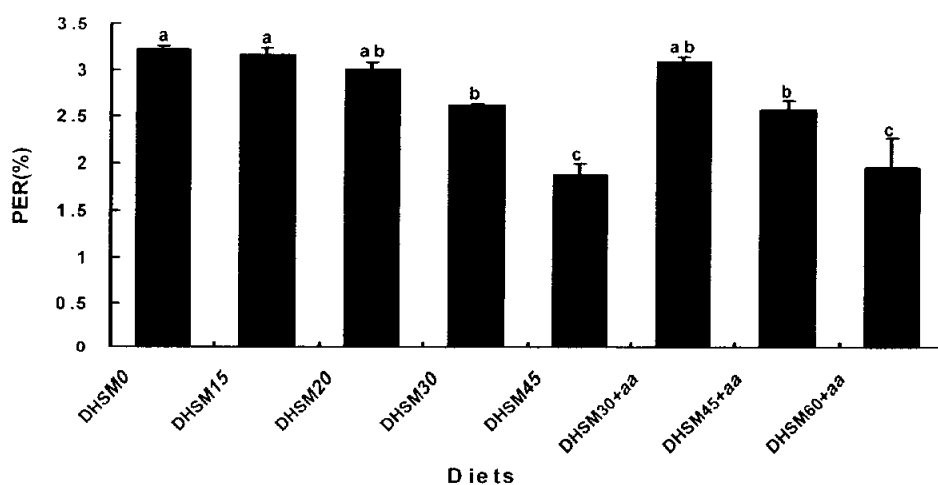


Fig.4. Average protein efficiency ratio (PER, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

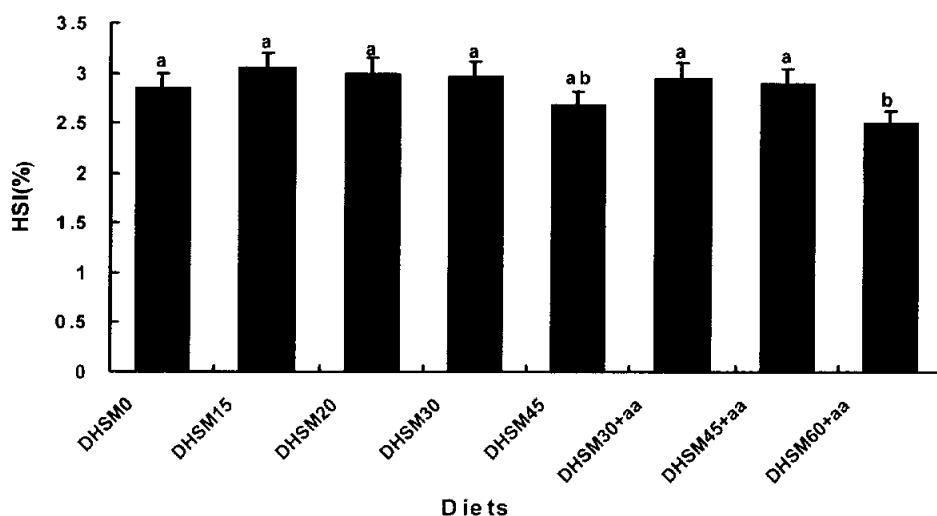


fig.5. Average hepatosomatic index (HSI, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

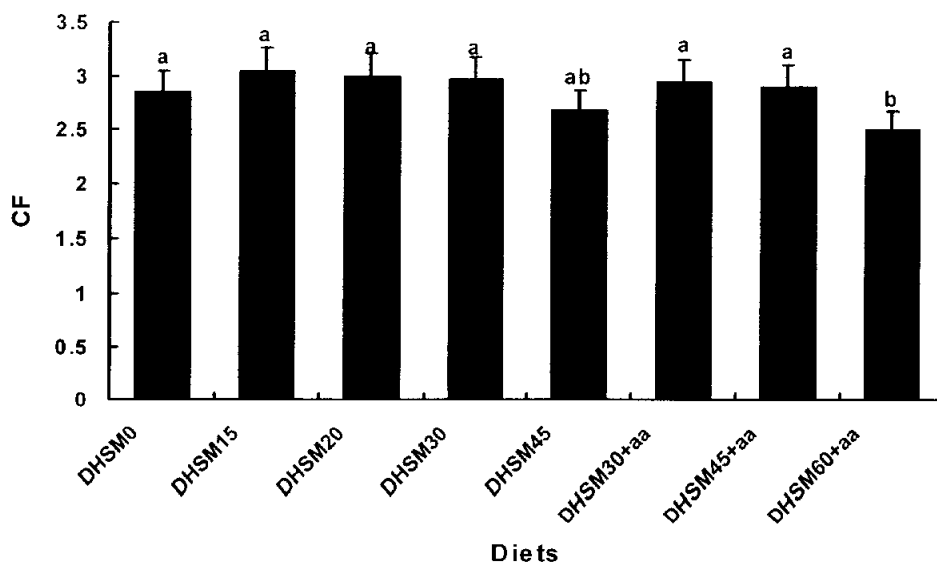


fig.6. Average condition factor (CF) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

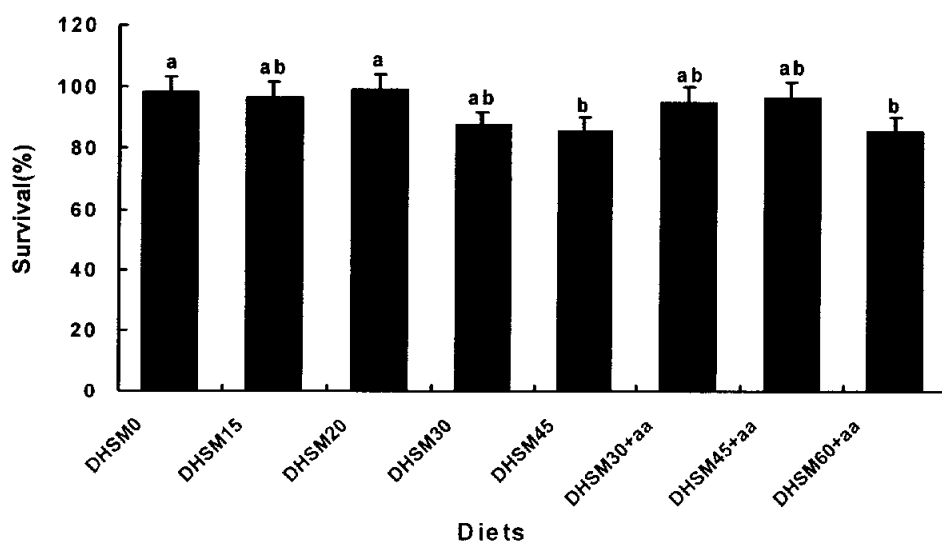


Fig.7. Average Survival (%) from the fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing Korean rockfish

The second 10-week experiment are summarized in Table 14. There was no significant difference in weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and intraperitoneal fat (IPF) ratio among fish fed six experimental diets. WG in this experiment ranged from 270.4% for fish fed DHSM₂₀ diet to a high of 277.2% for fish fed DHSM₀ diet. Average protein efficiency ratio (PER) of fish fed DHSM₀ diet was significantly higher than those of fish fed DHSM_{20+AA} and DHSM_{30+AA} diets ($P<0.05$). However, there was no significant difference in PER among fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀ diets. Average hepatosomatic index (HSI) of fish fed DHSM₀, DHSM₁₅, DHSM_{20+AA} diets were significantly higher than those of fish fed DHSM₂₀ diets ($P<0.05$). However, there was no significant difference in HSI among fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM_{20+AA} and DHSM_{30+AA} diets. Hematological and serological characteristics are shown in Table 15. Hemoglobin (Hb) content of fish fed DHSM_{20+AA} diet was significantly higher than those of fish fed DHSM₀, DHSM₁₀, DHSM₁₅ and DHSM₂₀ diets ($P<0.05$). However, there was no significant difference in Hb among fish fed DHSM_{20+AA} and DHSM_{30+AA} diets. Triglyceride (TG) content of fish fed DHSM_{30+AA} diet was significantly higher than those of fish fed DHSM_{20+AA} diet ($P<0.05$). However, there was no significant difference in TG among fish fed DHSM₀, DHSM₁₀, DHSM₁₅, DHSM₂₀ and DHSM_{30+AA} diets. There was no significant difference in Hematocrit (PCV), total protein (TP), glucose contents among fish fed the six experimental diets.

