

## Hybrid sturgeon 'AL' (*Acipenser naccarii* × *Acipenser baeri*) diets: the use of alternative plant protein sources

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### Abstract

In this experimentation, corn gluten (CG) and pea meal (PM) were tested as potential protein sources in hybrid sturgeon 'AL'. One hundred and twenty-eight hybrid sturgeons 'AL' (*Acipenser naccarii* × *Acipenser baeri*) (initial body weight  $364.8 \pm 4.5$  g) were utilized with four experimental diets and four replicates each; the experimental design was  $4 \times 4$ . Four isonitrogenous [crude protein = 40% dry matter (DM)] and isoenergetic (gross energy =  $20 \text{ MJ kg}^{-1}$  DM) diets were formulated containing different levels of CG with or without PM and tested against a control diet that was fish meal (FM) based. Diets were as follows: CG55 contained 55% of corn gluten; diet CG55PM25 contained 55% CG and 25% PM; diet CG45PM25 contained 45% of corn meal and 25% of PM; and diet FM was control, based on FM. Fish fed with the PM diet showed lower values of feed conversion ratio (FCR) and specific growth rate (SGR) (FCR =  $4.53 \pm 2.51$  in the CG55PM25 diet; FCR =  $4.09 \pm 1.45$  in the CG45PM25 diet; SGR =  $0.20 \pm 0.07$  in the CG55PM25 diet; SGR =  $0.19 \pm 0.11$  in the CG55PM25 diet). The results of tissue proximate composition confirmed the results obtained from productive traits. This study indicates that CG meal but not PM could be utilized as a substitute of FM in hybrid sturgeon 'AL' nutrition.

**Keywords:** sturgeon feeding, corn gluten, pea meal, growth performances, body composition

### Introduction

Sturgeon has been traditionally farmed mainly for caviar production and has been farmed for flesh

production only in the last decade. In Italy, which is the largest European sturgeon producer, the production has expanded from 280 tonnes year<sup>-1</sup> of the 1980s to the 1350 tonnes year<sup>-1</sup> in 2007, for a global business value of 12.15 million of Euros (ISMEA 2008). In sturgeon farming, commercial non-purified rainbow trout diets and marine fish diets are often used and their results are acceptable in terms of growth and survival. However, these diets are considered to be sub-optimal because prolonged feeding with these may result in malformations and physiological disorders (Gisbert & Williot 2002). In general, carbohydrates are inexpensive fish diet ingredients and they can spare protein, and the scientific literature on sturgeon nutrition is mainly focused on the physiological effects of dietary carbohydrates (Lin, Cui, Hung & Shiao 1997; Forneris, Gai, Gasco, Lussiana, Palmegiano, Sicuro & Zoccarato 2001) or sturgeon larval nutrition (Deng, Koshio, Yokoyama, Bai, Shao, Cui & Hung 2003). These studies demonstrated that in sturgeon feed, there is the possibility of partial substitution of fish meal (FM) with plant ingredients. In this species, there are few studies on FM substitution; Médale, Blanc and Kaushik (1991) studied the utilization of non-protein energy in *Acipenser baeri* and in the early 1990s Médale, Corraze and Kaushik (1995) drew up the first review on sturgeon feeding, while Kaushik (1995) studied the efficiency of the substitution of FM protein by soybean meal on the growth performances and body composition of sturgeon. Furthermore, other authors studied *Spirulina* meal introduction in Siberian (Palmegiano, Agradi, Forneris, Gai, Gasco, Rigamonti, Sicuro & Zoccarato 2005) and white sturgeon (Palmegiano, Gai, Daprà, Gasco, Pazzaglia & Peiretti 2008) nutrition. Among plant feedstuffs, corn gluten (CG) is a promising

ingredient in fish feed due to its high nutrient content and its increased availability as a bioethanol production by-product. The CG is currently used in the aquafeed industry for salmon and some marine species (Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg, Souza, Stone, Wilson & Wurtele 2007) and it has already been tested in several fish species such as sea bass (Ballestrazzi, Lanari, D'Agaro & Mion 1994) and sea bream (Robaina, Moyano, Izquierdo, Socorro, Vergara & Montero 1997). Considering other fish species, several plant protein sources have been tested as partial substitutes of FM (Krogdahl, Bakke-McKellep & Baevefjord 2003; De Francesco, Parisi, Medale, Lupi, Kaushik & Poli 2004; De Francesco, Parisi, Pérez-Sánchez, Gómez-Réqueni, Médale, Kaushik, Mecatti & Poli 2007) but no data are available on sturgeon nutrition. Hybridization is a common practice in sturgeon rearing and, until now, seven sturgeon hybrids have been utilized in Italian aquaculture; among them, hybrid 'AL' (*Acipenser naccarii* × *A. baeri*) seems to be more promising for flesh production (Cataudella & Bronzi 2001).

