Thesis for the Degree of Doctor of Philosophy

Evaluation of the optimum dietary protein level and the optimum protein to energy ratio in sterlet sturgeon, *Acipenser ruthenus*

by

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Pukyong National University

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스텔렛 철갑상어의 사료내 적정 단백질 수준 및
에너지에 대한 적정 단백질 비 평가

Advisor : Sungchul C. Bai

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Sung Ryul Lim

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Contents

스텔렛 철갑상어의 사료내 적정 단백질 수준 및 에너지에 대한 단백질 비 평가

치어기 스텔렛 철갑상어에 있어서 사료내 적정 단백질 요구수준과 적정 단백질 대 에너지 비를 규명하기 위하여 세 가지 실험이 수행되었다. 치어기 및 성장기 스텔렛 철갑상어의 사료내 적정 단백질 요구량은 증체량을 지표로 broken-line 및 second order polynomial 분석결과 각각 44.3%보다는 높아야 하지만 45.6% 수준이면 충분하며, 42.7%보다는 높아야 하지만 44.6% 수준이면 충분한 것으로 나타났다. 치어기 스텔렛 철갑상어의 사료내 적정 단백질 대 에너지 비는 사료내 단백질 함량 45%에서 21.9mg protein/ kJ energy로 나타났다.

1. 치어기 (7~40g)및 성장기 (38~90g) 스텔렛 철갑상어의 사료내 적정 단백질 수준

(1) 치어기 스텔렛 철갑상어(7~40g)의 적정 단백질 수준

본 실험은 스텔렛 철갑상어 치어에 있어서 사료내 적정 단백질 요구량을 평가하기 위해 실시하였다. 실험사료는 주단백질원으로 북양어분 (white fish meal, WFM), 카제인 (Casein), 콘글루텐밀 (corn gluten meal, CGM), 대두박 (soybean meal, SBM)을 사용하였으며, 조단백질 함량은 사료내 각각 30, 40, 45, 50, 55 및 60%, 총에너지는 20.7kJ/g으로 동일하게 맞추어 설계하였다. 2주간의 예비사육을 거친후 주 사육실험은 8주간 실시하였으며 실험어는 평균무게 7.02±0.02g (mean±SD)으로 100ℓ PVC 수조

에 각 실험구 당 각각 30마리씩 3반복으로 무작위 배치하였고, 일일 사료 공급량은 어체중의 3~5%로 1일 3회 공급하였다.

증체율에 있어서 단백질함량 45, 50%구는 30, 40, 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 하지만 45, 50, 55%구간에는 유의차가 나타나지 않았다 (*P*<0.05). 사료효율에 있어서 40, 45, 50, 55%구는 30, 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 일간성장률에 있어서 50%구는 30, 40, 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 하지만 45, 50, 55%구간에는 유의차가 나타나지 않았다 (*P*<0.05). 단백질효율은 사료내 단백질 함량이 증가함에 따라 감소하는 경향을 보였다. 단백질 효율에 있어서 30%구는 40, 45, 50, 55 및 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 단백질축적효율에 있어서 30%구는 40, 45, 50, 55 및 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 간중량 지수에 있어서는 단백질함량 45%구가 다른 모든 구보다 유의하게 높은 값을 보였고 40%와 50%구 사이에서는 유의차가 나타나지 않았다 (*P*<0.05). 비만도와 생존율에 있어서는 전 구간에 유의차가 나타나지 않았다 (*P*<0.05).

전어체 수분함량에 있어서 단백질함량 50%구는 30, 55 및 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 하지만, 40, 45, 40%구간에는 유의차가 나타나지 않았다 (*P*<0.05). 전어체 단백질함량에 있어서 50%구는 30, 40, 55 및 60%구보다 유의하게 높게 나타났다 (*P*>0.05). 하지만, 45, 50%구간에는 유의차가 나타나지 않았다 (*P*<0.05). 전어체 지질함량에 있어서 단백질함량 40, 50%구는 30, 45, 55 및 60%구보다 유의하게 높게 나타났다 (P>0.05). 하지만, 40, 50%구간에는 유의차가 나타나지 않았다 (*P*<0.05). 전어체 회분함량에 있어서는 모든 구간에 유의차가 나타나지 않았다 (*P*<0.05).

전어체 구성아미노산 분석결과 각 아미노산 간에 예외는 있었지만 증

체량과 비례하여 총 구성아미노산 축적이 높게 나타났다. 필수아미노산과 비필수아미노산의 수치 또한 총 구성아미노산과 같은 경향을 보였다. 필수 아미노산 및 비필수 아미노산 함량에 있어서 단백질 함량 50%구는 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05).

전어체 유리아미노산 조성에 있어서 L-Glutamic acid, L-Alanine, L-Isoleucine, L-Leucine, L-Tyrosine, L-Phenylalanine, β-Alanine, L-Lysine, 1-Methyl-L-Histidine 함량은 단백질 함량 40%구가 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05). Taurine 함량은 단백질 함량 55%구가 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05). L-Glycine 함량은 단백질 함량 30 및 55%구가 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05). L-Ornithine 함량은 단백질 함량 30%구가 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05). 총유리아미노산 함량에 있어서 40%구는 다른 모든 구보다 유의하게 높게 나타났다 (P>0.05).

헤모글로빈에 있어서 단백질 함량 30, 40, 45, 50 및 55%구는 60%구보다 유의하게 높게 나타났다 (P>0.05). 헤마토크리트는 유의차가 나타나지 않았다. Glucose 함량은 사료내 단백질함량의 증가에 따라 에너지원인 dextrin의 함량 감소에 비례하여 감소하는 경향을 보였다. 30, 40, 45 및 50%구는 60%구보다 유의하게 높게 나타났다 (P>0.05). 하지만, 30, 40, 45 , 50 및 55%구간에는 유의차가 나타나지 않았다. GOT와 GPT에 있어서 단백질함량 30%구는 50, 55 및 60%구보다 유의하게 높게 나타났다 (P>0.05). 하지만 30, 40 및 45%구간에는 유의차가 나타나지 않았다. 총단백질 함량에 있어서 30 및 40%구는 60%구보다 유의하게 높게 나타났다 (P>0.05). 하지만, 30, 40, 45, 50 및 55%구간에는 유의차가 나타나지 않았다. 알부민 함량에 있어서는 40%구가 60%구에 비해 유의하게 높게 나타났다 (P>0.05). 하지만, 30, 40, 45, 50 및 55%구간에는 유의차가 나타나지

않았다. 총콜레스테롤함량에 있어서는 모든 구간에 유의차가 나타나지 않았다.

본 연구결과 증체량을 지표로 broken line 및 second order polynomial 분석결과 치어기 스텔렛 철갑상어 (약 7.0~43.0g)의 사료내 적정 단백질 요구량은 주 단백질원으로 북양어분과 카제인을 사용했을 때 44.3%보다는 높아야 하지만 45.6% 수준이면 충분한 것으로 나타났다.

(2) 성장기 스텔렛 철갑상어 (38-90g)의 적정 단백질 수준

본 실험은 치어기 스텔렛 철갑상어에 있어서 사료내 적정 단백질 요구량을 평가하기 위해 실시하였다. 실험사료는 주단백질원으로 카제인 (casein) 및 젤라틴 (gelatin)을 사용하였으며, 탄수화물원으로 전분 (starch), 덱스트린 (dextrin) 및 밀가루 (wheat meal)를 사용하였고 지질원으로 어유 (fish oil) 및 대두유 (soybean oil)를 사용하였으며 첨가제로 철갑상어 근육분 (sturgeon muscle meal, SMM)을 사용하였다. 조단백질 함량은 사료내 각각 30, 35, 40, 45, 50 및 55%, 총에너지는 약 19.0kJ/g으로 동일하게 맞추어 설계하였다. 2주간의 예비사육을 거친 후 주 사육실험은 8주간 실시하였으며 실험어는 평균무게 37.7±0.1g (mean±SD)으로 100ℓ PVC 수조에 각 실험구 당 각각 15마리씩 3반복으로 무작위 배치하였고, 일일 사료공급량은 어체중의 4~6%로 1일 3회 공급하였다.

증체율에 있어서 단백질함량 45%구는 30, 35, 50 및 55%구보다 유의하게 높게 나타났다 ($P>0.05$). 하지만 40 및 45%구간에는 유의차가 나타나지 않았다 ($P<0.05$). 사료효율에 있어서 40 및 45%구는 30%구보다 유의하게 높게 나타났다 ($P>0.05$). 하지만 35, 40, 45, 50 및 55% 구간에는 유의차가 나타나지 않았다 ($P<0.05$). 일간성장률에 있어서 45%구는 30,

35, 50 및 55%구보다 유의하게 높게 나타났다 (P>0.05). 하지만 40 및 45%구간에는 유의차가 나타나지 않았다 (P<0.05). 단백질 효율에 있어서 30%구는 35, 40, 45, 50 및 55%보다 유의하게 높게 나타났다 (P>0.05). 하지만 50 및 55%구간에는 유의차가 나타나지 않았다 (P<0.05). 단백질축적효율에 있어서 30 및 35%구는 40, 45, 50 및 55%구보다 유의하게 높게 나타났다 (P>0.05). 하지만 30 및 35%구간에는 유의차가 나타나지 않았다 (P<0.05).

본 연구결과 증체량을 지표로 broken line 및 second order polynomial 분석결과 치어기 스텔렛 철갑상어 (약 37.7~93.6g)의 적정 단백질 요구량은 주 단백질원으로 카제인과 젤라틴을 사용했을 때 42.7%보다는 높아야 하지만 44.6% 수준이면 충분한 것으로 나타났다.

2. 치어기 스텔렛 철갑상어 (5.5~33.5g)의 적정 단백질 대 에너지 비

본 실험은 스텔렛 철갑상어 치어에 있어서 사료내 적정 단백질 대 에너지 비를 규명하기 위해 실시하였다. 실험사료는 주단백질원으로 북양어분 (white fish meal, WFM), 카제인 (casein), 대두박 (soybean meal, SBM)을 사용하였으며, 조단백질 함량은 사료내 각각 40 및 45%, 조지질 함량은 각각 10, 15 및 20%로 총 6가지의 사료를 제작하였고 총에너지는 각각 19.4, 18.5, 17.6, 21.9, 20.8 및 19.8kJ/g으로 설계하였다. 2주간의 예비사육을 거친후 주 사육실험은 8주간 실시하였으며 실험어는 평균무게 5.48±0.04g (mean±SD)으로 100ℓ PVC 수조에 각 실험구 당 각각 30마리씩 3반복으로 무작위 배치하였고, 일일 사료공급량은 어체중의 4~6%로 1일 3회 공급하였다.

증체율에 있어서 단백질/지질 45/10구는 40/10구보다 유의하게 높게

나타났다 ($P>0.05$). 하지만 45/10, 45/15, 45/20, 40/15 및 40/20구간에는 유의차가 나타나지 않았다 ($P<0.05$). 또한 40/10, 40/15 40/20, 45/15 및 45/20구간에도 유의차가 나타나지 않았다 ($P<0.05$). 사료효율에 있어서는 모든 구간에 유의차가 나타나지 않았다 ($P<0.05$). 일간성장률에 있어서 단백질/지질 45/10구는 40/10구보다 유의하게 높게 나타났다 ($P>0.05$). 하지만 45/10, 45/15, 45/20, 40/15 및 40/20구간에는 유의차가 나타나지 않았다 ($P<0.05$). 또한 40/10, 40/15 40/20, 45/15 및 45/20구간에도 유의차가 나타나지 않았다 ($P<0.05$). 단백질효율에 있어서 40/10 및 40/15구는 45/10, 45/15 및 45/20구보다 유의하게 높게 나타났다 ($P>0.05$). 하지만 40/10, 40/15 및 40/20구간에는 유의차가 나타나지 않았고 (P<0.05), 45/10, 45/15 및 45/20구간에도 유의차가 나타나지 않았다 ($P<0.05$). 생존율에 있어서는 전 구간에 유의차가 나타나지 않았다 ($P<0.05$).

본 연구결과 치어기 스텔렛 철갑상어의 사료내 적정 단백질 대 에너지비는 사료내 단백질 함량 45%에서 21.9mg protein/kJ energy로 나타났다.

Evaluation of the optimum dietary protein level and the optimum protein to energy ratio in sterlet sturgeon, *Acipenser ruthenus*

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Abstract

Three experiments were conducted to evaluate the optimum dietary protein level and the optimum protein to energy ratio in sterlet sturgeon, *Acipenser ruthenus* fed the experimental diets. In the first experiment, the results indicated that the optimum dietary protein requirement level could be greater than 44.3%, but less than 45.6% in fingerling sterlet sturgeon (7 to 40g) under our experimental conditions. In the second experiment, the results indicated that the optimum dietary protein requirement level could be greater than 42.7%, but less than 44.6% in

juvenile sterlet sturgeon (38 to 90g) under our experimental conditions. In the third experiment, the results indicated that the optimum dietary protein to energy ratio could be 21.9mg protein/kJ diet at 45% dietary protein levels in fingerling sterlet sturgeon (5.5～33.5g)

First Experiment : The optimum dietary protein level in fingerling sterlet sturgeon (7～40g), *Acipenser ruthenus*

This study was carried out to determine optimum protein requirement in fingerling sterlet sturgeon, *Acipenser ruthenus*. Fingerling sterlet sturgeon averaging 7.02 ± 0.02g (mean ± SD) were fed one of six experimental diets containing 30, 40, 45, 50, 55 and 60% crude protein (CP) with isocaloric gross energy of 20.7 kJ/g for 8 weeks. After the feeding trial, average weight gain (WG) of fish fed 45 and 50% CP diets were significantly higher than those of fish fed 30, 40 and 60% CP diets ($P<0.05$), however there were no significant differences in WG among fish fed 45, 50 and 55% CP diets. Average feed efficiency (FE) of fish fed 40, 45, 50 and 55% CP diets were significantly higher than those of fish fed 30 and 60% CP diets ($P<0.05$), meanwhile there was no significant differences in FE between fish fed 30 and 60% CP diets. Average specific growth rate (SGR) of fish fed 50% CP diet was significantly higher than those of fish fed 30, 40 and 60% CP diets ($P<0.05$), but no significant differences were observed in SGR among fish fed 45, 50 and 55% CP diets. Average protein efficiency ratio and protein retention were reduced with the increasing dietary protein levels. Average hepatosomatic index (HSI) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 40, 50, 55 and 60% CP diets (P<0.05), meanwhile no significant differences in HSI was observed between fish fed 40 and 50% CP diets. There were no significant differences in condition factor and in survival rate among fish fed the six experimental diets.

Based on the one-way ANOVA test, the broken-line regression analysis and the second order polynomial analysis model on weight gain, the optimum dietary protein requirement level could be greater than 44.3%, but less than 45.6% in fingerling sterlet sturgeon under our experimental conditions.

Second Experiment : The optimum dietary protein level in juvenile sterlet sturgeon (38～90g), *Acipenser ruthenus*

This study was carried out to determine optimum protein requirement in juvenile sterlet sturgeon, *Acipenser ruthenus*. Juvenile sterlet sturgeon averaging 37.7 ± 0.1g (mean ± SD) were fed one of six experimental diets containing 30, 35, 40, 45, 50 and 55% crude protein (CP) with isocaloric gross energy of 19.0 kJ/g for 10 weeks.

Casein and gelatin are major protein source in the experimental diet. After 10 weeks of feeding trial, average weight gain (WG) of fish fed 45% CP diets were significantly higher than those of fish fed 30, 35, 50 and 55% CP diets ($P<0.05$), however there were no significant differences in WG among fish fed 40 and 45% CP diets. Average feed efficiency (FE) of fish fed 40 and 45% CP diets were significantly higher than those of fish fed 30% CP diets ($P<0.05$), meanwhile there was no significant differences in FE among fish fed 35, 40, 45, 50 and 55% CP diets. Average specific growth rate (SGR) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 35, 50 and 55% CP diets ($P<0.05$), but no significant differences were observed in SGR between fish fed 40 and 45% CP diets. Average protein retention efficiency (PRE) of fish fed 30 and 35% CP diets were significantly higher than those of fish fed 40, 45, 50 and 55% CP diets ($P<0.05$), however there were no significant differences among fish fed 30 and 35% CP diets.

Based on the one-way ANOVA test, the broken-line regression analysis, the second order polynomial analysis on weight gain, the optimum dietary protein requirement level could be greater than 42.7%, but less than 44.6% in juvenile sterlet sturgeon under our experimental conditions.

Third Experiment: The optimum dietary protein to energy ratio in fingerling sterlet sturgeon (5.5～33.5g), *Acipenser ruthenus*

This experiment was conducted to evaluate optimum dietary protein to energy (PE) ratio in fingerling sterlet sturgeon, *Acipenser ruthenus* for 8 weeks. White fish meal, casein & soybean meal were used as protein sources. Prior to the feeding trial, fish were fed commercial diet for 2 weeks to adjust to the experimental diets and conditions. Fish averaging 5.48 ± 0.04g (Mean ± SD) were distributed to each aquarium as a group of 30 fish reared in the flow through system. Fish of triplicate groups were fed one of six diets containing 40 and 45% crude protein and 10, 15 and 20% crude lipid, the PE ratio was 19.4, 18.5, 17.6, 21.9, 20.8 and 19.8kJ gross energy/g diet, respectively.

Average weight gain (WG) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in WG among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets ($P>0.05$). Average feed efficiency (FE) of fish fed all of diet was no significant difference ($P>0.05$). Average specific growth rate (SGR) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in SGR among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets (*P*>0.05). Average protein efficiency ratio (PER) of fish fed 40/10 and 40/15 diets were significantly higher than those of fish fed 45/10, 45/15 and 45/20 diets (*P*<0.05). However, there was no significant difference in PER among fish fed 40/10, 40/15 and 40/20 diets (*P*>0.05).