Whole body proximate composition are shown in Table 16. There was no significant difference in whole body proximate composition among fish fed six experimental diets. Dorsal muscle amino acid composition during the second 12-weeks experiment are shown in Table 17. Amino acids in dorsal muscle was 85.1% - 81.7%. There was no significant difference in amino acids in dorsal muscle among fish fed the six experimental diets and there was no significant difference in amino acids composition in dorsal muscle among different dietary treatment.

Table 14. Effects of the experimental diets for the second 12 weeks in growing Korean rockfish¹

	Diets ²						Pooled SEM ³
	DHSM ₀	DHSM ₁₀	DHSM ₁₅	DHSM ₂₀	DHSM _{20+AA}	DHSM _{30+AA}	
WG(%) ⁴	277.2	275.1	271.3	270.4	274.3	271.6	0.93
FE(%) ⁵	83.9	83.5	83.2	82.0	83.2	81.5	0.48
SGR(%) ⁶	1.58	1.57	1.56	1.56	1.57	1.56	0.003
PER ⁷	1.75 ^a	1.74 ^{ab}	1.73 ^{ab}	1.71 ^{ab}	1.70 ^b	1.70 ^b	0.003
HSI ⁸	3.05 ^a	2.96 ^{ab}	3.11 ^a	2.56 ^b	3.23 ^a	2.93 ^{ab}	0.06
IPF ratio ⁹	2.96	2.96	3.40	2.94	3.63	3.23	0.11
CF ¹⁰	1.65	1.66	1.59	1.59	1.65	1.64	0.02
Survival(%)	100	100	100	100	100	100	0

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/ \sqrt{n} .

⁴ WG(%) = Weight gain (%) : [(final wt. - initial wt.) / initial wt.] \times 100.

⁵ FE = Feed efficiency : (wet weight gain / dry feed intake) \times 100.

⁶ SGR = Specific growth rate : [(log_e final wt. - log_e initial wt.)/days] \times 100.

⁷ PER =Protein efficiency ratio : wet wt. gain / protein intake.

⁸ HSI = Hepatosomatic index : (liver weight \times 100/ body weight).

⁹ CF = Condition factor : (wet weight / total length³) \times 100.

Table 15. Hematological and serological characteristics of growing Korean rockfish (*Sebastes schlegeli*) fed six experimental diets for the second 12 weeks¹

Diets ²	Hemoglobin (g/100ml)	Hematocrit (%)	Protein (g/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)
DHSM ₀	10.2 ^{bc}	40.7	3.6	99.7 ^{ab}	42.7
DHSM ₁₀	11.1 ^b	40.5	3.8	96.3 ^{ab}	39.3
DHSM ₁₅	10.4 ^{bc}	38.0	3.7	95.0 ^{ab}	53.3
DHSM ₂₀	9.6 ^c	39.7	3.5	102.3 ^{ab}	62.0
DHSM _{20+AA}	12.6 ^a	39.7	3.7	79.3 ^b	58.3
DHSM _{30+AA}	11.3 ^{ab}	40.3	3.9	115.3 ^a	46.0
Pooled SEM ³	0.27	0.48	0.06	3.96	3.29

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different ($P < 0.05$).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

Table 16. Whole body proximate composition (%) of growing Korean rockfish (*Sebastes schlegeli*) fed six experimental diets for the second 12 weeks¹

Diet ²	Moisture	Crude protein	Crude lipid	Ash
DHSM ₀	67.2	18.1	9.2	4.2
DHSM ₁₀	67.8	18.1	9.5	4.8
DHSM ₁₅	67.0	18.3	8.9	3.8
DHSM ₂₀	71.4	18.0	9.2	4.4
DHSM _{20+AA}	66.7	18.0	9.3	4.2
DHSM _{30+AA}	69.0	18.0	9.2	4.6
Pooled SEM ³	0.41	0.4	0.27	0.32

¹ Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different ($P < 0.05$).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

Table 17. Dorsal muscle amino acid composition (mg/100 mg) of growing Korean rockfish (*Sebastes schlegeli*) fed 6 experimental diets for 12 weeks (% of dry matter basis)¹

Amino Acid	Diets ²					
	DHSM ₀	DHSM ₁₀	DHSM ₁₅	DHSM ₂₀	DHSM 20+AA	DHSM 30+AA
Aspartic acid	9.28	9.06	8.98	9.38	9.02	9.17
Threonine	3.98	3.90	3.84	3.99	3.86	3.91
Serine	3.69	3.59	3.55	3.69	3.61	3.60
Glutamic acid	13.2	12.8	12.7	13.2	12.6	12.7
Glycine	3.79	3.71	3.64	3.85	3.72	3.72
Alanine	5.31	5.25	5.14	5.41	5.18	5.18
Cystine	1.26	0.91	1.28	0.84	1.29	1.24
Valine	4.08	3.97	3.97	4.14	3.97	3.97
Methionine	2.72	2.47	2.62	2.28	2.62	2.37
Isoleucine	4.21	4.23	4.08	4.30	4.11	4.05
Leucine	6.89	6.71	6.58	6.87	6.54	6.63
Tyrosine	3.03	2.95	2.94	2.98	2.96	2.90
Phenylalanine	3.84	3.87	3.21	3.96	3.87	3.83
Histidine	2.72	2.81	2.84	2.86	2.78	2.85
Lysine	8.46	8.26	8.15	8.45	8.21	8.23
Arginine	5.18	5.15	4.93	5.02	4.78	4.88
Total AA	85.1	83.2	81.7	84.6	82.5	82.7

¹ Means of triplicated groups, values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD / \sqrt{n} .

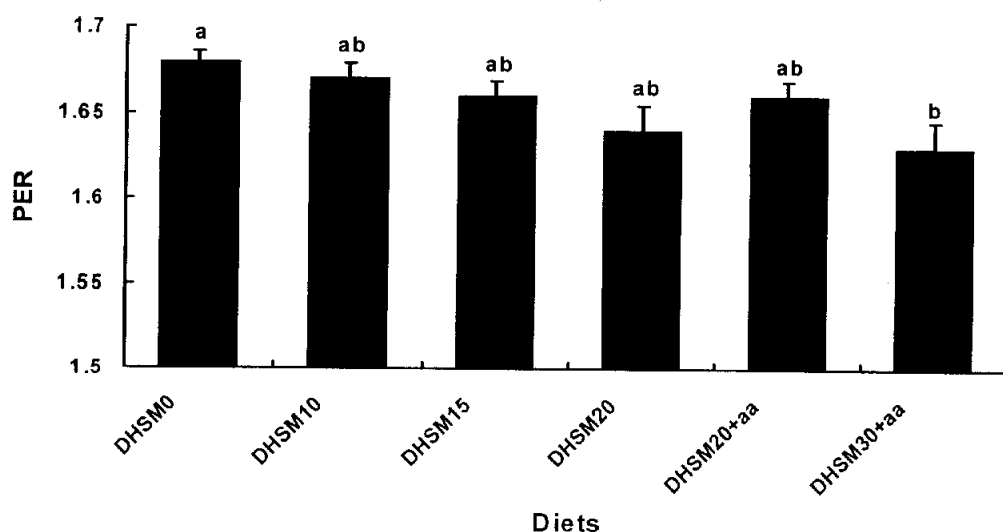


Fig.8. Average protein efficiency ratio (PER) from fish fed 6 diets for the second 12 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