The aim of this research was to evaluate CG and pea meal (PM) as sources of nutrients and potential substitutes for FM in hybrid sturgeon 'AL' diets.

## Material and methods

### Fish and rearing conditions

A stock of 128 hybrid sturgeons 'AL' (*Acipenser naccarii* × *A. baeri*) (initial weight  $364.8 \pm 4.5$  g) were kept in 16 fibreglass tanks (1 × 1 × 0.4 m) and fed by four isoproteic and isoenergetic diets (Table 1) with four replicates (4 × 4). Sturgeons were acclimated to the experimental tanks for 1 week before the commencement of the growth trial. Water temperature was artificially maintained constant by a heating system at  $20.3 \pm 1$  °C, dissolved oxygen  $4.8 \pm 0.3$  mg L<sup>-1</sup> and water flow rate of 3 L min<sup>-1</sup>. Fish were bulk weighed fortnightly in order to adjust the feeding rate and individually at the end of the experiment. The experimentation lasted 60 feeding days and fish were fed twice a day by hand 7 days week<sup>-1</sup> (feeding ratio 2% of body weight).

### Diets

Four isonitrogenous [crude protein, 40% dry matter (DM)] and isoenergetic (20 MJ kg<sup>-1</sup> DM) diets were formulated to contain one of the following as the

**Table 1** Ingredients and proximate composition of the experimental diets

	FM	CG55	CG55PM25	CG45PM25
Ingredients (g/100 g <sup>-1</sup> )				
Herring meal	54.0	8.0	2.0	6.0
Corn gluten meal	3.5	55.0	55.0	45.0
Pea meal	–	–	25.0	25.0
Merigel	18.0	10.0	5.0	5.0
Tosted rice	7.5	–	–	–
Wheat bran	3.5	–	–	–
Corn oil	7.5	8.0	6.0	6.0
Corn starch	–	10.0	2.0	5.0
Brewer's yeast	–	3.0	2.0	2.0
Mineral mix*	3.0	3.0	1.0	3.0
Vitamin mix†	2.0	2.0	1.0	2.0
Lignum sulphyte	1.0	1.0	1.0	1.0
Proximate composition (% DM)				
Dry matter %	92.3	92.6	92.6	92.5
Crude protein	42.9	40.7	40.5	40.6
Ether extract	12.8	8.1	5.0	6.1
Ash	10.8	4.4	4.3	5.0
Gross energy (MJ kg <sup>-1</sup> DM)‡	20.6	20.6	19.9	20.1

\*Vitamin mixture (IU or mg kg<sup>-1</sup> diet): DL- $\alpha$  tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15 000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B<sub>12</sub>, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (Granda Zootecnica, Cuneo, Italy).

†Mineral mixture (g or mg kg<sup>-1</sup> diet): bicalcium phosphate 500 g, calcium carbonate 215 g, sodium salt 40 g, potassium chloride 90 g, magnesium chloride 124 g, magnesium carbonate 124 g, iron sulphate 20 g, zinc sulphate 4 g, copper sulphate 3 g, potassium iodide 4 mg, cobalt sulphate 20 mg, manganese sulphate 3 g, sodium fluoride 1 g (Granda Zootecnica).

‡Calculated values.

major protein source: FM, CG and PM, with different inclusion levels.

Diets formulation and proximate composition were analysed according to standard methods (AOAC 1995) (Table 1).

### Sampling and chemical analyses

At the end of the feeding trial, the fish were starved for 1 day, and then the fish tanks were weighed for the final mean body weight. Productive traits and morphometric indexes were calculated as follows:

$$\begin{aligned} \text{WG (weight gain, g)} \\ &= [\text{FBW (final body weight, g)} \\ &\quad - \text{IBW (initial body weight, g)}] \end{aligned}$$

$$\text{SGR (specific growth rate, \%)} = \frac{[(\ln \text{FBW} - \ln \text{IBW}) / \text{number of feeding days}] \times 100$$

$$\text{FCR (feed conversion ratio)} = \frac{[\text{Total feed supplied (g DM)} / \text{WG (weight gain, g)}]$$

$$\text{PER (protein efficiency ratio)} = \frac{[\text{WG (weight gain, g)} / \text{total protein fed (g DM)}]$$

$$\text{TGC (Thermal growth coefficient)} = \frac{(\text{Final body weight}^{(1/3)} - \text{initial body weight}^{(1/3)}) / \sum \text{temperature} \times \text{day}}$$

$$\text{HSI} = \text{Liver weight} / \text{total body weight};$$

$$\text{VSI} = \text{Viscera weight} / \text{total body weight}$$

Samples of flesh, skin, liver and viscera tissues were obtained following anatomic descriptions reported by Daprà, Gai, Palmegiano, Sicuro, Falzone, Cabiale and Galloni (2009). All tissue samples were freeze dried before proximate composition analysis and gross energy was calculated.