Therefore, these results indicated that the optimum dietary protein to energy ratio in fingerling sterlet sturgeon could be 21.9mg protein/kJ diet at 45% dietary protein levels.

Chapter I. General Introduction

Sturgeon are one of the oldest living teleost, this originate early jurassic. In Family Acipenseridae, total 27 species exist including Beluga, Kaluga, Siberian, Russian and Sterlet sturgeon etc. They distributed in Mediterranean basin such as the black, Azop, Caspian seas and some of species inhabit Brackish water and others are freshwater species. In natural, most of them are carnivorous fish, but others are omnivorous bottom feeder fish which eat shells, crustacean and small fish. The world catch production of sturgeon was about 19,600 MT in 1950 and increased up to 32,078 MT in 1977. However, the catch production of 2006 was dramatically decreased 940 MT because of overfishing, illegal fishing, waste releasing, water pollution etc (Fig. 1). In the contrary, the aquaculture production of sturgeon increased up to 21,319 metric tons in the last 25 years from 150 metric tons in 1984 (Fig. 2).

Sterlet sturgeon, *Acipenser ruthenus* is a kind of small freshwater species which is distributed in Black sea. Generally, it is usually used as the broodstock source of hybrid because of its desirable characteristics for culture including the high tolerance to water temperature change, faster growth than other species.

Protein is not only vital factor which is composed of 60~75% body tissue composition of organism, but also nutrient which is used as energy source if carbohydrate and lipid are insufficient. Especially, fish consume protein to gain amino acid. Protein which is digested or hydrolyzed is released to free amino acid, it is absorbed to intestine tract and distributed to tissue and organ throughout blood vessel. When the dietary protein level is insufficient, retarded growth is observed while high dietary protein level is result in using of energy source. (Wilson, 2002). Therefore, determination of optimum dietary protein requirement is not only important in order to gain the maximum and optimum growth in terms of fish feed nutrition, but also to reduce loss of expensive protein ingredients. For this reason, many studies have been conducted to determine optimum dietary protein requirement of many species, Asian seabass (Boonyaratpalin, 1997), Atlantic salmon (Grisdale-Helland and Helland, 1997), Channel catfish (Garling and Wilson, 1976), Tilapia (Jauncey, 1982). Japanese eel (Nose and Arai, 1973), river puffer (Bai et al., 1999), olive flounder (Kim et al., 2002), Korean rockfish (Kim et al., 2004) etc.

Energy is not a nutrient. It is released during metabolic oxidation of carbohydrates, fats and amino acids. And it need to keep the normal life and metabolism. Therefore, energy level should be the first nutritional consideration in diet formulation. Also, dietary protein to energy ratio is should be kept in balance to meet the maintenance and normal growth of fish. In relation to this, many researches have been conducted to determine the optimum dietary protein to energy ratio of fish such as black catfish (Salhi et al., 2004), olive flounder (Kim et al., 2002), Korean rockfish (Kim et al., 2004), Japanese eel (Okorie et al., 2007) etc.

However, there are only a few studies for nutritional information of sterlet sturgeon. Therefore, the purpose of these experiments are to determine the optimum dietary protein level in fingerling and juvenile sterlet sturgeon, *Acipenser ruthenus*, and to evaluate the optimum dietary protein to energy ratio in fingerling sterlet sturgeon.

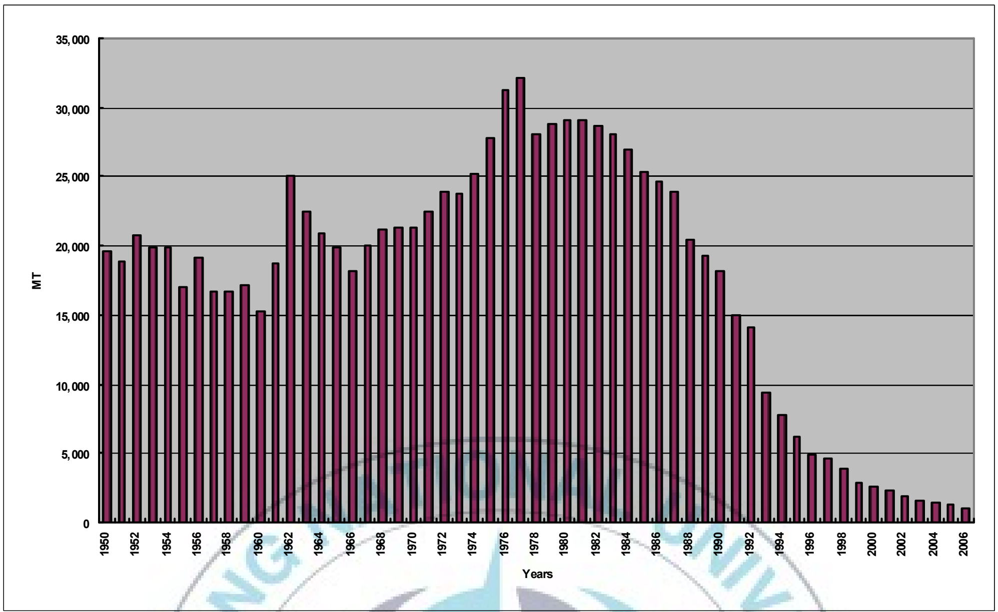

Fig.1. The world sturgeon catch 1950-2006 (FAO statistics)

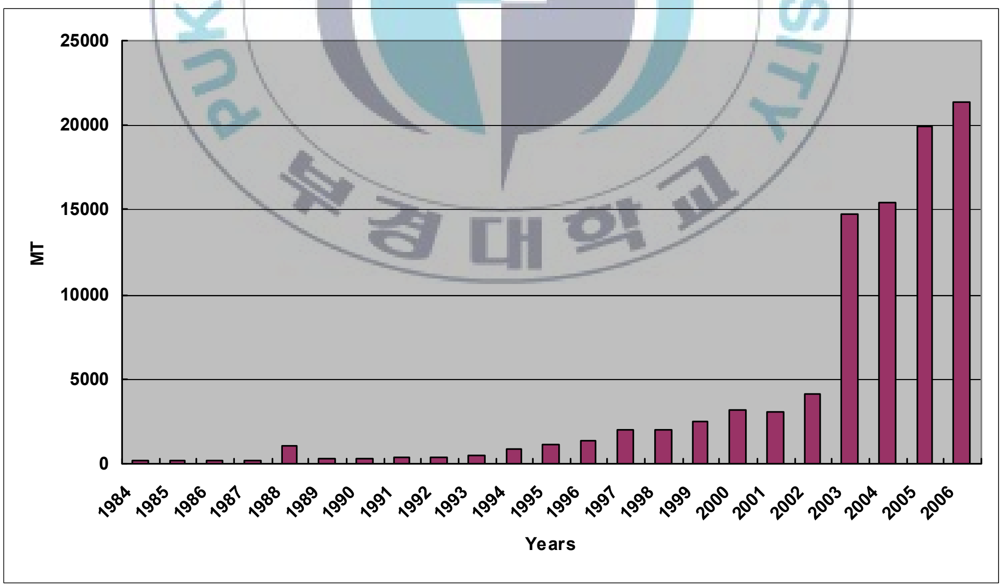

Fig.2. The world sturgeon aquaculture production 1984-2006 (FAO statistics)

Chapter II. The optimum dietary protein level in fingerling and juvenile sterlet stergeon, *Acipenser ruthenus*

Abstract

This study was carried out to determine optimum protein requirement in fingerling and juvenile sterlet sturgeon, *Acipenser ruthenus*. First experiment, fingerling sterlet sturgeon averaging 7.02 ± 0.02g (mean ± SD) were fed one of six experimental diets containing 30, 40, 45, 50, 55 and 60% crude protein (CP) with isocaloric gross energy of 20.7 kJ/g for 8 weeks. After the feeding trial, average weight gain (WG) of fish fed 45 and 50% CP diets were significantly higher than those of fish fed 30, 40 and 60% CP diets ($P<0.05$), however there were no significant differences in WG among fish fed 45, 50 and 55% CP diets. Average feed efficiency (FE) of fish fed 40, 45, 50 and 55% CP diets were significantly higher than those of fish fed 30 and 60% CP diets ($P<0.05$), meanwhile there was no significant differences in FE between fish fed 30 and 60% CP diets. Average specific growth rate (SGR) of fish fed 50% CP diet was significantly higher than those of fish fed 30, 40 and 60% CP diets ($P<0.05$), but no significant

differences were observed in SGR among fish fed 45, 50 and 55% CP diets. Average protein efficiency ratio and protein retention efficiency were reduced with the increasing of dietary protein levels. Average hepatosomatic index (HSI) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 40, 50, 55 and 60% CP diets (P<0.05), meanwhile no significant differences in HSI was observed between fish fed 40 and 50% CP diets. There were no significant differences in condition factor and in survival rate among fish fed the six experimental diets.

Based on the one-way ANOVA test, the broken-line regression analysis and the second order polynomial analysis on weight gain, the optimum dietary protein requirement level could be greater than 44.3%, but less than 45.6% in fingerling sterlet sturgeon under our experimental conditions.

Second experiment, juvenile sterlet sturgeon averaging 37.7 ± 0.1g (mean ± SD) were fed one of six experimental diets containing 30, 35, 40, 45, 50 and 55% crude protein (CP) with isocaloric gross energy of 19.0 kJ/g for 10 weeks. Casein and gelatin are major protein source in the experimental diet. After 10 weeks of feeding trial, average weight gain (WG) of fish fed 45% CP diets were significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (P<0.05), however there were no significant differences in WG among fish fed 40 and 45% CP diets. Average feed efficiency (FE) of fish fed 40 and 45% CP diets were significantly higher than those of fish fed 30% CP diets (*P*<0.05), meanwhile there was no significant differences in FE among fish fed 35, 40, 45, 50 and 55% CP diets. Average specific growth rate (SGR) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (*P*<0.05), but no significant differences were observed in SGR between fish fed 40 and 45% CP diets. Average protein retention efficiency (PRE) of fish fed 30 and 35% CP diets were significantly higher than those of fish fed 40, 45, 50 and 55% CP diets (*P*<0.05), however there were no significant differences between fish fed 30 and 35% CP diets.

Based on the one-way ANOVA test, the broken-line regression analysis, the second order polynomial analysis on weight gain, the optimum dietary protein requirement level could be greater than 42.7%, but less than 44.6% in juvenile sterlet sturgeon under our experimental conditions.

1. Introduction

Protein synthesis is to provide essential amino acid and nitrogen for synthesis non essential amino acids. Protein in fish play the role in following factors. It is energy source for maintenance and growth, the hormones to keep homeostasis of organism. And protein is used as enzyme and catalyst in life cycle and transportation of oxygen in blood. Also, it is indispensible to immune system.

Protein is the most expensive ingredient in aquaculture feed and the factor which control the success of aquaculture industry. Because of overfishing, illegal fishing, pollution of the habitat, the quantity of protein source such as fish meal is decreasing recently. Accordingly, plant protein source such as soybean meal, corn gluten meal, rapeseed meal and animal by product such as poultry by product are used in fish feed.

The protein requirements, meaning the minimum amount needed to meet requirements for amino acids and to achieve maximum growth. Usually the protein requirements mainly obtained from dose response curves in which graded amounts of high quality protein were fed in partially defined diets. The response measured was weight gain, and the values are expressed as a percentage of dry diet. The proper concentration of dietary protein can achieve the maximum growth and normal metabolism status of fish. However, too much protein in the diet resulted in wasting expensive ingredient. In addition to, this leads water pollution because of an excess quantity of nitrogen releasing into the cultured environment. Also, the unbalance of dietary protein to energy ratio result in adverse effects on growth. Therefore, too much protein in the diet is result in wasting expensive ingredient. In addition to, this leads water pollution because of an excess of quantity nitrogen releasing. Also, the unbalance of dietary protein to energy ratio result in adverse effects.

Therefore, many researchers have made a lot of efforts to determine the optimum dietary protein level in fresh, brackish and sea water fish species such as carp (Ogino and Saito., 1970), rainbow trout (Satia., 1974), channel catfish (Garling and Wilson., 1976), Japanese eel (Nose and Arai., 1972), river puffer (Bai et al., 1999), Atlantic salmon (Lall and Bishop, 1977), parrot fish (Ikeda et al., 1988), red drum (Serrano et al., 1992), olive flounder (Kim et al, 2002) Korean rockfish (Lee et al., 1993) and sturgeon (Kaushik et al., 1991 ; Moore et al., 1988).

However, there are only a few study for nutritional information in sterlet sturgeon. Therefore, the objectives of two experiments are to determine the optimum dietary protein level in fingerling and juvenile sterlet sturgeon.

2. Materials and Methods

(1) The optimum dietary protein level in fingerling sterlet sturgeon (7～40g), *Acipenser ruthenus*

Experimental design and diets

Six experimental diets were formulated to be isocaloric to contain 30, 40, 45, 50, 55 and 60% crude protein and gross energy level of 20.7kJ g^{-1}. White fish meal, casein, soybean meal and corn gluten meal were the major protein sources. Fish oil and soybean oil were used as lipid sources. Dextrin and wheat meal were used as carbohydrate sources. Cellulose was also included in the diets to match CP and energy levels. Procedures for diet preparation and storage were as previously described by Bai and Kim (1997). After thoroughly mixing the dry ingredients, fish oil and soybean oil were added with filtered tap water. The experimental diets were pelleted by using a laboratory pellet machine and stored -20℃ until used. The gross energy was 20.7kJ/g. Composition of the experimental diets is shown in Table 1. Amino acid (AA) composition of the experimental diets is shown in Table 2.

Experimental fish and feeding trial

Before the feeding trial, fish were fed commercial diet for two week to acclimate them to the experimental diet and conditions. And the feeding trials were conducted at the Gyeonggi Province Freshwater Fisheries Research Institute (Gwangtan-ri, Gyeonggi-do, Korea). At the beginning, fingerling sterlet sturgeon, *Acipenser ruthenus,* averaging 7.02 ± 0.02g (Mean ± SD) was divided into six groups and randomly distributed into each aquarium as a group of 30 fish. The feeding trial was conducted in 100ℓ aquariums with a water flow rate of 1-2ℓ min^{-1}. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5 ±0.5 ppm. The water temperature was maintained at 22 ± 3.0 ℃ (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. Feeding trial was conducted for 8-weeks. Fish were fed at a rate of 3～5% (dry matter basis) of total body weight per day. The fish were fed three time a day at 08:00, 13:00 and 17:00h. Total weight of fish in each tank was measured every 2 weeks, and the feeding rate was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing. Feeding trial was carried out at the Gyeonggi Province Freshwater Fisheries Research Institute.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with AQUES

(Handong Co. LTD. KOREA), 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 aqarium 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 form human blood (Sigma Chemical, St. Louis, Missouri) was used. The amino acids were quantified by amino acid analyzer S433 (Sykam, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4㎜ × 150㎜, Absorbance; 570㎚ 440㎚, Reagent flow rate; 0.25 1㎖/min, Buffer flow rate; 0.45㎖/min, Reactor temperature; 120, Reactor Size; 15m Fish ere stored at -20℃ until analysis. 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

All 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 $P < 0.05$. Broken-line analysis (Robbins et al. 1979) was used to determine the optimum dietary protein level in fingerling sterlet sturgeon by using SAS version 6.12 software (SAS Institute, Inc., Cary, North Carolina, USA). And the second-order polynomial regression analysis (Zeitoun et al. 1976) was introduced to determine the optimum protein requirement of fingerling stelret sturgeon using SAS version 6.12 software (SAS Institute, Inc., Cary, North Carolina, USA).

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

Ingredients	Dietary Protein levels (%)					
	30	40	45	50	55	60
White fish meal[2]	19.0	33.7	41.1	48.5	55.8	63.2
Casein-vita free[3]	10.0	10.0	10.0	10.0	10.0	10.0
Corn gluten meal[2]	6.0	6.0	6.0	6.0	6.0	6.0
Soybean meal[2]	5.0	5.0	5.0	5.0	5.0	5.0
Dextrin[3]	42.3	28.8	22.0	15.1	8.3	1.4
Wheat meal[2]	5.0	5.0	5.0	5.0	5.0	5.0
Fish oil[2]	4.5	3.5	3.0	2.4	1.9	1.4
Soybean oil[2]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[5]	1.0	1.0	1.0	1.0	1.0	1.0
Carboxymethylcellulose[3]	2.0	2.0	2.0	2.0	2.0	2.0
Proximate analysis						
Moisture	13.0	11.5	12.1	10.7	10.5	11.8
Crude protein	29.1	39.1	46.8	49.2	53.7	57.6
Crude lipid	9.3	10.0	10.6	9.2	9.6	9.5
Crude ash	6.4	9.5	12.1	13.1	15.1	16.2
Gross energy(kJ/g diet)	20.6	20.7	21.0	21.1	21.0	20.8

[1] The samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

[2,] Suhyup Feed Co. Korea.

[3] United States Biochemical, Cleveland, Ohio 44122.

[4] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ; Choline bitatrate, 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; B_{12}, 0.06.