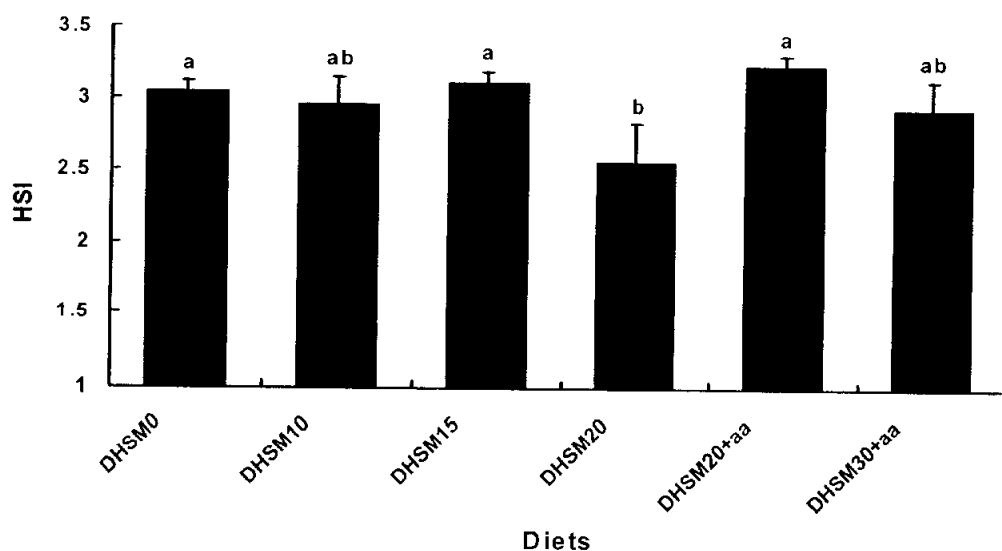


Fig.9. Average hepatosomatic index (HSI) from the fish fed 6 diets for the second 12 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

Experiment 2 : Evaluation of dehulled soybean meal as a fish meal replacer in olive flounder *Paralichthys olivaceus*

(1) Evaluation of dehulled soybean meal as a fish meal replacer in juvenile olive flounder

Weight gain (WG, %), feed efficiency (FE, %), specific growth rate (SGR, %), protein efficiency ratio (PER, %), hepatosomatic index (HSI, %), condition factor (CF), hematocrit (PCV, %), hemoglobin (Hb, g/100ml) and survival (%) during the 8-week experiment are shown in Table 18. There was no significant difference in weight gain (WG) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, WG of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than those of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets ($P<0.05$). There was no significant difference in FE among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, FE of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than those of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets ($P<0.05$). There was no significant difference in SGR among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, SGR of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. There was no significant difference in PER among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, average PER

of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than those of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets ($P<0.05$). There was no significant difference in HSI among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, average HSI of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than those of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diet ($P<0.05$). There was no significant difference in CF among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, CF of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than those of fish fed DHSM_{40+AA} and DHSM_{40+AA+Att} diets. PCV of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA}, DHSM_{30+AA+Att} and DHSM_{40+AA} diets were significantly higher than those of fish fed DHSM_{40+AA+Att} diet ($P<0.05$). Hb showed the same trend as PCV. However, there was no significant difference the same trend as PCV. However, there was no significant difference in survival among fish fed all of diets ($P>0.05$). Whole body proximate composition are shown in Table 19. There was no significant difference in crude protein (CP) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, CP of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM₄₀ and DHSM_{40+Att} diet ($P<0.05$). There was no significant difference in crude lipid among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, crude lipid of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA},

DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly higher than that of fish fed DHSM₄₀ and DHSM_{40+Att} diet ($P<0.05$). There was no significant difference in crude ash among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets. However, crude lipid of fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+AA}, DHSM_{30+AA} and DHSM_{30+AA+Att} diets were significantly lower than that of fish fed DHSM₄₀ and DHSM_{40+Att} diet ($P<0.05$). There was no significant difference in moisture among fish fed six experimental diets.

Table 18. Effects of the experimental diets for the first 8 weeks in juvenile olive flounder (*Paralichthys olivaceus*)¹

	Diets ²								Pooled SEM ³
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM 20+AA	DHSM 30+AA	DHSM 30+AA+AlI	DHSM 40+AA	DHSM 40+AA+AlI	
WG(%) ⁴	549.8 ^a	546.2 ^a	541.2 ^a	544.7 ^a	542.1 ^a	543.4 ^a	502.1 ^b	505.8 ^b	1.63
FE(%) ⁵	103.1 ^a	102.7 ^a	101.9 ^a	102.0 ^a	101.8 ^a	102.0 ^a	97.7 ^b	97.9 ^b	0.15
SGR(%) ⁶	3.34 ^a	3.33 ^a	3.32 ^a	3.33 ^a	3.32 ^a	3.32 ^a	3.21 ^b	3.22 ^b	0.005
PER ⁷	2.06 ^a	2.05 ^a	2.04 ^a	2.04 ^a	2.04 ^a	2.04 ^a	1.95 ^b	1.96 ^b	0.003
HSI ⁸	2.00 ^a	1.98 ^a	1.97 ^a	2.00 ^a	1.97 ^a	1.98 ^a	1.91 ^b	1.92 ^b	0.01
CF ⁹	1.40 ^a	1.39 ^a	1.38 ^a	1.39 ^a	1.38 ^a	1.38 ^a	1.31 ^b	1.33 ^b	0.007
PCV ¹⁰	22.9 ^a	22.3 ^{ab}	22.2 ^{ab}	22.7 ^a	22.1 ^{ab}	22.2 ^{ab}	21.3 ^{ab}	20.7 ^b	0.02
Hb ¹¹	5.00 ^a	4.98 ^a	4.89 ^a	4.94 ^a	4.80 ^a	4.90 ^a	4.65 ^{ab}	4.36 ^b	0.03
Survival(%)	100	99.2	99.2	99.2	100	100	98.3	99.2	0.23

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

⁴ WG(%) = Weight gain (%) : [(final wt. - initial wt.) / initial wt.] × 100.

⁵ FE = Feed efficiency : (wet weight gain / dry feed intake) × 100.

⁶ SGR = Specific growth rate : [(log_e final wt. - log_e initial wt.)/days] × 100.

⁷ PER =Protein efficiency ratio : wet wt. gain / protein intake.

⁸ HSI = Hepatosomatic index : (liver weight × 100/ body weight).

⁹ CF = Condition factor : (wet weight / total length³) × 100.

¹⁰PCV = Hematocrit (%).

¹¹Hb = Hemoglobin (g/100ml).

Table 19. Whole body proximate composition (%) of juvenile olive flounder (*Paralichthys olivaceus*) fed eight experimental diets for the first 8 weeks¹

Diet ²	Moisture	Crude protein	Crude lipid	Ash
DHSM ₀	74.6	17.5 ^a	10.1 ^a	3.70 ^b
DHSM ₁₀	74.5	17.5 ^a	9.9 ^a	3.72 ^b
DHSM ₂₀	74.8	17.4 ^a	9.8 ^a	3.73 ^b
DHSM _{20+AA}	74.4	17.5 ^a	9.9 ^a	3.72 ^b
DHSM _{30+AA}	74.3	17.4 ^a	9.8 ^a	3.72 ^b
DHSM _{30+AA+Att}	74.4	17.4 ^a	9.8 ^a	3.71 ^b
DHSM _{40+AA}	75.8	17.0 ^b	8.7 ^b	4.15 ^a
DHSM _{40+AA+Att}	75.8	17.0 ^b	8.8 ^b	4.11 ^a
Pooled SEM	0.17	0.1	0.11	0.05

¹ Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/ \sqrt{n} .