[5] Contains (as mg/kg in diets) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$. 1379.8; $NaH_2P_4 \cdot 2H_2O$, 877.8; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

Table 2. Amino acid composition of the six experimental diets (% of as is basis)[1]

	Protein levels(%)					
	30	40	45	50	55	60
Asp	1.91	2.77	3.25	3.52	3.66	3.93
Thr	0.96	1.44	1.67	1.75	1.85	2.01
Ser	1.07	1.46	1.74	1.84	1.89	2.04
Glu	4.66	6.26	7.09	7.20	7.70	8.34
Pro	1.15	1.43	1.49	1.53	1.61	1.98
Gly	0.84	1.22	1.58	1.65	1.98	2.28
Ala	1.13	1.66	1.96	2.12	2.39	2.57
Val	1.12	1.93	2.11	2.13	2.15	2.35
Ile	1.42	1.67	2.09	2.19	2.22	2.38
Leu	2.45	3.13	3.69	3.79	3.93	4.36
Tyr	0.98	1.49	1.59	1.64	1.72	2.01
Phe	1.40	1.69	1.85	1.91	2.01	2.25
His	0.72	0.88	0.96	1.05	1.35	1.37
Lys	1.42	2.22	2.67	2.84	3.41	3.33
Arg	1.20	1.37	2.04	2.11	2.79	2.40
Cys	0.12	0.10	0.42	0.46	0.47	0.54
Met	0.44	0.59	0.72	0.70	0.73	0.85
Total	23.0	31.3	36.9	38.4	41.9	45.0

[1] Amino acid samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

(2) The optimum dietary protein level in juvenile sterlet sturgeon (38～90g), *Acipenser ruthenus*

Experimental design and diets

Six experimental diets were formulated to be isocaloric to contain 30, 35, 40, 45, 50 and 55% crude protein and gross energy level of 19.0kJ/g. Casein and gelatin were the major protein sources. Fish oil and soybean oil were used as lipid sources. Wheat meal, dextrin and starch were used as carbohydrate sources. α-cellulose was also included in the diets to match CP and energy levels. And sturgeon muscle meal was added into the diet as feed additive. Procedures for diet preparation and storage were as previously described by Bai and Kim(1997). After thoroughly mixing the dry ingredients, fish oil and soybean oil were added with filtered tap water. The experimental diets were pelleted by using a laboratory pellet machine and stored -20℃ until used. Gross energy of experimental diets were adjusted to have 19.0kJ/g. Composition of the experimental diets is shown in Table 3. Amino acid (AA) composition of the experimental diets is shown in Table 4.

Experimental fish and feeding trial

Before the feeding trial, fish were fed commercial diet for one week to acclimate them to the experimental diet and conditions. And the feeding trials were conducted at the Gyeonggi Province Freshwater Fisheries Research Institute (Gwangtan-ri, Gyeonggi-do, Korea). At the beginning, juvenile sterlet sturgeon, *Acipenser ruthenus* averaging 37.7 ± 0.1g (Mean ± SD) was divided into six groups and randomly distributed into each aquarium as a group of 15 fish. The feeding trial was conducted in 100ℓ aquariums with a water flow rate of 1-2ℓ min^{-1}. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.8 ±0.7 ppm. The water temperature was maintained at 20 ± 3.0 ℃ (mean ± SD). and the pH was maintained at 7.4 ± 0.3 (mean ± SD) and photoperiod of 12h light : 12h dark (06:00 to 18:00) was used throughout the experimental periods. Feeding trial was conducted for 10-weeks. Fish were fed at a rate of 4～6% (dry matter basis) of total body weight per day. The fish were fed three times a day at 08:00, 13:00 and 17:00h. Total weight of fish in each tank was measured every 2 weeks, and the feeding rate was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with AQUES(Handong Co. LTD. KOREA), 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 form human blood (Sigma Chemical, St. Louis, Missouri) was used. The amino acids were quantified by amino acid analyzer S433 (Syknm, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4㎜ × 150㎜, Absorbance; 570㎚ 440㎚, Reagent flow rate; 0.25 1㎖/min, Buffer flow rate; 0.45㎖/min, Reactor temperature; 120, Reactor Size; 15m Fish ere stored at -20℃ until analysis. 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

All 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 $P < 0.05$. Broken-line analysis (Robbins et al. 1979) was used to determine the optimum dietary protein level in juvenile sterlet sturgeon by using

SAS version 6.12 software (SAS Institute, Inc., Cary, North Carolina, USA). And the second-order polynomial regression analysis (Zeitoun et al. 1976) was introduced to the optimum protein requirement of juvenile stelret sturgeon using SAS version 6.12 software (SAS Institute, Inc., Cary, North Carolina, USA).

Table 3. Composition and proximate analysis of the six experimental diets (% of dry matter basis)[1]

Ingredients	Dietary Protein levels (%)					
	30	35	40	45	50	55
Casein-vita free[3]	21.6	25.6	29.5	33.5	37.6	41.5
Gelatin[3]	6.0	7.1	8.2	9.3	10.4	11.5
Wheat meal[2]	12.8	12.8	12.8	12.8	12.8	12.8
Starch[2]	24.6	19.4	14.3	9.3	4.2	0
Dextrin[3]	19.0	19.0	19.0	19.0	19.0	18.1
Fish oil[2]	6.0	6.0	6.0	6.0	6.0	6.0
Soybean oil[2]	6.0	6.0	5.9	5.9	5.8	5.8
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[5]	1.0	1.0	1.0	1.0	1.0	1.0
Sturgeon muscle meal[6]	1.5	1.5	1.5	1.5	1.5	1.5
α-cellulose[3]	0.6	0.6	0.8	0.7	0.8	0.8
Proximate analysis						
Moisture	12.8	12.3	12.0	11.7	11.6	11.3
Crude protein	29.4	36.3	40.9	44.8	51.2	55.9
Crude lipid	11.3	11.9	11.0	10.3	10.1	10.1
Crude ash	1.7	1.8	1.8	1.8	1.9	2.0
Gross energy(kJ/g diet)	19.1	19.2	19.0	19.0	19.1	19.0

[1] The samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

[2,] Suhyup Feed Co. Korea.

[3] United States Biochemical, Cleveland, Ohio 44122.

[4] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ; Choline bitatrate, 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; B_{12}, 0.06.

[5] Contains (as mg/kg in diets) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$. 1379.8; $NaH_2P_4 \cdot 2H_2O$, 877.8; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

[6] Gyeonggi Province Freshwater Fisheries Research Institute, Gwangtan-ri, Gyeonggi-do, Korea.

Table 4. Amino acid composition of the six experimental diets (% of as is basis)[1]

	Protein levels(%)					
	30	35	40	45	50	55
Asp	2.03	2.26	2.41	2.71	3.02	3.25
Thr	1.03	1.13	1.24	1.41	1.55	1.99
Ser	1.41	1.65	1.81	2.08	2.20	2.31
Glu	5.89	6.65	7.33	8.35	9.30	9.98
Pro	3.18	3.38	3.80	4.66	4.27	4.52
Gly	1.90	2.24	2.31	2.77	2.90	3.16
Ala	1.25	1.39	1.56	1.80	1.80	1.94
Val	1.57	1.71	1.95	2.23	2.29	2.49
Ile	1.25	1.38	1.56	1.78	1.86	2.01
Leu	2.29	2.53	2.82	3.24	3.39	3.62
Tyr	1.11	1.28	1.40	1.64	1.78	1.86
Phe	0.98	1.31	1.44	1.68	1.91	1.99
His	0.73	0.93	1.01	1.13	1.05	1.20
Lys	1.92	2.18	2.42	2.74	2.89	3.15
Arg	1.35	1.49	1.57	1.91	1.97	2.13
Cys	0.16	0.20	0.21	0.22	0.23	0.26
Met	0.58	0.76	0.84	0.91	1.07	1.18
Total	28.6	32.5	35.7	41.3	43.5	47.1

[1] Amino acid samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

3. Results

(1) The optimum dietary protein level in fingerling sterlet sturgeon (7～40g), *Acipenser ruthenus*

Average weight gain (WG, %), feed efficiency (FE, %), specific growth rate (SGR, %), protein efficiency ratio (PER, %), protein retention efficiency (PRE, %), hepatosomatic index (HSI, %), condition factor (CF) and survival rate (%) for the 8-week experiment are shown in Table 5. Average weight gain of fish fed 45 and 50% CP iets were significantly higher than those of fish fed 30, 40 and 60% CP diets ($P < 0.05$), however there were no significant differences among fish fed 45, 50 and 55% CP diets. Feed efficiency (FE) of fish fed 40, 45, 50 and 55% CP diets were significantly higher than those of fish fed 30 and 60% CP diets ($P < 0.05$), meanwhile there was no significant differences in FE between fish fed 30 and 60% CP diets. Specific growth rate (SGR) of fish fed 50% CP diet was significantly higher than those of fish fed 30, 40 and 60% CP diets ($P < 0.05$), but no significant differences were observed in SGR among fish fed 45, 50 and 55% CP diets. Protein efficiency ratio and protein retention efficiency were reduced with the high dietary protein levels. Hepatosomatic index (HSI) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$), meanwhile no significant differences in HSI was observed between fish fed 40 and 50% CP diets. There were no significant differences in condition factor and in survival rate among fish fed the six experimental diets.

The whole-body proximate composition of fingerling sterlet sturgeon during the 8-week experiment is shown in Table 6. Moisture (%) among fish fed 60% CP diet was significantly higher than that of fish fed 30, 40, 45, 50 and 55% CP diets (P < 0.05). however Moisture (%) among fish fed 40, 45 and 50 % CP diets had no significant difference (P > 0.05). Crude protein (%) among fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 55 and 60 % CP diets (P < 0.05). however, no significant difference in crude protein was observed among fish fed 45 and 50% CP diets (P > 0.05). Also no significant difference in crude protein was observed among fish fed 30, 40, 45, 55 and 60% CP diets (P > 0.05). Crude lipid (%) of fish fed 40 and 50% CP diets was significantly higher than that of fish fed 30, 45, 55 and 60% CP diets (P < 0.05). However, no significant difference in crude lipid was observed among fish fed 30, 45 and 55% CP diets (P > 0.05). There was no significant difference in crude ash (%) among fish fed all of the diets (P > 0.05).

The whole body amino acid composition of fingerling sterlet sturgeon fed 6 experimental diets for 8 weeks is shown in Table 7. Threonine (%) of fish fed 60% CP diet was significantly higher than that of fish fed 30, 40, 45, 50 and 55% CP diets (P < 0.05). however, no significant difference in threonine (%) was observed among fish fed 40 and 45% CP diets ($P > 0.05$). Valine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). however, no significant difference in valine (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). Isoleucine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in isoleucine (%) was observed among fish fed 45 and 60% CP diets ($P > 0.05$). And no significant difference in isoleucine (%) was observed among fish fed 40 and 60% CP diets ($P > 0.05$). Also, no significant difference in isoleucine (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). Also, no significant difference in isoleucine (%) was observed among fish fed 30 and 55% CP diets ($P > 0.05$). Leucine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). however, no significant difference in leucine (%) was observed among fish fed 40 and 55% CP diets ($P > 0.05$). Also, no significant difference in valine (%) was observed among fish fed 30, 55 and 60% CP diets ($P > 0.05$). Phenylalanine (%) of fish fed 30, 45 and 50% CP diet was significantly higher than that of fish fed 40, 55 and 60% CP diets ($P < 0.05$). however, no significant difference in phenylalanine (%) was observed among fish fed 30, 45 and 50% CP diets ($P > 0.05$). Also, no significant difference in phenylalanine (%)

was observed among fish fed 40, 55 and 60% CP diets ($P > 0.05$). Histidine (%) of fish fed 45% CP diet was significantly higher than that of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in histidine (%) was observed among fish fed 30, 50 and 60% CP diets ($P > 0.05$). And no significant difference in histidine (%) was observed among fish fed 30, 55 and 60% CP diets ($P > 0.05$). Also, no significant difference in histidine (%) was observed among fish fed 40, 55 and 60% CP diets ($P > 0.05$). Lysine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in lysine (%) was observed among fish fed 40 and 60% CP diets ($P > 0.05$). And no significant difference in lysine (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). Arginine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in arginine (%) was observed among fish fed 30, 55 and 60% CP diets ($P > 0.05$). Methionine (%) of fish fed 45% CP diet was significantly higher than that of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in methionine (%) was observed among fish fed 30, 40 and 55% CP diets ($P > 0.05$). And no significant difference in methionine (%) was observed among fish fed 40 and 50% CP diets ($P > 0.05$).

The whole body essential amino acids composition (%) of fish fed

50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in whole body essential amino acids composition (%) was observed among fish fed 30 and 45% CP diets ($P > 0.05$). And no significant difference in whole body essential amino acids composition (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). Also, no significant difference in whole body essential amino acids composition (%) was observed among fish fed 55 and 60% CP diets ($P > 0.05$).

Aspartic acids (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in aspartic acid (%) was observed among fish fed 30 and 45% CP diets ($P > 0.05$). And no significant difference in aspartic acid (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). Also, no significant difference in aspartic acid (%) was observed among fish fed 40 and 55% CP diets ($P > 0.05$). Serine (%) of fish fed 40 and 60% CP diets was significantly higher than that of fish fed 30, 45, 50 and 55% CP diets ($P < 0.05$). Meanwhile, no significant difference in serine (%) was observed among fish fed 45 and 50% CP diets ($P > 0.05$). And no significant difference in serine (%) was observed among fish fed 30 and 55% CP diets ($P > 0.05$). Glutamic acid (%) of fish fed 45, 50 and 55% CP diets was significantly higher than that of fish fed 30, 40 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in glutamic acid (%) was observed among fish fed 40 and 60% CP diets ($P > 0.05$). Proline (%) of fish fed 45% CP diet was significantly higher than that of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in proline (%) was observed among fish fed 30, 40 and 50% CP diets ($P > 0.05$). And no significant difference in proline (%) was observed among fish fed 55 and 60% CP diets ($P > 0.05$). Glycine (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in glycine (%) was observed among fish fed 30 and 40% CP diets ($P > 0.05$). And no significant difference in glycine (%) was observed among fish fed 45 and 60% CP diets ($P > 0.05$). Alanine (%) of fish fed 45% CP diet was significantly higher than that of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in alanine (%) was observed among fish fed 40, 55 and 60% CP diets ($P > 0.05$). And no significant difference in alanine (%) was observed among fish fed 30 and 50% CP diets ($P > 0.05$). Tyrosine (%) of fish fed 30% CP diet was significantly higher than that of fish fed 40, 45, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in tyrosine (%) was observed among fish fed 45 and 50% CP diets ($P > 0.05$). Cysteine (%) of fish fed 45% CP diet was significantly higher than that of fish fed 30, 40, 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in cysteine (%)

was observed among fish fed 55 and 60% CP diets ($P > 0.05$). And no significant difference in cysteine (%) was observed among fish fed 40, 50 and 55% CP diets ($P > 0.05$).

The whole body non-essential amino acids composition (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in whole body non-essential amino acids composition (%) was observed among fish fed 30, 40, 55 and 60% CP diets ($P > 0.05$).

The whole body total amino acids composition (%) of fish fed 50% CP diet was significantly higher than that of fish fed 30, 40, 45, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in whole body total amino acids composition (%) was observed among fish fed 30, 40 and 60% CP diets ($P > 0.05$). And no significant difference in whole body total amino acids composition (%) was observed among fish fed 40, 55 and 60% CP diets ($P > 0.05$).

The whole body free amino acid composition of fingerling sterlet sturgeon fed 6 experimental diets for 8 weeks is shown in Table 8.

Taurine (%) of fish fed 55% CP diet was significantly higher than that of fish fed 30, 40, 45, 50 and 60% CP diets ($P < 0.05$). L-glutamic acid (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 40, 45, 50 and 55% CP diets ($P < 0.05$). L-glycine (%) of fish fed 30 and 55% CP diet was significantly higher than that of fish fed 40, 45, 50 and 60% CP diets ($P < 0.05$).

however, no significant difference in L-glycine (%) was observed among fish fed 30 and 55% CP diets (P > 0.05). L-alanine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). however, no significant difference in L-alanine (%) was observed among fish fed 50 and 60% CP diets (P > 0.05). L-isoleucine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in L-isoleucine (%) was observed among fish fed 55 and 60% CP diets (P > 0.05). And no significant difference in L-isoleucine (%) was observed among fish fed 30 and 45% CP diets (P > 0.05). L-leucine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in L-leucine (%) was observed among fish fed 45 and 60% CP diets (P > 0.05). L-tyrosine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in L-tyrosine (%) was observed among fish fed 30 and 45% CP diets (P > 0.05). L-phenylalanine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in L-phenylalanine (%) was observed among fish fed 45 and 60% CP diets (P > 0.05). L-alanine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in L-alanine (%) was observed among fish fed 45 and 60% CP diets (P > 0.05). And no significant difference in L-alanine (%) was observed among fish fed 30 and 50% CP diets (P > 0.05). L-ornithine (%) of fish fed 30% CP diet was significantly higher than that of fish fed 40, 45, 50, 55 and 60% CP diets (P < 0.05). L-lysine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). 1-Methyl-L-Histidine (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in 1-Methyl-L-Histidine (%) was observed among fish fed 30, 55 and 60% CP diets (P > 0.05).

The whole body total free amino acids composition (%) of fish fed 40% CP diet was significantly higher than that of fish fed 30, 45, 50, 55 and 60% CP diets (P < 0.05). Meanwhile, no significant difference in whole body total free amino acids composition (%) was observed among fish fed 45 and 60% CP diets (P > 0.05).

The hematological and serological characteristics of fingerling sterlet sturgeon fed 6 experimental diets for 8 weeks is shown in Table 9.

The hemoglobin (g/dL) of fish fed 30, 40, 45, 50 and 55% CP diet was significantly higher than that of fish fed 60% CP diets (P < 0.05). Meanwhile, no significant difference in hemoglobin (g/dL) was observed among fish fed 30, 40, 45, 50 and 55% CP diets (P > 0.05).