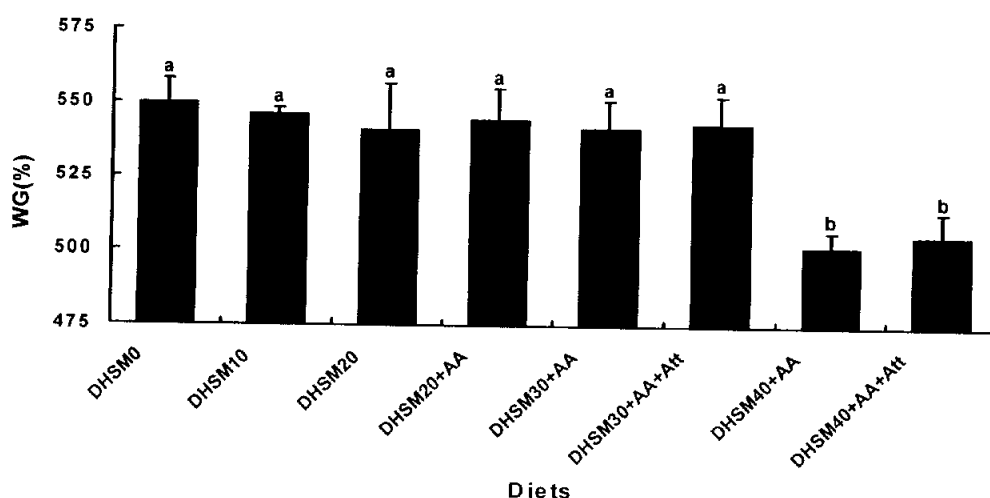


Fig.10. Average weight gain (WG, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

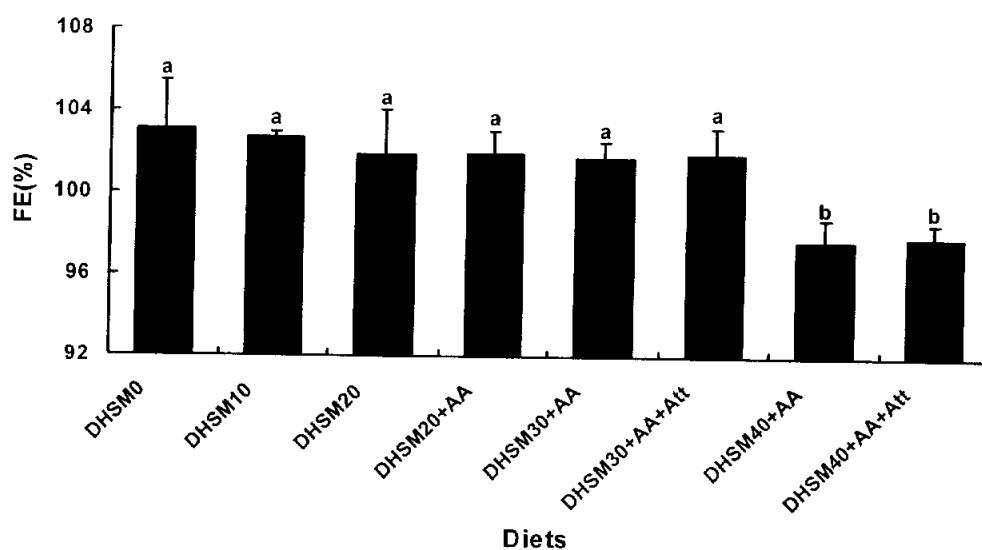


Fig.11. Average feed efficiency (FE, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

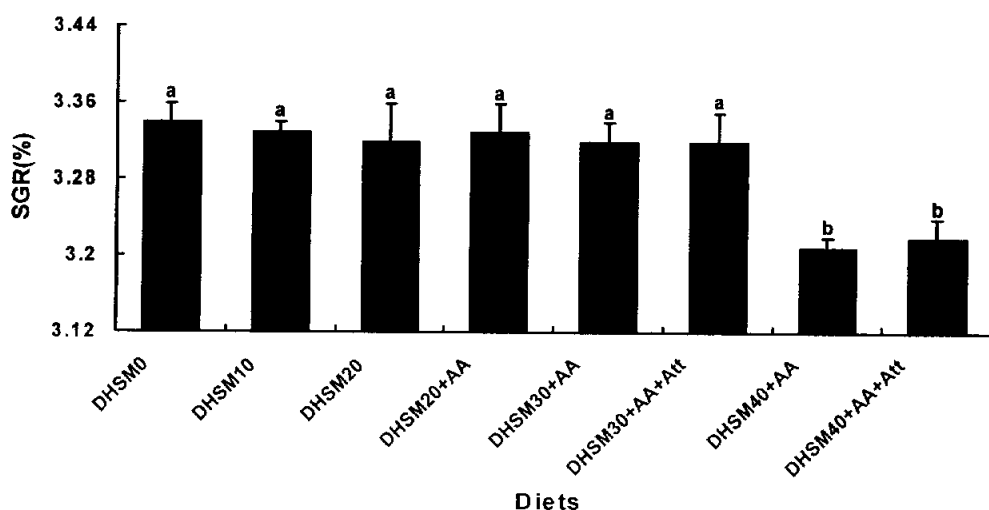


Fig.12. Average specific growth rate (SGR, %) from fish fed 8 diet for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

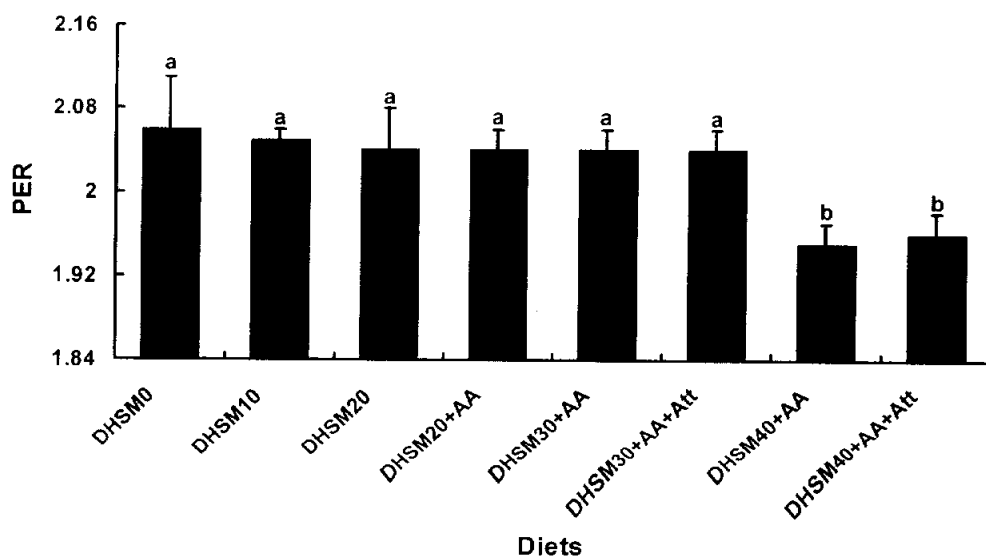


Fig.13. Average protein efficiency ratio (PER, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

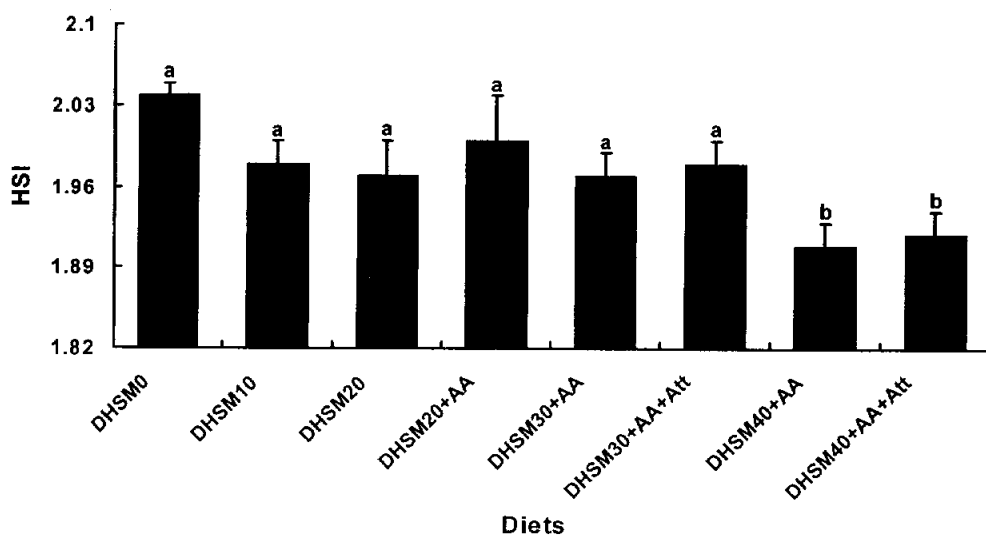


Fig.14. Average hepatosomatic index (HSI, %) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

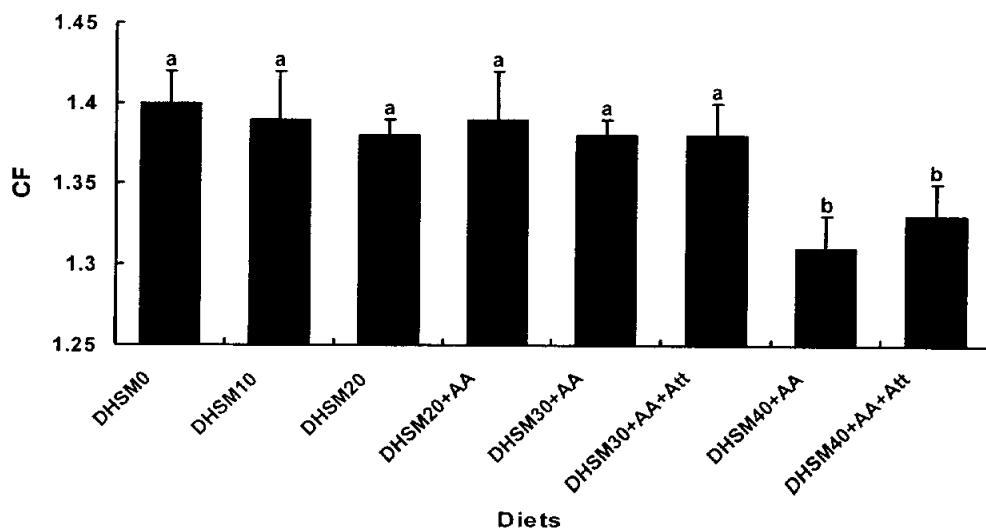


Fig.15. Average condition factor (CF) from fish fed 8 diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

(2) Evaluation of dehulled soybean meal as a fish meal replacer in growing olive flounder

The second 10-week experiment are summarized in Table 20. There was no significant difference in weight gain (WG) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets. However, WG of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While WG of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in feed efficiency (FE) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, FE of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While FE of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in specific growth rate (SGR) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets. However, SGR of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While SGR of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in protein efficiency ratio (PER) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, PER of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While PER of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in hepatosomatic index (HSI) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, HSI of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher

than those of fish fed DHSM₃₀ ($P<0.05$). While HSI of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in condition factor (CF) among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, CF of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than those of fish fed DHSM₃₀ ($P<0.05$). While CF of fish fed DHSM_{20+Att} diet was highest among all the dietary treatments. There was no significant difference in survival among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+Att} and DHSM_{30+Att} diets. Hematological and serological characteristics are shown in Table 21. There was no significant difference in hemoglobin (Hb), hematocrit (PCV), triglyceride (TG), glucose, GOT, GPT contents among fish fed the six experimental diets. There was no significant difference in total protein (TP) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀, DHSM_{20+Att} and DHSM_{30+Att} diets. However, TP content of fish fed DHSM₂₀ diet was significantly higher than that of fish fed DHSM_{30+AA} diet ($P<0.05$). Whole body proximate composition are shown in Table 22. There was no significant difference in crude protein (CP) among fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets. However, CP of fish fed DHSM₀, DHSM₁₀, DHSM₂₀ and DHSM_{30+Att} diets were significantly higher than that of fish fed DHSM₃₀ diet ($P<0.05$). While, CP of fish fed DHSM_{20+Att} diet was the highest among all the dietary treatment. There was no significant difference in crude lipid among fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets. However, crude lipid of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly higher than that of fish fed DHSM₃₀ diet ($P<0.05$). While, Crude lipid of fish fed DHSM₀, DHSM₁₀ and DHSM₂₀ diets were significantly lower than that of fish fed

DHSM_{20+Att} and DHSM_{30+Att} diets. There was no significant difference in crude ash among fish fed DHSM₀, DHSM₁₀ and DHSM_{20+Att} diets. However, crude ash of fish fed DHSM₀, DHSM₁₀ and DHSM_{20+Att} diets were significantly lower than that of fish fed DHSM₂₀, DHSM₃₀ and DHSM_{30+Att} diets ($P<0.05$). There was no significant difference in moisture among fish fed six experimental diets. Dorsal muscle amino acid composition during the second 12-weeks experiment are shown in Table 23. Amino acids in dorsal muscle was 85.1% - 81.7%. There was no significant difference in amino acids in dorsal muscle among fish fed the six experimental diets and there was no significant difference in amino acids composition in dorsal muscle among different dietary treatment.

Table 20. Effects of the experimental diets for the second 10 weeks in growing olive flounder

	Diets ²						Pooled SEM ³
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM ₃₀	DHSM _{20+Att.}	DHSM _{30+Att.}	
WG(%) ⁴	142.0 ^b	141.4 ^b	140.1 ^b	118.6 ^c	155.3 ^a	144.5 ^b	2.7
FE(%) ⁵	100.8 ^b	100.0 ^b	99.2 ^b	87.2 ^c	103.7 ^a	101.0 ^b	2.0
SGR(%) ⁶	1.26 ^b	1.26 ^b	1.25 ^b	1.12 ^c	1.34 ^a	1.28 ^b	0.02
PER ⁷	2.02 ^b	2.00 ^b	1.98 ^b	1.74 ^c	2.07 ^a	2.02 ^b	0.04
HSI ⁸	2.12 ^{bc}	2.10 ^{bc}	2.08 ^c	1.99 ^d	2.20 ^a	2.15 ^{ab}	0.07
CF ⁹	1.20 ^{bc}	1.18 ^c	1.16 ^c	1.04 ^d	1.26 ^a	1.22 ^b	0.07
Survival(%)	100 ^a	95.5 ^a	97.8 ^a	84.5 ^b	100 ^a	97.8 ^a	1.4

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

⁴ WG(%) = Weight gain (%) : [(final wt. - initial wt.) / initial wt.] × 100.

⁵ FE = Feed efficiency : (wet weight gain / dry feed intake) × 100.

⁶ SGR = Specific growth rate : [(log_e final wt. - log_e initial wt.)/days] × 100.

⁷ PER =Protein efficiency ratio : wet wt. gain / protein intake.

⁸ HSI = Hepatosomatic index : (liver weight × 100/ body weight).

⁹ CF = Condition factor : (wet weight / total length³) × 100.