There was no significant difference in hematocrit (%) among fish fed all of the diets ($P > 0.05$). The Glucose (%) of fish fed 30, 40, 45 and 50% CP diet was significantly higher than that of fish fed 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in glucose (mg/dL) was observed among fish fed 30, 40, 45, 50 and 55% CP diets ($P > 0.05$). And no significant difference in glucose (mg/dL) was observed among fish fed 55 and 60% CP diets ($P > 0.05$). The glutamic oxaloacetic transaminase (GOT, IU/dL) of fish fed 30% CP diet was significantly higher than that of fish fed 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in GOT (IU/dL) was observed among fish fed 30, 40 and 45% CP diets ($P > 0.05$). And no significant difference in GOT (IU/dL) was observed among fish fed 40, 45, 50, 55 and 60% CP diets ($P > 0.05$). The glutamic pyruvic transaminase (GPT, IU/dL) of fish fed 30% CP diet was significantly higher than that of fish fed 50, 55 and 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in GPT (IU/dL) was observed among fish fed 30, 40 and 45% CP diets ($P > 0.05$). And no significant difference in GPT (IU/dL) was observed among fish fed 40, 45, 50, 55 and 60% CP diets ($P > 0.05$). Total protein (TP, g/dL) of fish fed 30 and 40% CP diet was significantly higher than that of fish fed 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in TP (g/dL) was observed among fish fed 30, 40, 45, 50 and 55% CP diets ($P > 0.05$). And no significant difference in TP (g/dL) was observed among fish fed 45, 50, 55 and 60% CP diets ($P > 0.05$). Albumin (ALB, g/dL) of fish fed 40% CP diet was significantly higher than that of fish fed 60% CP diets ($P < 0.05$). Meanwhile, no significant difference in ALB (g/dL) was observed among fish fed 30, 40, 45, 50 and 55% CP diets ($P > 0.05$). And no significant difference in ALB (g/dL) was observed among fish fed 30, 45, 50, 55 and 60% CP diets ($P > 0.05$). There was no significant difference in total cholesterol (mg/dL) among fish fed all of the diets ($P > 0.05$).

Table 5. Effects of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 8 weeks

Parameters	Diets[1] (Protein levels, %)						
	30	40	45	50	55	60	Pooled SEM
Initial mean wt. (g)	7.03	7.03	7.03	7.03	7.03	7.03	0.03
Final mean wt. (g)	30.7	39.9	42.6	43.4	41.8	29.6	1.94
Wt. gain (%)[2]	337^{c}	468^{b}	507^{a}	518^{a}	495^{ab}	321^{c}	27.4
FE (%)[3]	76.7^{b}	97.8^{a}	97.9^{a}	101.0^{a}	99.1^{a}	74.6^{b}	4.07
SGR (%)[4]	2.63^{c}	3.10^{b}	3.22^{ab}	3.25^{a}	3.18^{ab}	2.56^{c}	0.10
PER[5]	2.64^{a}	2.50^{b}	2.09^{c}	2.05^{c}	1.85^{d}	1.29^{e}	0.09
PRE[6]	17.3^{a}	14.4^{b}	12.9^{c}	11.4^{d}	10.0^{e}	8.4^{f}	0.59
HSI[7]	1.75^{d}	2.79^{b}	3.49^{a}	2.91^{b}	2.38^{c}	1.65^{d}	0.95
CF[8]	0.38	0.38	0.39	0.39	0.39	0.37	0.54
Survival rate(%)	93.3	100	96.7	94.4	98.9	98.9	4.69

[1] Values are means from triplicate groups of fish where the means in each row with a different letters are significantly different (P<0.05).
[2] Percent weight gain; (final wt. - initial wt.) × 100 / initial wt.
[3] Feed conversion ratio; (wet weight gain / dry feed intake).
[4] Specific growth rate; (loge final wt. - loge initial wt.) / days.
[5] Protein efficiency ratio; (wet weight gain / protein intake) × 100.
[6] Protein retention efficiency; 100× [(final weight × final body crude protein) -(initial weight ×initial body crude protein) in (g)]/total food × dietary crude protein in (g).
[7] Hepatosomatic index; (liver weight / body weight) × 100.
[8] Condition factor; [fish wt. (g) / fish length (cm)^{3}] × 100.

Table 6. Whole body proximate composition (%) of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 8 weeks (% of as is basis)[1]

Diet	Protein levels(%)						
	30	40	45	50	55	60	Pooled SEM[2]
Moisture(%)	76.5[bc]	75.9[cd]	75.8[cd]	75.3[d]	76.8[b]	77.9[a]	0.32
Crude protein(%)	12.6[b]	12.5[b]	12.9[ab]	13.3[a]	12.4[b]	12.6[b]	0.27
Crude lipid(%)	7.0[b]	7.9[a]	6.9[b]	7.9[a]	6.4[b]	5.0[c]	0.24
Ash(%)	2.6	2.6	2.9	2.7	2.8	2.7	0.19

[1] Refer to the table 5.
[2] Pooled standard error of mean: SD/√n.

Table 7. Whole body amino acid composition (mg/100mg) of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 8weeks[1]

	Protein levels(%)						
	30	40	45	50	55	60	Pooled SEM
Thr	0.44[d]	0.41[e]	0.41[e]	0.52[b]	0.49[c]	0.54[a]	0.11
Val	0.85[c]	0.85[c]	0.87[b]	0.90[a]	0.75[d]	0.74[e]	0.19
Ile	0.54[de]	0.55[cd]	0.57[b]	0.59[a]	0.54[e]	0.56[bc]	0.13
Leu	0.88[c]	0.90[b]	0.85[d]	0.94[a]	0.89[bc]	0.88[c]	0.21
Phe	0.51[a]	0.44[b]	0.49[a]	0.51[a]	0.44[b]	0.44[b]	0.11
His	0.43[bc]	0.40[d]	0.47[a]	0.44[b]	0.41[cd]	0.42[bcd]	0.10
Lys	0.94[d]	0.95[cd]	0.91[e]	1.02[a]	0.98[b]	0.96[c]	0.23
Arg	0.79[c]	0.82[b]	0.75[d]	0.87[a]	0.78[c]	0.79[c]	0.19
Met	0.39[c]	0.38[cd]	0.45[a]	0.38[d]	0.39[c]	0.41[b]	0.09
Essential	5.34[bc]	5.30[c]	5.35[b]	5.63[a]	5.18[d]	5.20[d]	1.26
Asp	1.10[cd]	1.09[de]	1.11[c]	1.27[a]	1.08[e]	1.14[b]	0.27
Ser	0.64[c]	0.70[a]	0.67[b]	0.67[b]	0.65[c]	0.70[a]	0.16
Glu	1.75[b]	1.72[c]	1.81[a]	1.84[a]	1.84[a]	1.73[c]	0.42
Pro	0.82[b]	0.82[b]	0.86[a]	0.83[b]	0.77[c]	0.79[c]	0.19
Gly	1.14[b]	1.12[b]	1.08[d]	1.19[a]	1.09[c]	1.08[d]	0.26
Ala	0.70[c]	0.75[b]	0.84[a]	0.70[c]	0.75[b]	0.76[b]	0.18
Tyr	0.41[a]	0.34[e]	0.39[b]	0.39[b]	0.35[d]	0.37[c]	0.09
Cys	0.16[b]	0.15[d]	0.18[a]	0.15[d]	0.15[cd]	0.15[c]	0.04
Non-essential	6.71[c]	6.68[c]	6.95[b]	7.04[a]	6.69[c]	6.70[c]	1.60
Total	12.49[c]	12.39[cd]	12.72[b]	13.19[a]	12.35[d]	12.45[cd]	2.97

[1] Refer to the table 5.

Table 8. Whole body free amino acid composition (mg/100mg) of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the first 6 experimental diets for 8weeks[1]

	Protein levels(%)						
	30	40	45	50	55	60	Pooled SEM
Taurine	0.061^{f}	0.080^{d}	0.088^{b}	0.068^{e}	0.092^{a}	0.085^{c}	0.33
L-Glutamic acid	0.004^{f}	0.020^{a}	0.015^{d}	0.011^{e}	0.016^{c}	0.016^{b}	0.06
L-Glycine	0.015^{a}	0.013^{c}	0.010^{d}	0.009^{e}	0.015^{a}	0.014^{b}	0.05
L-Alanine	0.021^{c}	0.027^{a}	0.025^{b}	0.019^{d}	0.019^{e}	0.019^{d}	0.09
L-Isoleucine	0.005^{c}	0.007^{a}	0.005^{c}	0.004^{d}	0.006^{b}	0.006^{b}	0.02
L-Leucine	0.010^{c}	0.011^{a}	0.009^{d}	0.007^{e}	0.010^{b}	0.009^{d}	0.04
L-Tyrosine	0.006^{d}	0.007^{a}	0.006^{d}	0.005^{e}	0.006^{b}	0.006^{c}	0.02
L-Phenylalanine	0.005^{d}	0.008^{a}	0.006^{c}	0.004^{e}	0.007^{b}	0.006^{c}	0.02
β-Alanine	0.034^{d}	0.040^{a}	0.035^{c}	0.034^{d}	0.037^{b}	0.035^{c}	0.15
L-Ornithine	0.015^{a}	0.014^{b}	0.009^{f}	0.010^{e}	0.012^{c}	0.011^{d}	0.05
L-Lysine	0.010^{b}	0.011^{a}	0.007^{e}	0.006^{f}	0.010^{c}	0.009^{d}	0.04
1-Methyl-L-Histidine	0.029^{b}	0.036^{a}	0.027^{c}	0.023^{d}	0.029^{b}	0.029^{b}	0.12
Total	0.216^{d}	0.275^{a}	0.241c	0.201^{e}	0.259^{b}	0.245^{c}	0.99

[1] Refer to the table 5.

Table 9. Hematological and serological characteristics of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 8weeks[1]

Parameters	Diets (Protein levels, %)						
	30	40	45	50	55	60	Pooled SEM[2]
Hemoglobin (g/dL)	8.2[a]	8.0[a]	8.1[a]	8.0[a]	8.0[a]	6.8[b]	1.78
Hematocrit (%)	26.9	27.7	26.8	26.2	26.9	24.7	6.25
Glucose (mg/dL)	66.3[a]	63.2[a]	62.4[a]	62.0[a]	57.9[ab]	49.7[b]	14.2
GOT (IU/L)	667[a]	536[ab]	559[ab]	437[b]	475[b]	517[b]	125.4
GPT (IU/L)	204[a]	163[ab]	173[ab]	132[b]	143[b]	141[b]	37.6
Total Protein (g/dL)	1.44[a]	1.52[a]	1.43[ab]	1.46[ab]	1.48[ab]	1.26[b]	0.34
Albumin (g/dL)	0.37[ab]	0.42[a]	0.37[ab]	0.36[ab]	0.38[ab]	0.32[b]	0.09
Total cholesterol (mg/dL)	106	117	117	128	126	116	27.9

[1] Refer to the table 5.
[2] Pooled standard error of mean: SD/√n.

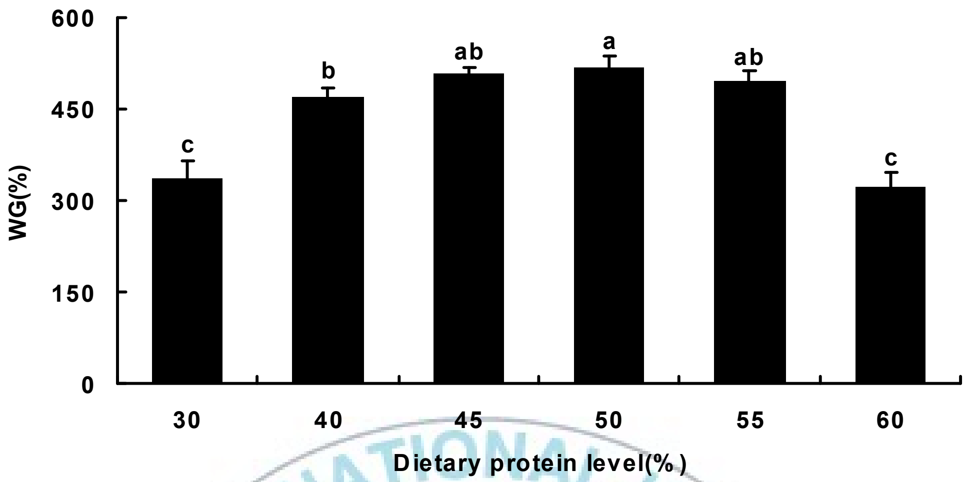

Fig.3. Average weight gain (WG, %) from fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

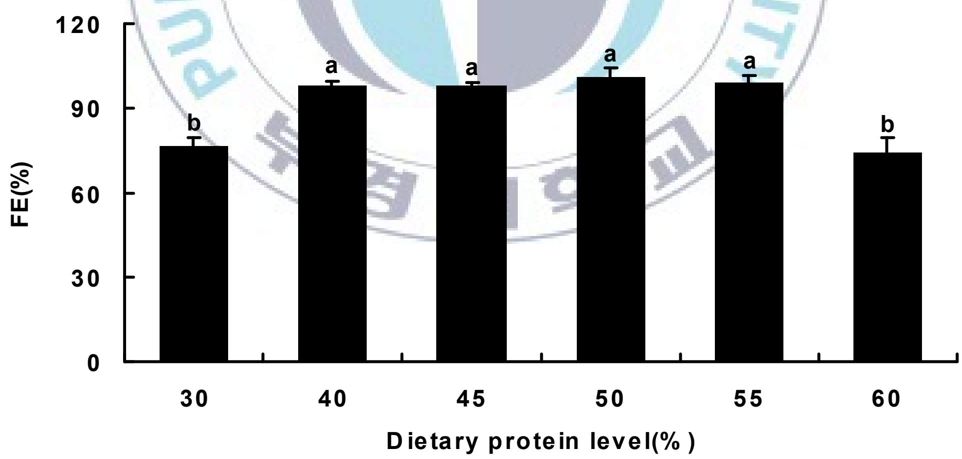

Fig.4. Average feed efficiency (FE, %) from fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

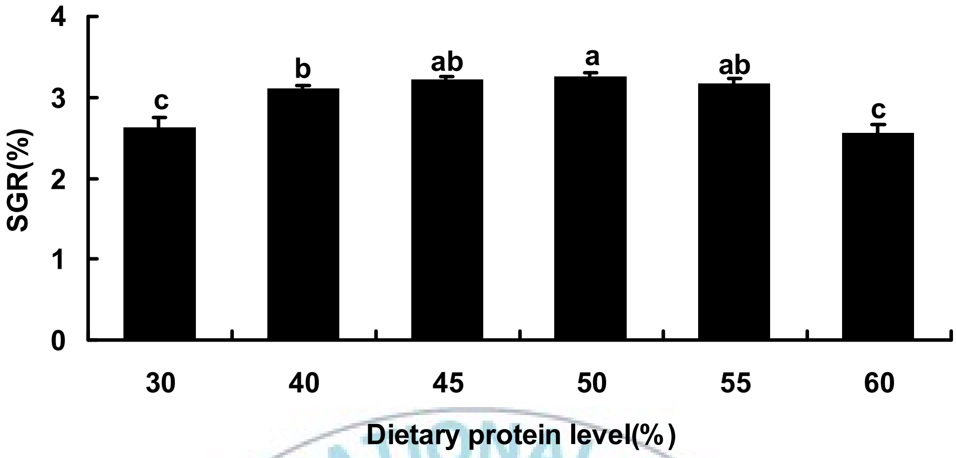

Fig.5. Average specific growth rate (SGR, %) from fish fed six diet for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

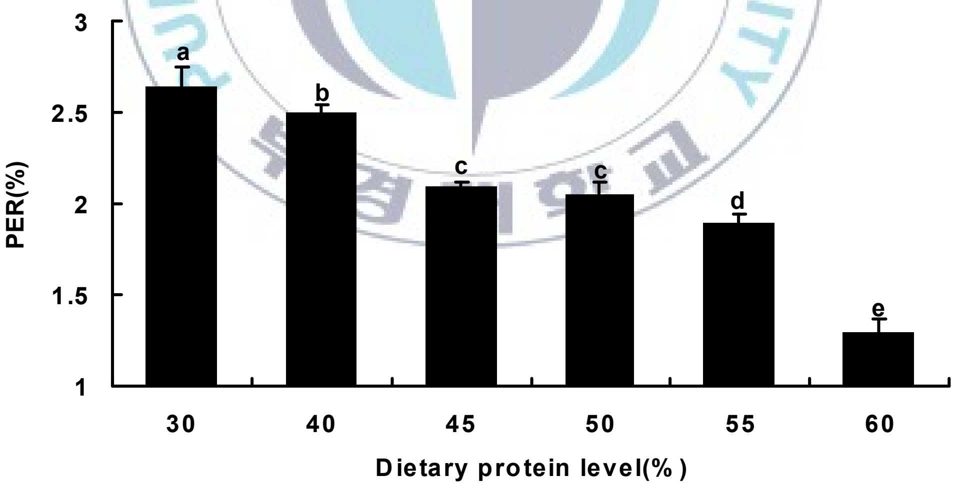

Fig.6. Average protein efficiency ratio (PER, %) from fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

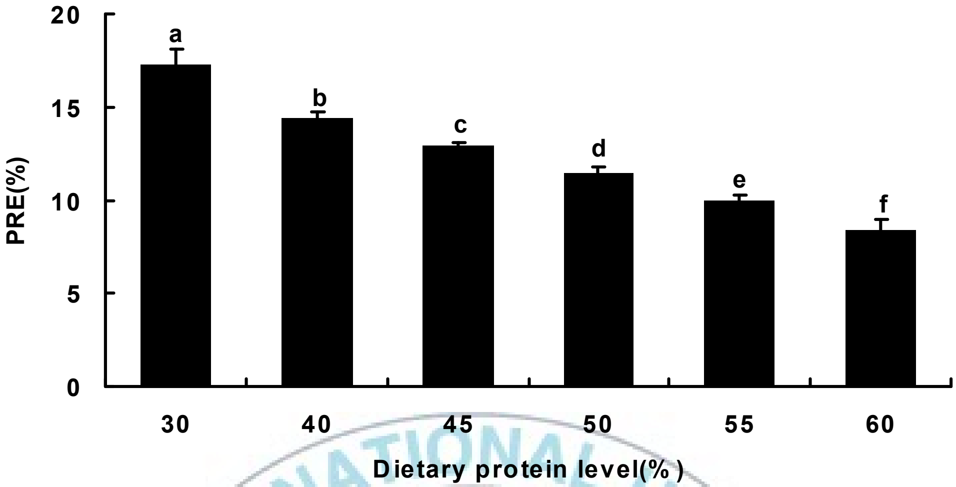

Fig.7. Average protein retention efficiency (PRE, %) from fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

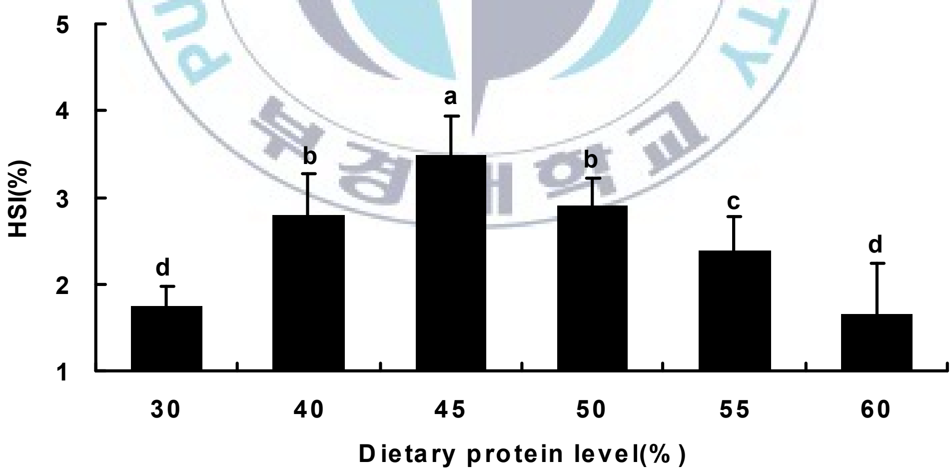

Fig.8. Average hepatosomatic index (HSI, %) from fish fed six diets for the first 8weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

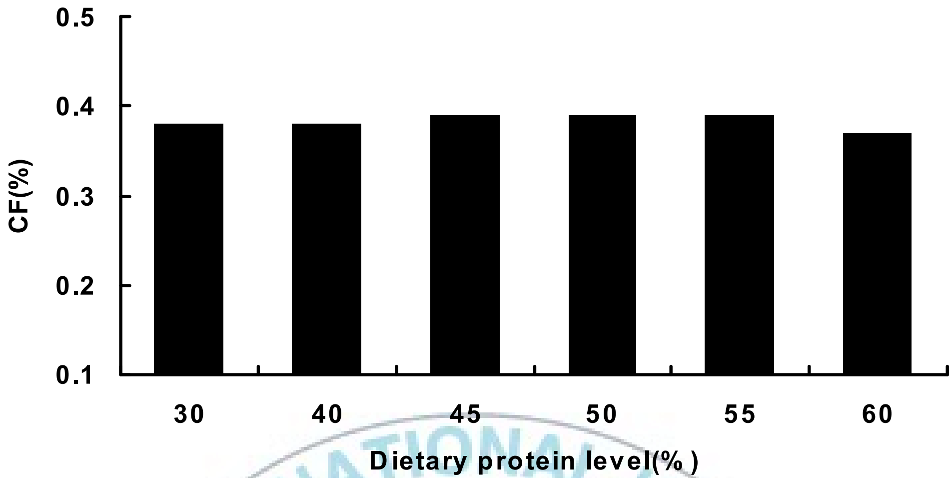

Fig.9. Average condition factor (CF, %) from the fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

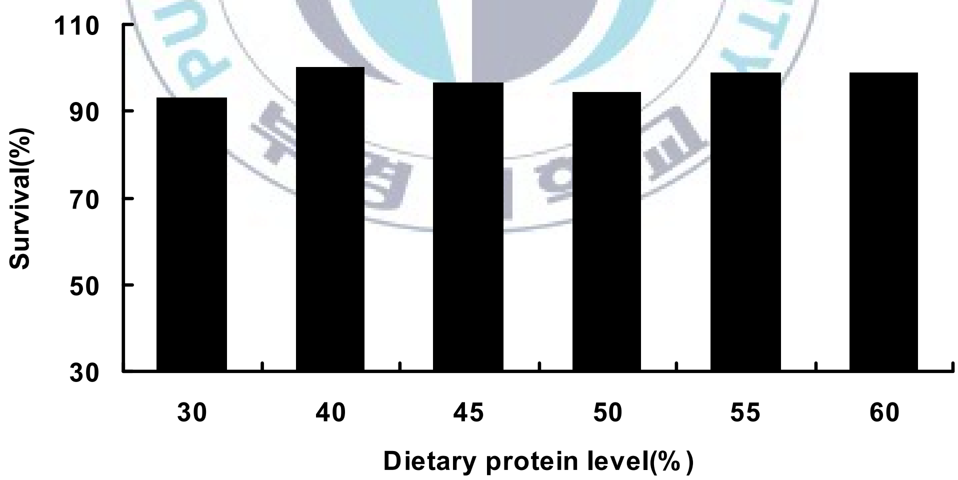

Fig.10. Average survival (%) from the fish fed six diets for the first 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P <0.05).

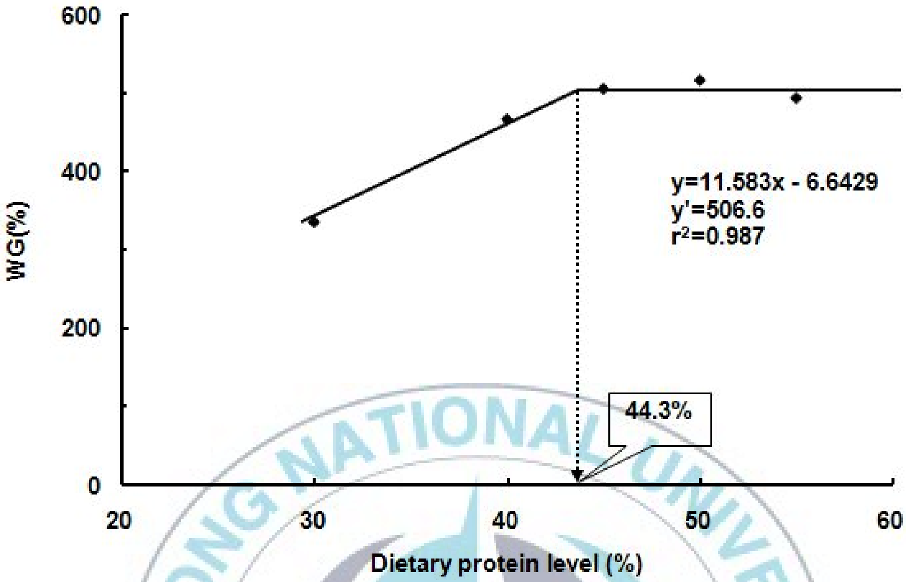

Fig.11. Broken-line model of WG(%) in fingerling sterlet sturgeon, *Acipenser ruthenus* fed different levels of dietary protein for 8 weeks. Values are mean ± SEM, n=3.

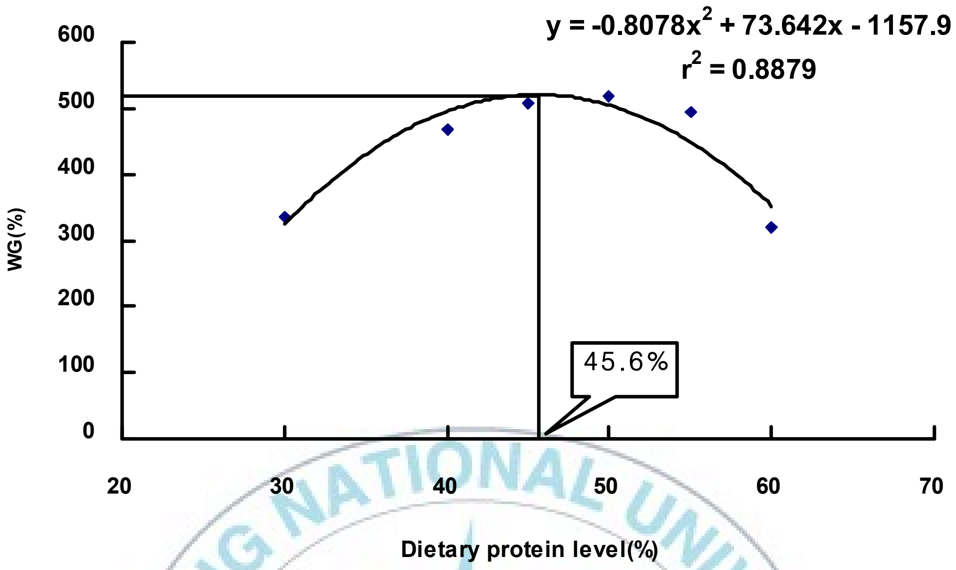

Fig.12. The second order polynomial analysis (solid curved line) of average weight gain and dietary protein levels for fingerling sterlet sturgeon, *Acipenser ruthenus* fed different levels of dietary protein for 8 weeks. Values are mean ± SEM, n=3.

(2) The optimum dietary protein level in juvenile sterlet sturgeon (38～90g), *Acipenser ruthenus*

Average weight gain (WG, %), feed efficiency (FE, %), specific growth rate (SGR, %), protein efficiency ratio (PER, %), protein retention efficiency (PRE, %), hepatosomatic index (HSI, %), condition factor (CF) and survival rate (%) during the 10-week experiment are shown in Table 10. After 10 weeks of feeding trial, average weight gain (WG) of fish fed 45% CP diets were significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (P < 0.05), however there were no significant differences in WG among fish fed 40 and 45% CP diets. Average feed efficiency (FE) of fish fed 40 and 45% CP diets were significantly higher than those of fish fed 30% CP diets (P < 0.05), meanwhile there was no significant differences in FE between fish fed 35, 40, 45, 50 and 55% CP diets. Average specific growth rate (SGR) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (P < 0.05), but no significant differences were observed in SGR among fish fed 40 and 45% CP diets. Average protein efficiency ratio (PER) of fish fed 30% CP diet was significantly higher than those of fish fed 35, 40, 45, 50 and 55% CP diets (P < 0.05), but no significant differences were observed in PER among fish fed 50 and 55% CP diets. Average protein retention efficiency (PRE) of fish fed 30 and 35% CP diets were significantly higher than those of fish fed 40, 45, 50 and 55% CP diets ($P < 0.05$), however there were no significant differences in PRE between fish fed 30 and 35% CP diets.

Based on the one-way ANOVA test, the broken-line regression analysis and second order polynomial relation model on weight gain, the optimum dietary protein requirement level could be greater than 42.7%, but less than 44.6% in juvenile sterlet sturgeon under our experimental conditions.

Table 10. Effects of juvenile sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diet for 10 weeks

Parameters	Diets[1] (Protein levels, %)						
	30	35	40	45	50	55	Pooled SEM
Initial mean wt. (g)	37.6	37.5	37.5	37.5	37.6	37.6	0.19
Final mean wt. (g)	73.1	83.1	90.4	93.6	87.3	83.3	1.69
Wt. gain (%)[2]	95[d]	121[c]	141[ab]	149[a]	133[bc]	122[c]	4.48
FE (%)[3]	81.7[b]	86.2[ab]	91.4[a]	92.6[a]	86.6[ab]	86.6[ab]	1.10
SGR (%)[4]	0.95[d]	1.14[c]	1.25[ab]	1.30[a]	1.20[bc]	1.14[c]	0.03
PER[5]	2.85[a]	2.37[b]	2.15[c]	1.83[d]	1.49[e]	1.36[e]	0.13
PRE[6]	13.6[a]	13.5[a]	11.9[b]	11.5[b]	11.4[b]	10.6[b]	0.31
HSI[7]	1.91[b]	2.27[ab]	2.76[a]	2.56[a]	2.31[ab]	2.28[ab]	0.27
CF[8]	0.35	0.35	0.36	0.35	0.35	0.35	0.20
Survival rate (%)	100	100	97.8	93.3	93.3	93.3	1.11

[1] Refer to the table 5.
[2] Percent weight gain; (final wt. - initial wt.) × 100 / initial wt.
[3] Feed conversion ratio; (wet weight gain / dry feed intake).
[4] Specific growth rate; (loge final wt. - loge initial wt.) / days.
[5] Protein efficiency ratio; (wet weight gain / protein intake) × 100.
[6] Protein retention efficiency; 100× [(final weight × final body crude protein) -(initial weight × initial body crude protein) in (g)]/total food × dietary crude protein in (g).
[7] Hepatosomatic index; (liver weight / body weight) × 100.
[8] Condition factor; [fish wt. (g) / fish length (cm)^3] × 100.

Table 11. Whole body proximate composition (%) of juvenile sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 10 weeks (% of as is basis)[1]

Diet	Protein levels(%)						
	30	35	40	45	50	55	Pooled SEM[2]
Moisture(%)	78.4[a]	76.8[b]	75.1[c]	75.0[c]	76.1[bc]	76.3[bc]	0.14
Crude protein(%)	13.5[d]	14.5[c]	15.0[ab]	15.1[a]	14.8[bc]	14.6[c]	0.32
Crude lipid(%)	5.1[c]	5.2[bc]	5.6[a]	5.7[a]	5.5[ab]	5.3[bc]	0.06
Ash(%)	2.43	2.32	2.30	2.28	2.33	2.32	0.04

[1] Refer to the table 5.
[2] Pooled standard error of mean: SD/√n.

Table 12. Whole body amino acid composition (mg/100mg) of juvenile sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 10 weeks[1] (% of as is basis)[1]

	Protein levels(%)						
	30	35	40	45	50	55	Pooled SEM
Thr	0.50^{c}	0.54^{a}	0.49^{c}	0.42^{e}	0.46^{d}	0.51^{b}	0.01
Val	0.55^{cd}	0.62^{a}	0.58^{bc}	0.47^{e}	0.53^{d}	0.60^{ab}	0.01
Ile	0.52^{b}	0.58^{a}	0.54^{b}	0.44^{d}	0.48^{c}	0.56^{ab}	0.01
Leu	0.89^{b}	0.99^{a}	0.92^{ab}	0.76^{c}	0.81^{c}	0.96^{ab}	0.02
Phe	0.51^{a}	0.55^{a}	0.51^{a}	0.42^{b}	0.52^{a}	0.53^{a}	0.01
His	0.39^{b}	0.43^{a}	0.40^{b}	0.34^{c}	0.35^{c}	0.41^{ab}	0.01
Lys	0.75^{bc}	0.86^{a}	0.79^{b}	0.66^{d}	0.74^{c}	0.83^{a}	0.02
Arg	0.86^{a}	0.87^{a}	0.84^{ab}	0.72^{c}	0.83^{b}	0.87^{a}	0.01
Met	0.20^{d}	0.22^{a}	0.21^{b}	0.20^{d}	0.22^{c}	0.11^{e}	0.01
Essential	5.17^{c}	5.66^{a}	5.28^{bc}	4.43^{e}	4.94^{d}	5.38^{b}	0.10
Asp	1.13^{c}	1.31^{a}	1.17^{bc}	0.96^{d}	1.11^{c}	1.23^{b}	0.03
Ser	0.59^{b}	0.62^{a}	0.59^{b}	0.51^{d}	0.56^{c}	0.61^{b}	0.01
Glu	1.79^{b}	1.94^{a}	1.80^{b}	1.54^{c}	1.74^{b}	1.88^{a}	0.03
Pro	0.71^{a}	0.62^{ab}	0.67^{ab}	0.58^{b}	0.67^{ab}	0.68^{ab}	0.01
Gly	1.33^{a}	1.11^{b}	1.11^{b}	1.09^{b}	1.25^{ab}	1.20^{ab}	0.03
Ala	0.87^{a}	0.87^{a}	0.86^{a}	0.76^{b}	0.89^{a}	0.91^{a}	0.01
Tyr	0.34^{b}	0.40^{a}	0.38^{a}	0.31^{b}	0.33^{b}	0.40^{a}	0.01
Cys	0.16^{c}	0.18^{a}	0.17^{b}	0.15^{d}	0.16^{c}	0.07^{e}	0.01
Non-essential	6.93^{ab}	7.05^{a}	6.75^{ab}	5.90^{c}	6.71^{b}	6.98^{ab}	0.10
Total	12.10^{bc}	12.71^{a}	12.03^{c}	10.33^{e}	11.65^{d}	12.36^{b}	0.19

[1] Refer to the table 5.