Table 21. Hematological and serological characteristics of growing olive flounder (*Paralichthys olivaceus*) fed six experimental diets for the second 10 weeks¹

Diets ²	Hb (g/100mℓ)	PCV (%)	T.P (g/dL)	TG ³ (mg/dL)	Glucose (mg/dL)	GOT	GPT
DHSM ₀	4.5	21.1	2.9 ^{ab}	588	41	6.7	8.0
DHSM ₁₀	4.3	21.3	2.9 ^{ab}	559	35	8.3	9.7
DHSM ₂₀	4.3	20.4	3.2 ^a	559	44	9.3	9.3
DHSM ₃₀	4.5	21.9	2.5 ^b	428	27	6.3	6.0
DHSM _{20+Att.}	4.5	21.2	2.6 ^{ab}	632	34	10.7	9.7
DHSM _{30+Att.}	4.3	22.2	2.7 ^{ab}	711	36	10.0	11.7
Pooled SEM ³	0.3	2.8	0.09	38	2.7	0.7	1.0

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

Table 22. Whole body proximate composition (%) of growing olive flounder (*Paralichthys olivaceus*) fed six experimental diets for the second 10 weeks¹

Diet ²	Moisture	Crude protein	Crude lipid	Ash
DHSM ₀	74.5	17.5 ^b	10.3 ^b	3.70 ^c
DHSM ₁₀	74.4	17.6 ^b	9.8 ^b	3.69 ^c
DHSM ₂₀	74.3	17.5 ^b	9.7 ^b	4.18 ^{ab}
DHSM ₃₀	75.4	17.0 ^c	8.5 ^c	4.75 ^a
DHSM _{20+Att}	73.8	18.2 ^a	12.0 ^a	3.72 ^c
DHSM _{30+Att}	74.0	17.5 ^b	11.6 ^a	3.89 ^b
Pooled SEM ³	0.3	0.09	0.51	0.1

¹ Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different ($P < 0.05$).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

Table 23. Dorsal muscle amino acid composition (mg/100 mg) of growing olive flounder (*Paralichthys olivaceus*) fed 6 experimental diets for 10 weeks (% of dry matter basis)¹

Amino Acid	Diets ²					
	DHSM ₀	DHSM ₁₀	DHSM ₂₀	DHSM ₃₀	DHSM _{20+Att}	DHSM _{30+Att}
Aspartic acid	9.90	10.0	9.68	9.60	9.64	9.79
Threonine	4.50	4.51	4.42	4.36	4.38	4.43
Serine	3.90	3.90	3.80	3.76	3.82	3.81
Glutamic acid	13.7	13.8	13.3	13.3	13.4	13.3
Glycine	3.70	3.76	3.62	3.55	3.63	3.63
Alanine	5.30	5.40	5.24	5.13	5.17	5.17
Cystine	1.30	0.88	0.95	1.32	1.33	1.28
Valine	4.50	4.56	4.39	4.39	4.39	4.39
Methionine	2.80	2.36	2.55	2.70	2.70	2.45
Isoleucine	4.50	4.59	4.52	4.37	4.40	4.34
Leucine	6.80	6.78	6.62	6.49	6.45	6.54
Tyrosine	3.40	3.35	3.32	3.31	3.34	3.27
Phenylalanine	4.40	4.52	4.43	3.77	4.43	4.39
Histidine	2.70	2.84	2.79	2.82	2.85	2.83
Lysine	8.10	8.09	7.90	7.79	7.85	7.87
Arginine	6.00	5.84	5.97	5.75	5.60	5.70
Total AA	85.5	85.1	83.5	82.4	83.3	83.2

¹ Means of triplicated groups, values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD / \sqrt{n} .

Table 13. Apparent dry matter digestibility (AD) and apparent protein digestibility (APD) in growing olive flounder (*Paralichthys olivaceus*) diets¹

Diets ²	AD(%)	APD(%)
DHSM ₀	84.1 ^b	92.4 ^b
DHSM ₁₀	82.4 ^b	91.5 ^b
DHSM ₂₀	80.3 ^b	90.2 ^b
DHSM ₃₀	76.7 ^c	86.3 ^c
DHSM _{20+Att}	85.3 ^a	93.7 ^a
DHSM _{30+Att}	82.7 ^b	91.8 ^b
Pooled SEM ³	0.75	0.70

¹ Means of triplicate groups; Values in the same row with different superscripts are significantly different (P<0.05).

² Refer to the table 1.

³ Pooled standard error of mean : SD/\sqrt{n} .

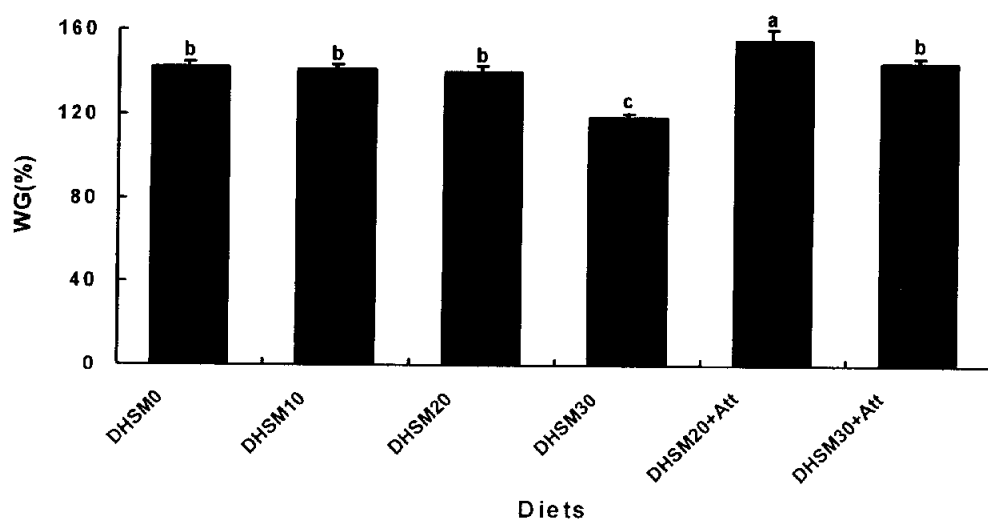


Fig.16. Average weight gain (WG, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

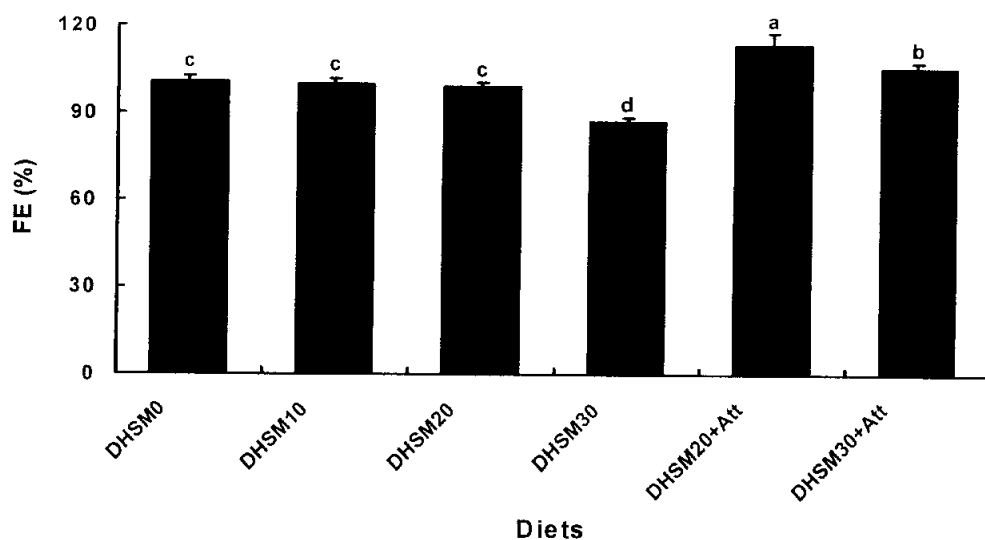


Fig.17. Average feed efficiency (WG, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

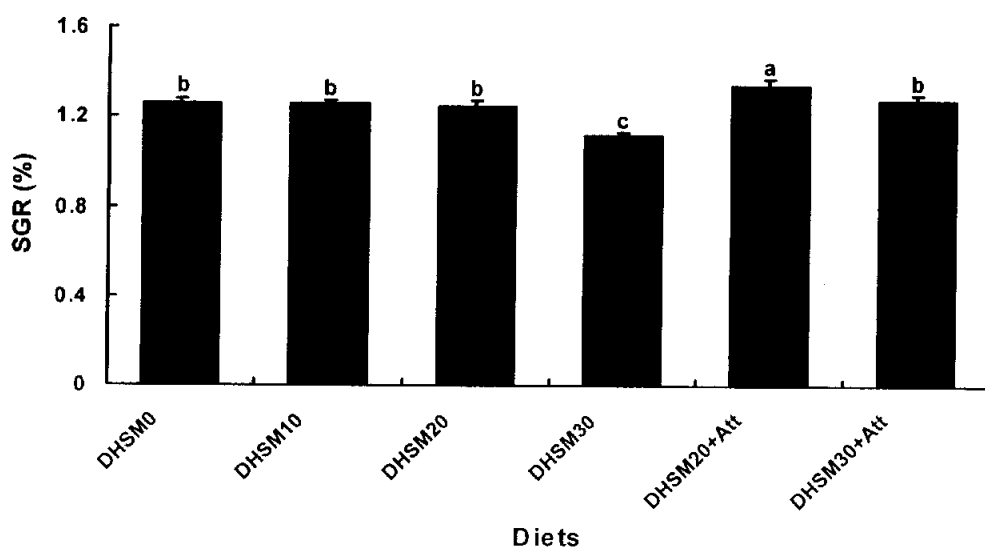


Fig.18. Average specific growth rate (SGR, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

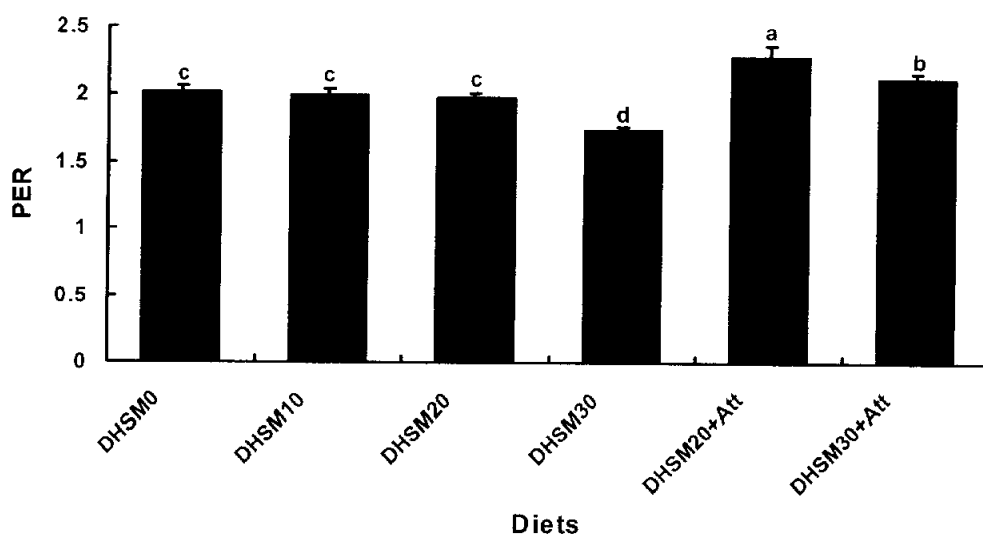


Fig.19. Average protein efficiency ratio (PER, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

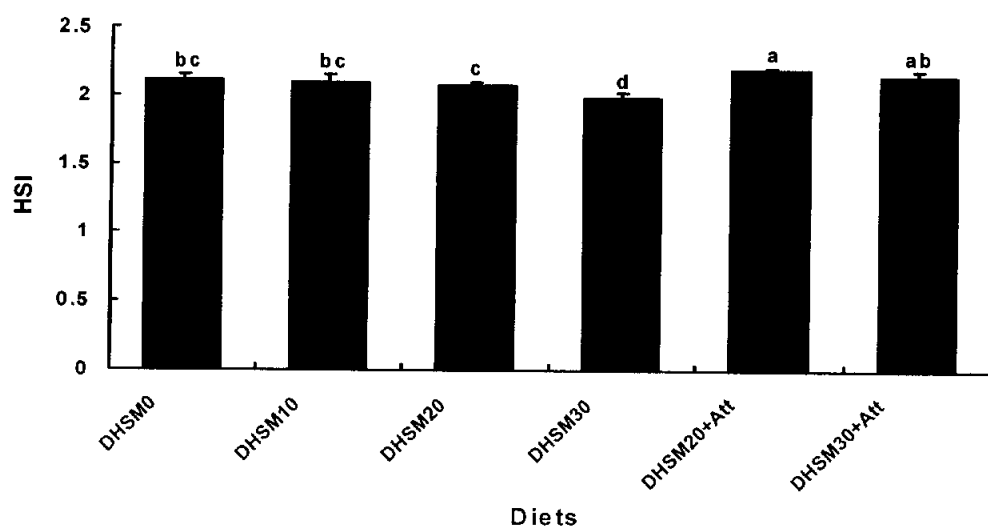


Fig.20. Average hepatosomatic index (HSI, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

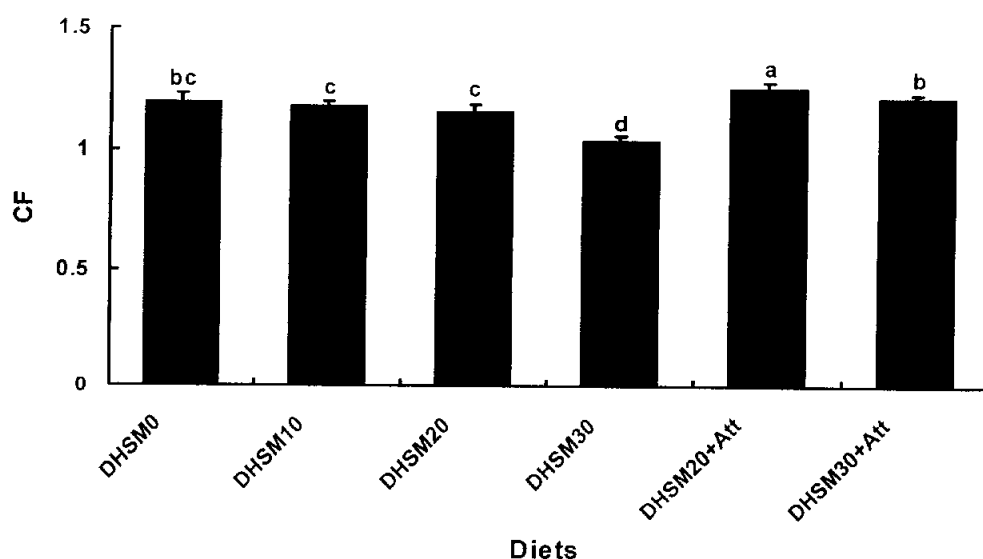


Fig.21. Average condition factor (CF) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

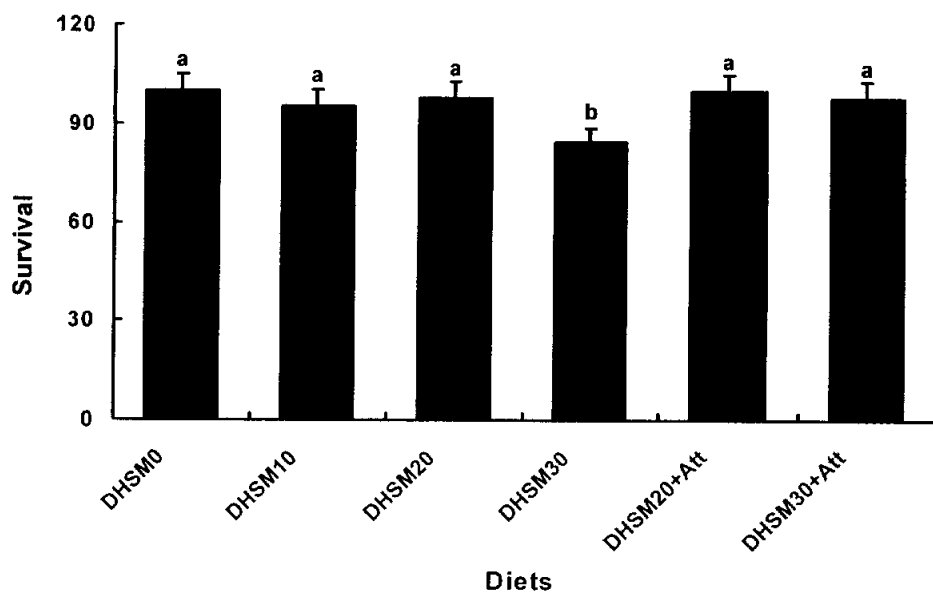


Fig.22. Average survival (Sur, %) from fish fed 6 diets for the second 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

IV. Discussions

The dietary protein was formulated to contain 48% & 50% crude protein based on the protein requirement of Korean rockfish and olive flounder (Kim, Wang & Bai 2001, 2002). When compared the analyzed proximate composition of diets and the composition of formulation, there was no significant differences. Therefore, it is impossible that the dietary protein content or the deficiency of one or more amino acids in the diets would affect the growth of fish in this experiment.