Table 13. Whole body free amino acid composition (mg/100mg) of juvenile sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 10 weeks[1]

	Protein levels(%)						
	30	35	40	45	50	55	Pooled SEM
Phosphoserine	0.006[b]	0.060[a]	0.005[c]	0.004[e]	0.004[d]	0.004[f]	0.0002
Taurine	0.025[b]	0.023[d]	0.027[a]	0.023[d]	0.026[b]	0.025[c]	0.0003
L-Threonine	0.003[c]	0.003[d]	0.004[a]	0.003[e]	0.002[a]	0.004[b]	0.0002
L-Serine	0.006[a]	0.005[e]	0.005[b]	0.005[c]	0.004[f]	0.005[d]	0.0002
L-Glutamic acid	0.011[a]	0.006[d]	0.009[b]	0.007[c]	0.006[e]	0.006[e]	0.0005
L-Glycine	0.011[c]	0.010[e]	0.013[b]	0.016[a]	0.010[d]	0.010[e]	0.0006
L-Alanine	0.019[a]	0.016[c]	0.018[b]	0.015[d]	0.016[cd]	0.016[c]	0.0003
L-Valine	0.005[a]	0.003[d]	0.004[b]	0.004[b]	0.003[d]	0.003[c]	0.0001
L-Isoleucine	0.003[a]	0.002[f]	0.003[d]	0.003[c]	0.003[b]	0.002[e]	0.0001
L-Leucine	0.006[a]	0.004[e]	0.005[b]	0.005[c]	0.005[c]	0.004[d]	0.0001
L-Tyrosine	0.003[a]	0.003[d]	0.003[b]	0.002[f]	0.003[c]	0.002[e]	0.0001
L-Phenylalanine	0.003[a]	0.003[c]	0.003[b]	0.003[c]	0.002[d]	0.003[b]	0.0001
L-Lysine	0.006[a]	0.004[c]	0.006[a]	0.004[d]	0.004[c]	0.004[b]	0.0002
L-Histidine	0.009[a]	0.007[b]	0.005[c]	0.005[d]	0.005[d]	0.005[d]	0.0004
Total	0.117[a]	0.096[d]	0.111[b]	0.099[c]	0.092[f]	0.094[e]	0.0022

[1] Refer to the table 5.

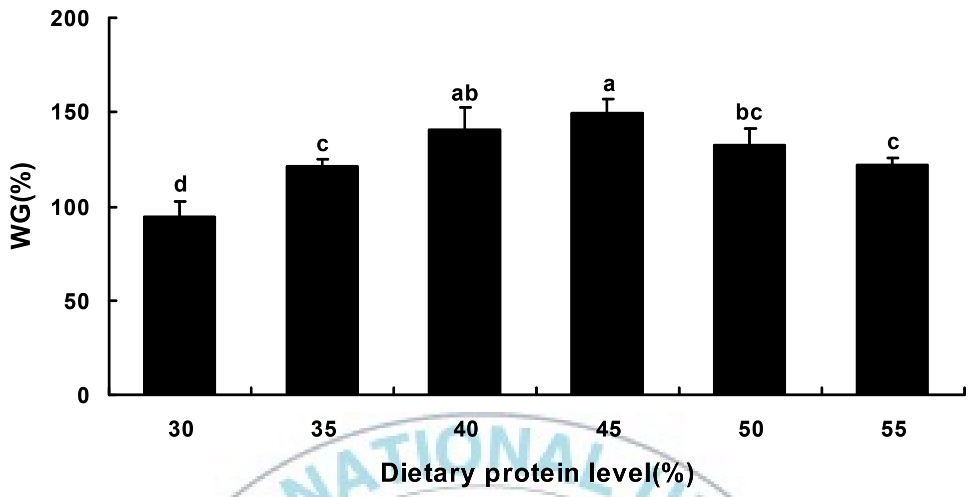

Fig.11. Average weight gain (WG, %) from fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

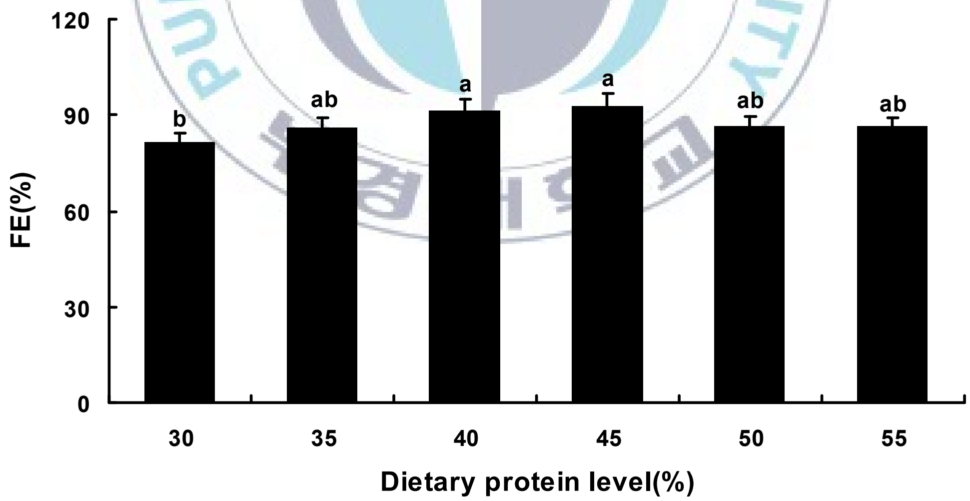

Fig.12. Average feed efficiency (FE, %) from fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

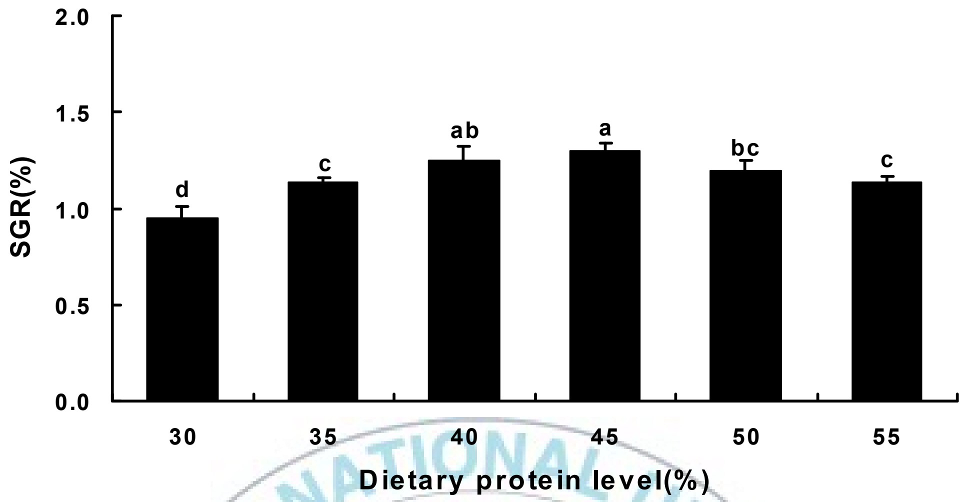

Fig.13. Average specific growth rate (SGR, %) from fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

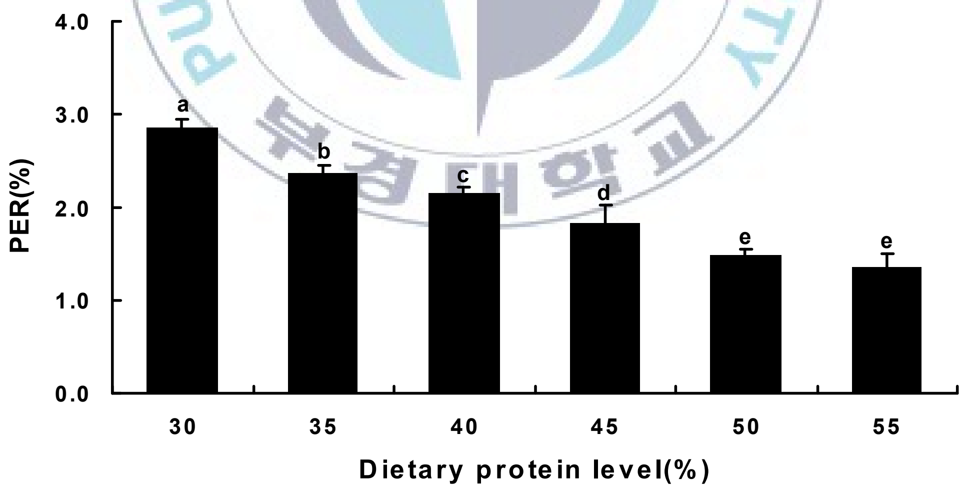

Fig.14. Average protein efficiency ratio (PER) from fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

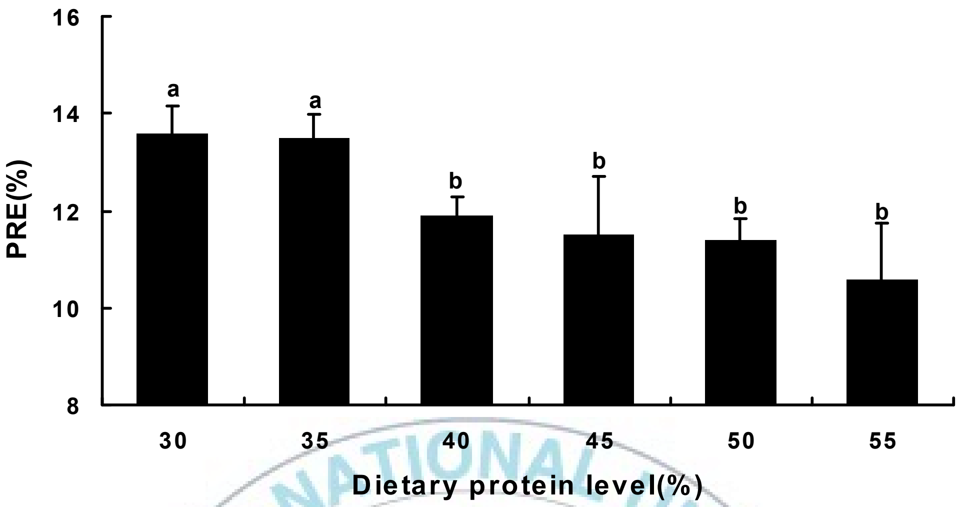

Fig.15. Average protein retention efficiency (PRE, %)) from the fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

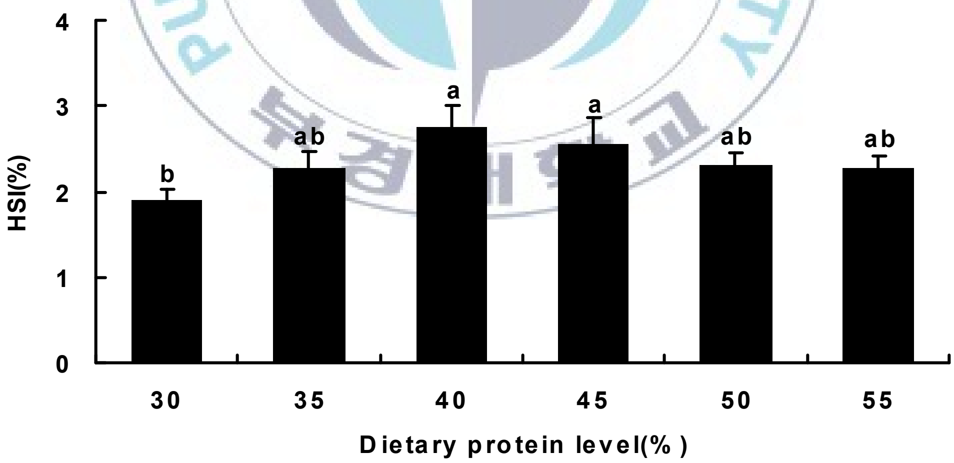

Fig.16. Average hepatosomatic index (HSI) from the fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

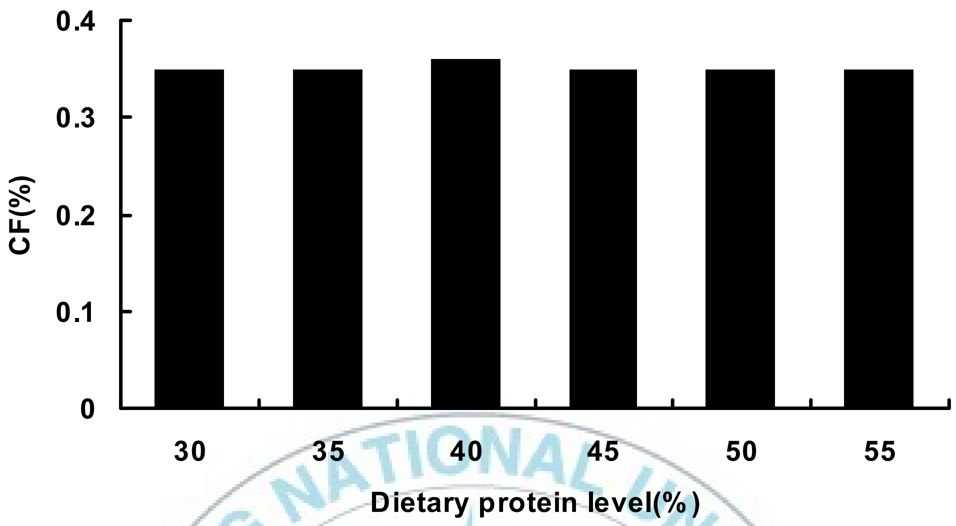

Fig.17. Average condition factor (CF) from fish fed six diets for 10 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$).

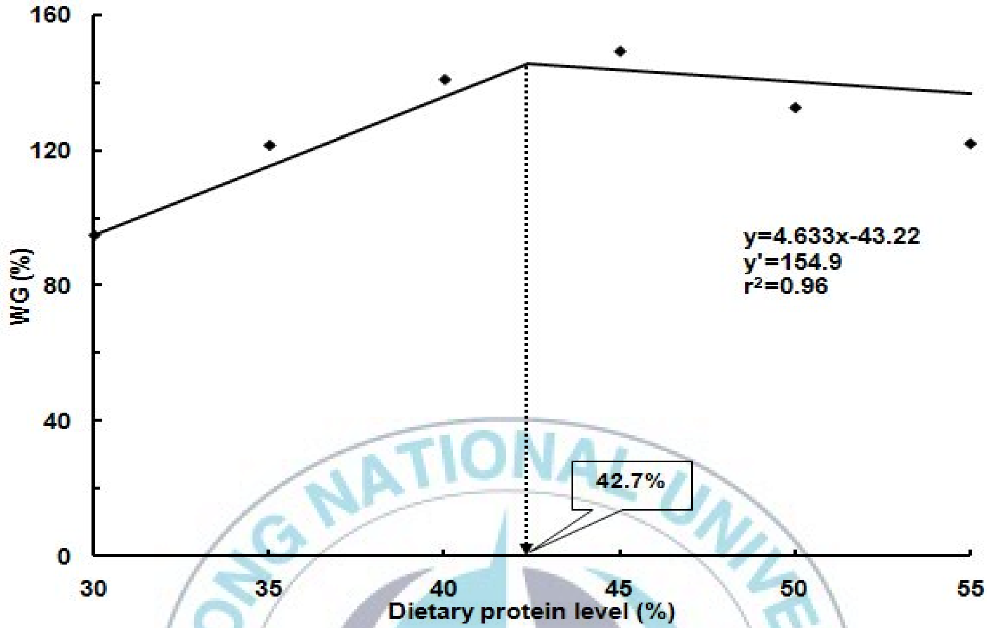

Fig.18. Broken-line model of WG (%) in juvenile sterlet sturgeon, *Acipenser ruthenus* fed six different levels of dietary protein for 10 weeks. Values are mean ± SEM, n=3.

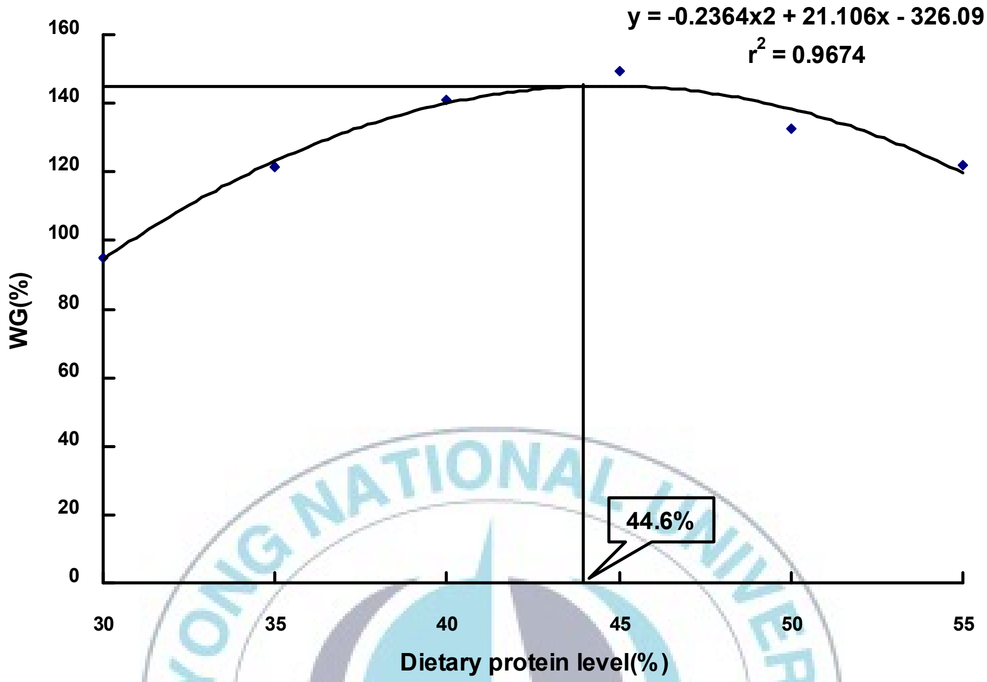

Fig.19. The second order polynomial analysis (solid curved line) of average weight gain and dietary protein levels for juvenile sterlet sturgeon, *Acipenser ruthenus* fed six different levels of dietary protein for 10 weeks. Values are mean ± SEM, n=3.

4. Discussion

In the fingerling experiment, after 8 weeks of the feeding trial, WG of fish fed 50% crude protein (CP) diet was not significantly different from that of fish fed 45and 55% CP diets, but significantly higher than those of fish fed 30, 40 and 60% CP diets (Table 5). WG of fish increased with increasing in dietary protein levels up to 50% and then decreased at higher protein levels. The similar trend has been observed in many other fish species irrespective of culture strategies (Jauncey 1982; Cho, Cowey & Watanabe 1985; Vergara, Fernandez-Palacios, Robaina, Jauncey, Higuera & Izquierdo 1996; Bai *et al.* 1999). This decrease in WG at protein levels above the optimum may be due to a reduction in available energy for growth due to inadequate non-protein energy necessary to deaminate and excrete excess absorbed amino acids (Jauncey 1982; Cho *et al.* 1985; Vergara *et al.* 1996). Cowey, Adron & Brown (1975) reported that weight gains of plaice (*Pleuronectes platessa*) given diets containing carbohydrate as well as protein and lipid were superior to those given diets lacking carbohydrate. This is also accordance with the present study; WG of fish fed 60% CP was decreased when little carbohydrate was included in the diet. When a broken-line model analysis was used (Fig. 11), the optimum dietary protein level for supporting maximum weight gain was 44.3% for fingerling sterlet sturgeon when fed at a fixed feeding rate of body weight per day. According to the second-order polynomial regression analysis (Fig. 12), the maximum % WG (Ymax) occurred at 45.6% CP (Xmax).

In the juvenile experiment, after 10 weeks of the feeding trial, WG of fish fed 45% CP diet was highest among all the dietary treatments, and the WG of juvenile sterlet sturgeon of fish fed 45% CP was not significantly different from that of fish fed 40% CP diet; but significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (Table 10).

When a broken-line model analysis was used (Fig. 18), the optimum dietary protein level for supporting maximum weight gain was 42.7% for juvenile sterlet sturgeon when fed at a fixed feeding rate of body weight per day. According to the second-order polynomial regression analysis (Fig. 19), the maximum % WG (Ymax) occurred at 44.6% CP (Xmax) for juvenile sterlet sturgeon.

It has already been demonstrated that estimates of quantitative nutrient requirement are influenced by not only the choice of response variable but also by the statistical methods used to evaluate the criterion response to different dietary nutrient levels (Zeitoun, Ullrey, Magae, Gill

& Bergen 1976; Baker 1986; Lovell 1989). In most of the protein requirement studies of fish, percent WG has been used as a response variable to dietary protein levels. In the first and second experiments, we use WG as the criteria to estimate the dietary protein requirement for fingerling and juvenile sterlet sturgeon. The broken line model and the second order polynomial regression analysis have been used to determine the protein requirements of several fish species (Zeitoun *et al.* 1976; Moore, Hung & Medranno 1988). The broken-line model is appropriate to use when the growth response increases linearly up to the minimum required nutrient level and then reaches a plateau above that level. On the other hand, the polynomial regression analysis had the advantage of being more accurate than other methods when the interval between dietary nutrient concentrations is wide and growth response is curvilinear (Zeitoun *et al.* 1976). Although the maximum percent WG (Y_{max}) occurred at 45.6% CP (X_{max}) in the first experiment and 44.6% CP (X_{max}) in the second experiment, it does not reflect the practically insignificant differences in percentage gain below and beyond the maximum. On the contrary, the selection of X_0 to X_1 on the polynomial regression as the minimum range of protein requirement is an economic decision; and this decision may be economically conservative as Zeitoun *et al.* (1976) has already described.

High protein requirements for Atlantic halibut and turbot have been reported by several researchers (Caceres-Martinez, Cadena-Roa & Metailler 1984; Hjertnes, Gulbrandsen, Johnsen & Opstvedt 1993). Caceres-Martinez *et al*. (1984) stated that the highest growth rate was obtained with a 69.8% protein diet for turbot. However, from the two experiments we conduct to measure the optimum dietary protein requirements for fingerling and juvenile sterlet sturgeon, we found that WG of fish was depressed when the fish were fed the 60% CP diet in the first experiment and 55% in the second experiment. Cowey, Pope, Adron & Blair (1972) and Helland & Grisdale-Helland (1998) reported that the dietary protein requirements for plaice *Pleuronectes platessa* and halibut *Hippoglossus hippoglossus* were 50% and 51%, respectively. Kikuchi *et al*. (1992) indicated that best growth was found in young flounder *Paralichthys olivaceus* fed 47% CP though no statistical analysis was performed. Lee *et al.* (2000) reported that the best WG was obtained from flounder fed a 50% protein diet with 3000 kcal kg^{-1}diet. Results from the present experiment also indicated that it is not necessary to include more than 55% protein in the diet containing gross energy 20.7 kJ g^{-1} for fingerling sterlet sturgeon and 50% protein containing gross energy 19.0 kJ g^{-1} for juvenile sterlet sturgeon in the diet. Lee *et al.* (2000) claimed that WG of flounder was decreased with increasing energy content from 3000 to 4000 kcal kg^{-1} diet at protein contents of 30, 40 and 50%. No growth plateaus or decreases were found in the study, and it indicated that the ranges of protein or energy in the study of Lee *et al*. (2000) were not large enough. Further study of the optimum protein to energy level in diets for the sterlet will be necessary to resolve this issue.

In general, the feed efficiency (FE) showed a similar trend to WG. In the fingerling experiment, fish fed the diet containing 50% CP had the highest FE (101%) among all the dietary treatments however there was no significant difference in FE among fish fed the 40, 45, 50 and 55% CP diets. However, Kikuchi *et al*.(1992) found that the FE of flounder increased with increasing levels of dietary protein from 21% to 60%, regardless of fish size. In specific growth rate (SGR), there was no significant difference among the fish fed the 45, 50 and 55% CP diets ($P > 0.05$) the lowest value (2.56) was found in fish fed 60% CP diet. And in the juvenile experiment, FE also showed a similar trend as WG. There was no significant different in FE among fish fed 35, 40, 45, 50 and 60% CP diets, and the FE of fish fed 40 and 50% CP diets were significantly higher than that of fish fed 30% CP diet in juvenile sterlet sturgeon.

In the fingerling experiment, protein efficiency ratio (PER) tended to decrease with increasing dietary protein levels. PER decreased from 2.64 to 1.29 with increasing protein levels from 30% to 60%. The highest value of PER was observed in fish fed the 30% CP diet. But no significant difference was found among fish fed the 45 and 50% CP diets. The PER data from the second experiment showed similar trend as described in first experiment. Similar results were reported by Parazo (1990) and Santinha, Gomes & Coimbra (1996) in other fish species. These researchers hypothesized that absorbed protein over the amount needed for protein synthesis was used as for energy. However, Kikuchi *et al.* (1992) and Lee *et al.* (2000) reported that PER values of flounder increased with increasing dietary protein levels.

Additionally, hepatosomatic index (HSI) increased with increasing dietary protein levels in both of the two experiments. Meanwhile, condition factor (CF) values and survival rate of fish fed the different experimental diets were not significantly different in these two experiments, respectively.

The proximate composition of whole body from the fingerling and juvenile experiment are shown in Table 6 and Table 11. In general, the crude protein of whole body increased with the increasing dietary protein levels, it was proved from the proximate composition data in the fingerling and juvenile experiments. In the fingerling experiment, there was no significant difference in whole body CP content of fish fed 45 and 50% CP diets. On the contrary, the crude lipid content in whole body decreased with increasing dietary protein levels. Fish fed the 50% CP diet had the highest lipid levels among all the dietary treatments (*P* < 0.05). And in the juvenile experiment, the crude protein content was also increased with the dietary protein contents up to 45% CP. A similar trend has been reported by Murai, Akiyama, Watanabe & Nose (1985). There were no significant differences existed in ash contents of whole body among the dietary treatments in this experiment.

Therefore, based on the broken-line analysis and second order polynomial regression analysis, the optimum dietary protein level was estimated to be greater than 44.3% but less than 45.6% in fingerling sterlet sturgon to support reasonable growth when dietary energy content was fixed at 20.7 kJ g^{-1} diet. Based on the broken-line analysis and second order polynomial regression analysis, the dietary protein requirement was estimated to be greater than 42.7% but less than 44.6% in juvenile sterlet sturgeon support reasonable growth when dietary energy content was fixed at 19.0 kJ g^{-1} diet.

Chapter Ⅲ. The optimum dietary protein to energy ratio in fingerling sterlet stergeon, *Acipenser ruthenus*

Abstract

This experiment was conducted to evaluate optimum dietary protein to energy ratio of fingerling sterlet sturgeon, *Acipenser ruthenus* for 8 weeks. White fish meal, casein & soybean meal were used as protein sources. Prior to the feeding trial, fish were fed control diet for 2 weeks to adjust to the experimental diets and conditions. Fish averaging 5.48 ± 0.04g (Mean ± SD) were distributed to each aquarium as a group of 30 fish reared in the flow through system. Fish of triplicate groups were fed one of six diets containing 40 and 45% crude protein and 10, 15 and 20% crude lipid, 19.4, 18.5, 17.6, 21.9, 20.8 and 19.8kJ gross energy/g diet, respectively.

Average weight gain (WG) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in WG among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets ($P>0.05$). Average feed efficiency (FE) of fish fed all of diet was no significant difference ($P>0.05$). Average specific

growth rate (SGR) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in SGR among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets ($P>0.05$). Average protein efficiency ratio (PER) of fish fed 40/10, 40/15 and 40/20 diets were significantly higher than those of fish fed 45/10, 45/15 and 45/20 diets ($P<0.05$). However, there was no significant difference in PER among fish fed 40/10, 40/15 and 40/20 diets ($P>0.05$).

Therefore these results indicated that the optimum dietary protein to energy ratio of fingerling sterlet sturgeon could be 21.9mg protein/kJ diet at 45% dietary protein levels.

1. Introduction

Energy is not a nutrient. It is released during metabolic oxidation of carbohydrates, fats and amino acids. And it need to keep the normal life and metabolism. Therefore, energy level should be the first nutritional consideration in diet formulation. Also, dietary protein to energy ratio is should be kept in balance to meet the maintenance and normal growth of fish. In relation to this, Many research have been conducted to determine the optimum dietary protein to energy ratio of fish such as black catfish (Salhi et al., 2004), olive flounder (Kim et al., 2004), Korean rockfish (Lee et al., 1993), Japanese eel (Okorie et al., 2007) etc.

However, there are only a few study for nutritional information in sterlet sturgeon. Therefore, the objective of this experiment is to determine the optimum dietary protein to energy ratio in fingerling sterlet sturgeon.

2. Materials and Methods

Experimental design and diets

Six experimental diets were formulated to be contained 40 and 45% crude protein and 10, 15 and 20% crude lipid and 19.4, 18.5, 17.6, 21.9, 20.8 and 19.8kJ gross energy g^{-1}diet, respectively. White fish meal, casein & soybean meal were used as protein sources. Fish oil and soybean oil were used as lipid sources. Wheat meal and dextrin were used as carbohydrate sources. α-cellulose were also included in the diets to match CP and energy levels. Procedures for diet preparation and storage were as previously described by Bai and Kim (1997). After thoroughly mixing the dry ingredients, fish oil and soybean oil were added with filtered tap water. The experimental diets were pelleted by using a laboratory pellet machine and stored -20℃ until used. Composition of the experimental diets is shown in Table 14. Amino acid (AA) composition of the experimental diets is shown in Table 15.

Experimental fish and feeding trial

Before the feeding trial, fish were fed commercial diet for one week to acclimate them to the commercial diet and conditions. And the feeding trials were conducted at the Gyeonggi Province Freshwater Fisheries Research Institute (Gwangtan-ri, Gyeonggi-do, Korea). At the beginning, fingerling sterlet sturgeon, *Acipenser ruthenus* averaging 5.48

± 0.04g (Mean ± SD) was divided into six groups and randomly distributed into each aquarium as a group of 30 fish. The feeding trial was conducted in 100ℓ aquariums with a water flow rate of 1-2ℓ min^{-1}. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.7 ±0.5 ppm. The water temperature was maintained at 20 ± 2.0 ℃ (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. Feeding trial was conducted for 8-weeks. Fish were fed at a rate of 4～6% (dry matter basis) of total body weight per day. The fish were fed three times a day at 08:00, 13:00 and 17:00h. Total weight of fish in each tank was measured every 2 weeks, and the feeding rate was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing.

Sample collections and analysis

At the end of the feeding trial, fish were anesthetized with AQUES(Handong Co. LTD. KOREA), 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 form human blood (Sigma Chemical, St. Louis, Missouri) was used. The muscle structural amino acids were quantified by amino acid analyzer S433 (Syknm, Germany) using ninhydrin method. Analysis conditions are as follows : Column size; 4㎜ × 150㎜, Absorbance; 570 ㎚ 440㎚, Reagent flow rate; 0.25 1㎖/min, Buffer flow rate; 0.45㎖ /min, Reactor temperature; 120, Reactor Size; 15m Fish ere stored at -20℃ until analysis. 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

All 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 14. Composition and proximate analysis of the six experimental diets (% of dry matter basis)[1]

Ingredients	Dietary Protein to lipid levels (%)					
	40/10	40/15	40/20	45/10	45/15	45/20
White fish meal[2]	35.0	35.0	35.0	41.0	41.0	41.0
Casein-vita free[3]	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal[2]	11.0	11.0	11.0	13.0	13.0	13.0
Dextrin[3]	28.5	23.5	18.5	21.0	16.0	11.0
Wheat meal[2]	6.5	6.5	6.5	6.5	6.5	6.5
Fish oil[2]	4.0	6.5	9.0	3.5	6.0	8.5
Soybean oil[2]	3.0	5.5	8.0	3.0	5.5	8.0
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[5]	1.0	1.0	1.0	1.0	1.0	1.0
Proximate analysis						
Moisture	10.9	10.8	11.1	13.4	12.2	13.1
Crude protein	40.1	40.2	40.1	45.3	45.2	45.1
Crude lipid	10.2	15.1	20.1	10.0	15.3	19.9
Crude fiber	2.0	2.2	2.4	1.3	1.5	1.7
Crude ash	9.9	10.2	9.9	11.6	11.7	12.0
Nitrogen free extract	26.9	21.5	16.4	18.4	14.1	8.2
Gross energy(kJ/g diet)	20.6	21.7	22.8	20.6	21.7	22.8
P/GE ratio(mg/kJ diet)	19.5	18.5	17.6	21.9	20.8	19.8

[1] The samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

[2] Suhyup Feed Co. Korea.

[3] United States Biochemical, Cleveland, Ohio 44122.

[4] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ;

Choline bitatrate, 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; B_{12}, 0.06.

[5] Contains (as mg/kg in diets) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$. 1379.8; $NaH_2P_4 \cdot 2H_2O$, 877.8; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

Table 15. Amino acid composition of the six experimental diets (% of as is basis)[1]

	Dietary Protein to lipid levels (%)					
	40/10	40/15	40/20	45/10	45/15	45/20
Asp	3.23	3.34	3.32	3.70	3.71	3.69
Thr	1.30	1.21	1.19	1.32	1.35	1.32
Ser	1.59	1.49	1.49	1.63	1.64	1.62
Glu	5.24	4.98	4.98	5.50	5.59	5.39
Pro	1.95	1.83	1.79	1.98	1.96	1.97
Gly	1.67	1.60	1.63	1.76	1.85	1.86
Ala	1.55	1.46	1.48	1.63	1.70	1.72
Val	1.50	1.44	1.46	1.63	1.77	1.62
Ile	1.34	1.29	1.27	1.46	1.51	1.47
Leu	2.45	2.36	2.38	2.60	2.68	2.62
Tyr	1.04	0.80	0.84	0.85	0.87	0.93
Phe	1.39	1.34	1.35	1.50	1.51	1.49
His	1.06	1.22	1.17	1.34	1.40	1.28
Lys	2.17	2.08	2.11	2.35	2.40	2.37
Arg	1.76	1.69	1.73	1.94	1.97	1.97
Cys	0.37	0.47	0.47	0.39	0.55	0.44
Met	1.94	2.82	2.55	1.88	2.16	2.27
Total	31.5	31.4	31.2	33.5	34.6	34.0

[1] Amino acid samples were analysed at Feeds & Foods Nutrition Research Center, Pukyong National University. Values are means of triplicate samples.

3. Results

Average weight gain (WG, %), feed efficiency (FE, %), specific growth rate (SGR, %), protein efficiency ratio (PER, %), protein retention efficiency (PRE, %), energy retention efficiency (ERE, %), hepatosomatic index (HSI, %), condition factor (CF) and survival rate (%) during the 8-week experiment are shown in Table 16. Average weight gain (WG) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets (P<0.05). However, there was no significant difference in WG among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets (P>0.05). Average feed efficiency (FE) of fish fed all of diet was no significant difference (P>0.05). Average specific growth rate (SGR) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets (P<0.05). However, there was no significant difference in SGR among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets (P>0.05). Average protein efficiency ratio (PER) of fish fed 40/10, 40/15 and 40/20 diets were significantly higher than those of fish fed 45/10, 45/15 and 45/20 diets (P<0.05). However, there was no significant difference in PER among fish fed 40/10, 40/15 and 40/20 diets (P>0.05).

Therefore these results indicated that the optimum dietary protein to energy ratios of juvenile sterlet sturgeon could be 21.9mg protein/kJ diet at 45% dietary protein levels.