Many studies have been done in soybean meal utilization in fresh water fish such as tilapia (Wee & Shu 1989), carp (Murai, Ogata, Kosutarak & Arai 1986), catfish (Wilson and Poe 1985) and grass carp (Dabrowski & Kozak 1979) and blue catfish (Webster, Goodgame-Tiu & Tidwell 1995). Also there were some researches of soybean meal utilization in marine fish. Lee, Kang & Lee (1991) and Shimeno, Mima, Imanaga & Tomaru (1993b) reported that soybean meal could replace up to 20% of fish meal in yellowtail. McGoogan & Gatlin (1997) found that soybean meal could replace fish meal up to 90% in red drum, and could replace up to 95% fish meal when supplemented with glycine and fish soluble. Day & Gonzalez (2000) reported that soybean protein concentration could replace up to 25% fish meal in turbot. Kikuchi (1999) found that soybean meal could replace up to 47% of fish meal in flounder. Carter & Hauler (2000) also described that soybean meal could replace 33% fish meal in pacific salmon.

In the present experiment, DHSM could replace up to 20% fish meal or could replace up to 30% FM with the supplementation of deficiency amino acids in Korean rockfish and olive flounder. Many researches have been shown that supplement the methionine and lysine, whose contents were lower in DHSM, or

phosphorus when DHSM was used in the diets could improve the growth of experimental fish (Shiau *et al.* 1988). The results from the present study also proved that improved growth was observed in those groups supplemented with limiting amino acid of Met and Lys when the same levels of DHSM was replaced. However, it must be considered when such kind of soybean was used as the diet ingredients. Firstly, there were several anti-nutrition elements in full fat or dehulled soybean meal such as hemagglutinin, goitrogen and protease inhibitor that could inhibit the activity of trypsin and chymotrypsin (Dabrowski, Poczyzynski, Kock & Berger 1989). Secondly, 0.6% phosphorus in soybean meal was the form of phytin that fish cannot digest, fish can only utilize approximately 1/3 of the phosphorus that contained in soybean, therefore, phosphorus must be supplemented with the increase of soybean meal used (Lovell 1982). Thirdly, with the increase using of soybean meal in fish diets, the balance of essential amino acid should be check and amino acid must be supplemented in the diets (Viola *et al.* 1983). Many researches have been done to determine the requirement of amino acid supplemented in soybean meal containing diets (Murai, Daozun & Ogata 1989); to remove the anti-nutrition elements in full fat and dehulled soybean meal (Wee & Shu 1989); to determine the utilization of full fat, solvent extracted and refined soybean meal (Pongmaneerat & Watanabe 1993a); phosphorus utilization and discharge (Sato, Porn-Ngam, Takeuchi & Watanabe 1993); to study the utilization of different forms of phosphorus in supplemented the deficient phosphorus in soybean meal (Watanabe, Shuichi & Toshio 1988); etc.

WG and FE reduced significantly with the increasing of DHSM in the diets in the present study ($P < 0.05$). The reason might be the difference in dietary palatability. In order to improve the palatability, amino acids such as glycine,

L-amino acids and betaine, etc., and some kinds of fatty acids have been tested in sea bream *Pagrus major* (Tokoru & Shirakawa 1990) and chinook salmon (Hughes 1985); and showed effective results when attractants were added (McGoogan & Gatlin 1997). From the present study, the improved growth was observed when supplemented the limiting amino acids methionine and lysine to balance the amino acid content in experimental diets.

The hemoglobin value was 9.4 ± 0.5 g/100ml, 10.4 ± 1.2 g/100ml in juvenile and growing Korean rockfish, respectively. The result from this study was higher than the result of 3.6 - 5.3 g/100ml in flounder as described by Kikuchi, Furuta & Honda (1994). The Hb value was 4.8 ± 0.2 g/100ml, 4.4 ± 0.3 g/100ml in juvenile and growing olive flounder, respectively. This result was similar to the result reported by Kikuchi *et al* (1994). It was reported that the hemoglobin of healthy fish should be approximately 10g/100ml (Post 1983), though the normal value of hemoglobin has not been proved in fish yet. However, many researchers found that hemoglobin varied according to the deficiency of essential nutrients, fish species, environmental conditions and growth status (Garrido, Chapuli & Andres 1990).

In this study, there is no significant difference in whole-body approximate composition. Zeitler, Kirchgessner & Schwarz (1984) reported that whole-body approximate composition correlated with fish species, water temperature, weight gain, feeding and the formulated diets. Also Murai, Akiyama & Takeuchi (1985) reported that the content of crude protein and crude ash decreased with the increase of crude fat content.

Apparent protein digestibility was $86.2 \pm 3.5\%$ in juvenile Korean rockfish. The digestibility in DHSM₀, DHSM₁₅, DHSM₂₀, DHSM₃₀, DHSM₄₅, DHSM_{30+AA}, DHSM_{45+AA} and DHSM_{60+AA} was 91.3%, 89.2%, 87.4%, 86.2%,

81.3%, 88.3%, 84.2% and 81.9%, respectively. Apparent protein digestibility was $91.0 \pm 2.6\%$ in growing olive flounder. The digestibility in DHSM₀, DHSM₁₀, DHSM₂₀, DHSM₃₀, DHSM_{20+Att} and DHSM_{30+Att} diets was 92.43%, 91.5%, 90.2%, 86.3%, 93.7% and 91.8%, respectively.

The digestibility value in each replacement level of diets in juvenile Korean rockfish was lower than those value reported by Lee & Jun (1996). The reason might be that we used satiation feeding in this study or the lower culture temperature resulted in the lower digestibility. The digestibility value in each diet except the amino acid supplemented diets decreased with the increasing levels of dehulled soybean meal replacement. These results were accordance with the digestibility of fish meal 87.9% and soybean meal 79% in Korean rockfish as described by Bai, Choi, Kim & Wang (2001). However, the digestibility of methionine and L-lysine·HCL supplemented diets was higher than those diets without amino acid supplementation in this study. We considered that methionine and lysine improved protein digestibility. Also based on the dietary digestibility we could concluded that DHSM could replace up to 20% of fish meal, and could replace up to 30% fish meal when supplemented with amino acids in maximum growth of Korean rockfish and olive flounder. The utilization of soybean meal varied greatly with the processing method of soybean meal. The extrusion of soybean meal could improve digestible energy and carbohydrate (Watanabe & Pongmaneerat 1993). However, an increase in digestible energy from carbohydrate does not necessarily result in an increase in growth performance (Pongmaneerat & Watanabe 1993b). It was depended on the utilization ability of different fish species (Grisdale-Helland & Helland 1997). Plant meals also contain various anti-nutritional factors of which trypsin inhibitors are of particular concern. However, extrusion reduces the efficacy of

the majority of these (Watanabe *et al.* 1993).

In conclusion, our results indicated that DHSM could replace FM up to 20% without amino acids supplementation and 30% with amino acids (Met, Lys) and/or attractant supplementation for the maximum growth of Korean rockfish and olive flounder.

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