Table 16. Effects of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diet for 8 weeks

Parameters	Diets[1] (Protein to lipid levels, %)						
	40/10	40/15	40/20	45/10	45/15	45/20	Pooled SEM
Initial mean wt. (g)	5.48	5.48	5.48	5.48	5.48	5.48	0.04
Final mean wt. (g)	27.1^{b}	29.3^{ab}	32.6^{ab}	33.5^{a}	29.0^{ab}	29.1^{ab}	1.84
Wt. gain (%)[2]	394^{b}	434^{ab}	494^{ab}	512^{a}	430^{ab}	432^{ab}	25.1
FE (%)[3]	75.6	80.5	81.5	81.8	78.2	79.5	3.93
SGR (%)[4]	2.84^{b}	2.98^{ab}	3.17^{ab}	3.23^{a}	2.98^{ab}	2.98^{ab}	0.09
PER[5]	1.82^{a}	1.82^{a}	1.79^{a}	1.50^{b}	1.47^{b}	1.53^{b}	0.08
Survival rate (%)	82	77	88	86	88	86	4.51

[1] Refer to the table 5.
[2] Percent weight gain; (final wt. - initial wt.) × 100 / initial wt.
[3] Feed conversion ratio; (wet weight gain / dry feed intake).
[4] Specific growth rate; ($\log_e$ final wt. - $\log_e$ initial wt.) / days.
[5] Protein efficiency ratio; (wet weight gain / protein intake) × 100.

Table 17. Whole body proximate composition (%) of fingerling sterlet sturgeon, *Acipenser ruthenus* fed the six experimental diets for 8 weeks (% of as is basis)[1]

Parameters	Dietary Protein to lipid levels (%)						
	40/10	40/15	40/20	45/10	45/15	45/20	Pooled SEM[2]
Moisture(%)	77.4[a]	76.2[b]	75.8[b]	72.8[c]	76.4[b]	76.5[b]	0.36
Crude protein(%)	12.1[d]	12.7[bc]	13.0[b]	13.5[a]	12.6[c]	12.7[bc]	0.11
Crude lipid(%)	6.3[e]	7.5[bc]	7.8[b]	9.3[a]	7.0[d]	7.3[cd]	0.23
Ash(%)	3.1[a]	2.5[bc]	2.4[bc]	2.4[c]	2.6[b]	2.6[b]	0.06

[1] Refer to the table 5.
[2] Pooled standard error of mean : SD/√ n.

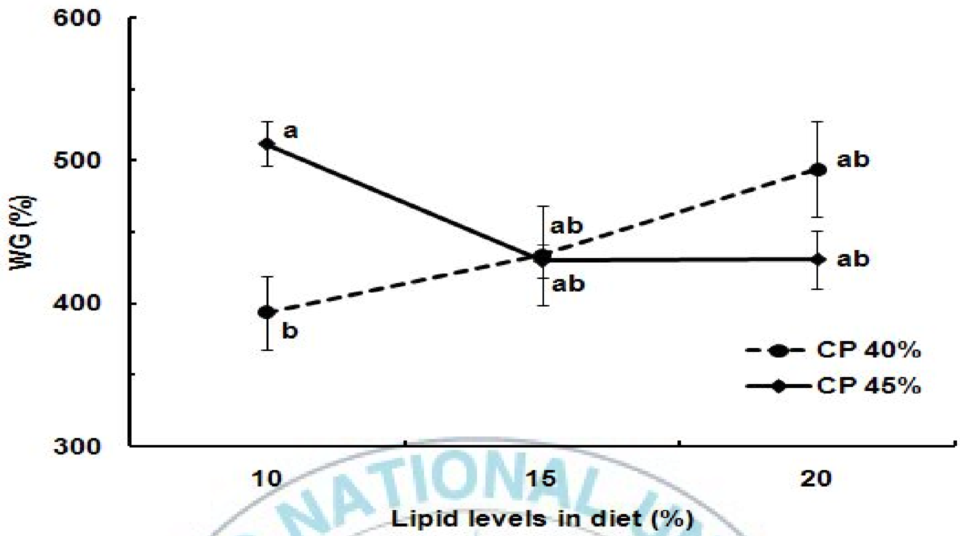

Fig.20. Average weight gain (WG, %) from fish fed six diets for 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$)

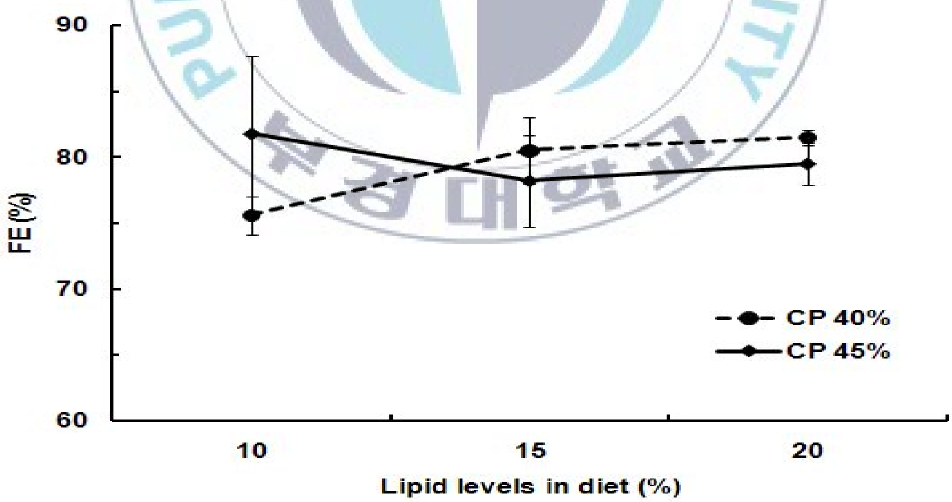

Fig.21. Average feed efficiency (FE, %) from fish fed six diets for 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different ($P < 0.05$)

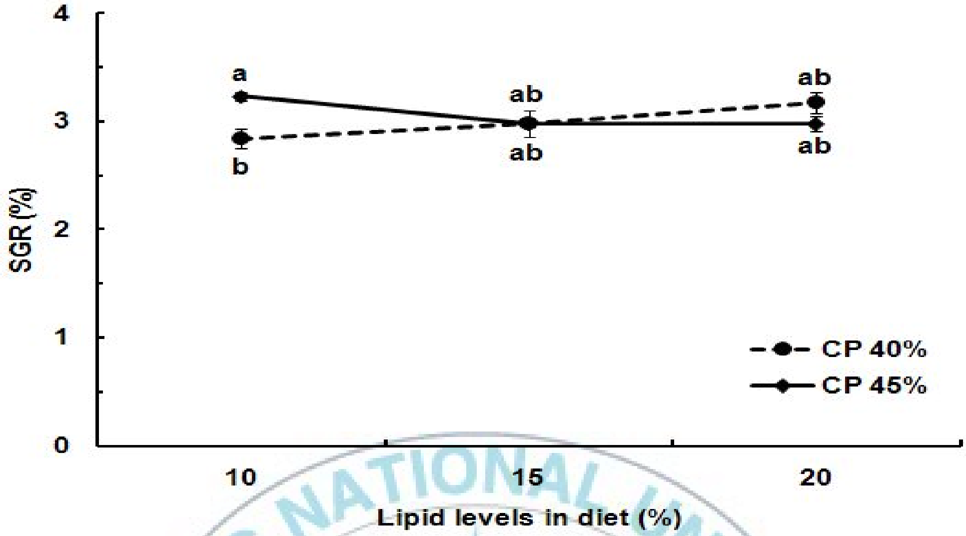

Fig.23. Average specific growth rate (SGR, %)) from fish fed six diets for 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

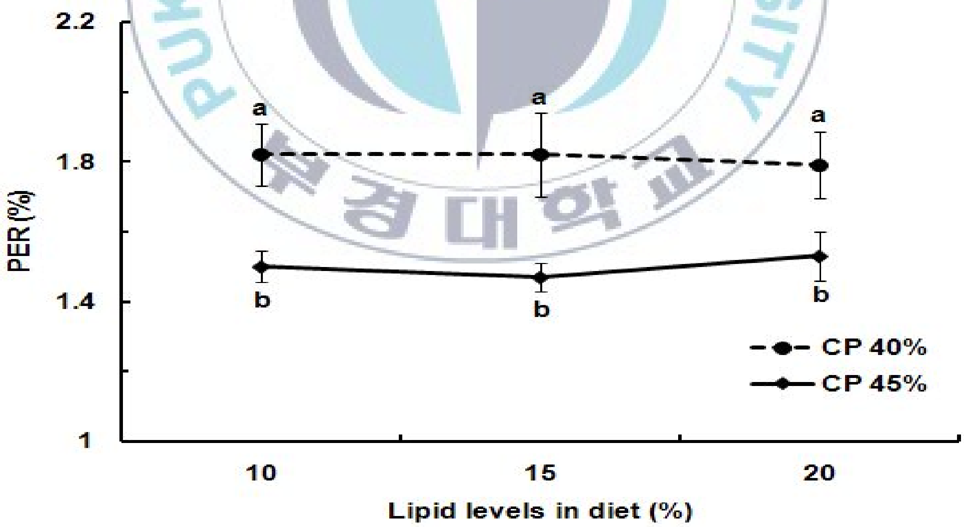

Fig.23. Average protein efficiency ratio (PER) from fish fed six diets for 8 weeks. Values are means from triplicate groups where the bar have different superscript are significantly different (P < 0.05).

4. Discussion

Dietary crude protein (CP) level of 45% which resulted from this experiment for the maximum weight gain (WG) of fingerling sterlet sturgeon estimated in this experiment was 45% CP with 21.9 mg kJ^{-1} which was higher than the value 40% CP with 18-20 mg kJ^{-1} energy reported by Mohseni et al. (2007). In this study, fish size (5.5 g) used in this study was smaller than the fish size (136.8 g) used in the study (Mohseni et al., 2007) which might have caused requirement of higher dietary CP level.

Improved growth performance with increasing dietary lipid levels at a relative lower dietary protein level has been observed in most of fish (NRC 1993). WG and specific growth rate (SGR) were improved with increasing dietary lipid levels in the present experiment with 40% CP diets. However, in 45% CP diet, WG and SGR of fish decreased with the increasing dietary lipid levels. When fish are fed a diet containing excess energy, growth may be reduced due to reduced feed consumption (Lovell 1989). On the other hand, when fish are fed a diet deficient in energy, dietary protein will be used as an energy source and this could elevate the production cost. In the present experiment, the optimum dietary protein level could be 45% with 10% lipid level (gross energy

21.9 mg protein kJ^{-1}). Kim et al., (2004) showed that the optimum dietary level in low energy diets (14.2 kJ/g diet) was higher than medium energy diets (18.6 kJ/g energy diet) and higher energy diets (20.9 kJ/g energy diets) in Korean rockfish. Furthermore, the comprehensive comparison of weight gain at 40, 45 and 50% CP with four different dietary energy levels in Korean rockfish (Kim et al., 2004) indicated weight gain of fish fed high energy diets were significantly higher than that of fish fed the low-energy diets at 40 and 45% CP levels. This phenomenon indicated that an increase in dietary energy through dietary lipid provided a more efficient utilization of dietary protein for growth. Such kind of improvement of growth performance in fish with increasing dietary energy levels has been observed in other fish such as channel catfish (Page and Andrew 1973), rainbow trout (LeGrow and Beamish 1986), tilapia (De Silva et al. 1991), salmon (Lee and Kim 2001). In the present study, WG at 40% CP was increased with increased lipid level, and WG at 45% CP was decreased with increased lipid level as indicated by one-way ANOVA test. These results showed similar tendency with the report by Lee and Kim (2001).

Body lipid content generally increased as the dietary lipid level increased at the 40% CP diets. This is in accordance with Shiau and

Lan (1996) in grouper and Bai et al. (1999) in yellow puffer. Also, the increased energy levels increased the body lipid contents. The difference in lipid content in the experimental diets (ranged from 10 to 20%) may well be the reason for the lipid variation in the fish. The increase of gross energy mainly resulted from the increase of dietary lipid levels also improved energy retention efficiency (ERE), which in accordance with previous results on sea bass (Morales and Oliva-Teles 1995; Dias et al. 1998; Peres and Oliva-Teles 1999).

As Garling and Wilson (1976) suggested, the concept of protein to energy (P/E) ratios must be restricted to diets containing adequate levels of protein and energy. Fish fed diets with approximately the same P/E ratios that differed greatly in crude protein and gross energy, were significantly different in terms of growth (Table 17).

Therefore these results indicated that the optimum dietary protein to energy ratio of fingerling sterlet sturgeon could be 21.9mg protein/kJ diet at 45% dietary protein levels.

Chapter IV. Summary and Conclusions

Three experiments were conducted to evaluate the optimum dietary protein level and the optimum protein to energy ratio in sterlet sturgeon, *Acipenser ruthenus* fed the experimental diets.

The first experiment was conducted to determine the optimum protein requirement by different analysis methods and to study the effects of dietary protein levels on growth performance and body composition in fingerling sterlet sturgeon, *Acipenser ruthenus* fed white fish meal and casein-based diets for 8 weeks. After 2 week conditioning period, one of six isocaloric diets containing 30, 40, 45, 50, 55 and 60% crude protein (CP) was fed to fish at approximately 3-5% of wet body weight on a dry matter basis to triplicate groups of 30 fish averaging 7.02 ± 0.02g (mean ± SD). After the feeding trial, average weight gain (WG) of fish fed 45 and 50% CP diets were significantly higher than those of fish fed 30, 40 and 60% CP diets ($P<0.05$), however there were no significant differences in WG among fish fed 45, 50 and 55% CP diets. Average feed efficiency (FE) of fish fed 40, 45, 50 and 55% CP diets were significantly higher than those of fish fed 30 and 60% CP diets ($P<0.05$), meanwhile there was no significant differences in FE between fish fed 30 and 60% CP diets. Average specific growth rate (SGR) of fish fed 50% CP diet was significantly higher than those of fish fed 30, 40 and 60% CP diets (*P*<0.05), but no significant differences were observed in SGR among fish fed 45, 50 and 55% CP diets. Average protein efficiency ratio and protein retention were reduced with the increasing dietary protein levels.

Based on the one-way ANOVA test, the broken-line regression analysis and the second order polynomial relation model on weight gain, the optimum dietary protein requirement level could be greater than 44.3%, but less than 45.6% in fingerling sterlet sturgeon under our experimental conditions.

The second experiment was conducted to determine the optimum protein requirement by different analysis methods and to study the effects of dietary protein levels on growth performance and body composition in juvenile sterlet sturgeon, *Acipenser ruthenus* fed casein and gelatin-based diets for 10 weeks. After 2 week conditioning period, one of six isocaloric diets containing 30, 35, 40, 45, 50 and 55% crude protein (CP) was fed to fish at approximately 4-6% of wet body weight on a dry matter basis to triplicate groups of 15 fish averaging 37.7 ± 0.1g (mean ± SD). After 10 weeks of feeding trial, average weight gain (WG) of fish fed 45% CP diets were significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (*P*<0.05), however there were no significant differences in WG among fish fed 40 and 45% CP diets. Average feed efficiency (FE) of fish fed 40 and 45% CP diets were significantly higher than those of fish fed 30% CP diets (*P*<0.05), meanwhile there was no significant differences in FE among fish fed 35, 40, 45, 50 and 55% CP diets. Average specific growth rate (SGR) of fish fed 45% CP diet was significantly higher than those of fish fed 30, 35, 50 and 55% CP diets (P<0.05), but no significant differences were observed in SGR between fish fed 40 and 45% CP diets. Average protein retention efficiency (PRE) of fish fed 30 and 35% CP diets were significantly higher than those of fish fed 40, 45, 50 and 55% CP diets (P<0.05), however there were no significant differences among fish fed 30 and 35% CP diets.

Based on the one-way ANOVA test, the broken-line regression analysis, the second order polynomial analysis on weight gain, the optimum dietary protein level could be greater than 42.7%, but less than 44.6% in juvenile sterlet sturgeon under our experimental conditions.

The third experiment was conducted to evaluate the optimum dietary protein to energy (PE) ratio in fingerling sterlet sturgeon, *Acipenser ruthenus* for 8 weeks. White fish meal, casein & soybean meal were used as protein sources. Prior to the feeding trial, fish were fed commercial diet for 2 weeks to adjust to the experimental diets and conditions. Fish averaging 5.48 ± 0.04g (mean ± SD) were distributed to each aquarium as a group of 30 fish reared in the flow through system. Fish of triplicate groups were fed one of six diets containing 40 and 45% crude protein and 10, 15 and 20% crude lipid, the PE ratio was 19.4, 18.5, 17.6, 21.9, 20.8 and 19.8kJ gross energy/g diet, respectively. Average weight gain (WG) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in WG among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets ($P>0.05$). Average feed efficiency (FE) of fish fed all of diet was no significant difference ($P>0.05$). Average specific growth rate (SGR) of fish fed 45/10 diet were significantly higher than those of fish fed 40/10 diets ($P<0.05$). However, there was no significant difference in SGR among fish fed 45/10, 45/15, 45/20, 40/15 and 40/20 diets ($P>0.05$). Average protein efficiency ratio (PER) of fish fed 40/10, 40/15 and 40/20 diets were significantly higher than those of fish fed 45/10, 45/15 and 45/20 diets ($P<0.05$). However, there was no significant difference in PER among fish fed 40/10, 40/15 and 40/20 diets ($P>0.05$).

Therefore, these results indicated that the optimum dietary protein to energy ratio in fingerling sterlet sturgeon could be 21.9mg protein/kJ diet at 45% dietary protein levels.

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