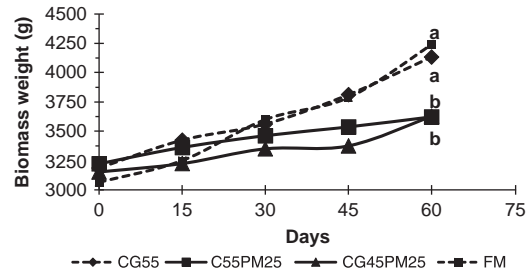
**Statistical analysis**

Data are presented as the mean, standard deviation and coefficient of variation. Before using parametric statistics, Bartlett’s test was utilized in order to test homogeneity of variance and to verify the applicability of analysis of variance (ANOVA). For inferential statistics, we used one-way ANOVA and the Kruskal–Wallis test (non-parametric ANOVA); non-parametric Tukey-type multiple comparisons were also carried out using Nemenyi’s test (Zar 1996). Statistical analyses were performed using the statistical package STATGRAPHICS by Manugistic International (1993).

**Results**

**Productive traits**

Fish meal and CG55 showed better productive indexes while the PM inclusion affected the fish growth (Fig. 1) and productive traits negatively. Particularly, lower protein efficiency ratio (PER) and specific growth rate (SGR) values were recorded in PM diets (Table 2a). A similar negative trend was found for FCR in the PM diets. In order to correctly consider



\* Different superscript letters indicates significantly different values (P < 0.05)

**Figure 1** Biomass trend in the experimental groups (FM, fish meal diet; CG55, 55% corn gluten diet; CG55PM25, 55% corn gluten and 25% pea meal diet; CG45PM25, 45% of corn meal and 25% of pea meal diet).

**Table 2a** Productive traits and somatic indexes (mean ± SD, n = 4)

	FM	CG55	CG55PM25	CG45PM25
SGR	0.43 ± 0.02 <sup>b</sup>	0.54 ± 0.04 <sup>a</sup>	0.20 ± 0.07 <sup>b</sup>	0.19 ± 0.11 <sup>b</sup>
FCR	1.64 ± 0.14 <sup>ab</sup>	1.30 ± 0.03 <sup>b</sup>	4.53 ± 2.51 <sup>a</sup>	4.09 ± 1.45 <sup>ab</sup>
PER	1.81 ± 0.05 <sup>a</sup>	1.50 ± 0.14 <sup>a</sup>	0.69 ± 0.22 <sup>b</sup>	0.66 ± 0.36 <sup>b</sup>
TGC	0.22 ± 0.01 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	0.07 ± 0.03 <sup>b</sup>	0.08 ± 0.04 <sup>b</sup>
VSI*	6.61 ± 1.60	6.60 ± 0.56	5.26 ± 2.10	5.01 ± 1.07
HSI*	1.83 ± 0.64	2.25 ± 0.23	1.42 ± 1.21	1.51 ± 0.37

Values in the same row with different superscript letters are significantly different (P < 0.05).

\*n = 12.

SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; TGC, thermal growth coefficient; HSI, hepatosomatic index; VSI, viscerosomatic index; FM, fish meal.

the effect of water temperature on fish growth, the thermal growth coefficient (TGC) index was calculated following Cho, Slinger and Bayley (1982). The TGC trend overlapped the SGR trend, thus confirming that small temperature differences among tanks did not interfere with the productive traits. Productive indexes indicate that the among tested plant proteins, CG is more efficaciously used by hybrid sturgeon ‘AL’. In terms of somatic indexes, fish fed PM diets shows lower values of VSI and HSI (Table 2a). Considering that the above-mentioned difference emerged from productive parameters, a successive step has been considered and productive parameters were grouped into two groups on the basis of PM presence in the diet. Before the use of ANOVA for data statistical elaboration, the variance homogeneity was tested using Bartlett’s test (for unbalanced data); there was no homogeneity of variances for

FCR and homogeneity ( $P < 0.05$ ) for other indexes (PER, SGR and TGC). Therefore, the Kruskal–Wallis test was used for FCR data elaboration. The analysis of these data confirmed the negative effect of PM in hybrid sturgeon 'AL' feeding. All the productive parameters showed a statistically significant difference between two groups (Table 2b).

### Fish body composition

Considering the fish body composition, fish fed with CG and PM diets showed a significant decrease in the viscera crude protein content, whereas muscle, liver and skin crude protein contents were not affected by the experimental diets (Table 3a). As far as the fat tissue content is concerned, higher fat deposition was found in fish fed FM and CG55 diets; this trend is clear in the liver even if there are not statistically significant differences. Moreover, considering the plant meal-based groups, it is possible to see a less fat deposition in the muscle and liver of fish fed with PM. Observing the tissue gross energy contents, a statistical increase was observed in the liver and viscera of fish fed FM and CG55 diets, while skin gross energy content was not affected by the dietary treatments. In all the tissues considered, ash content was not affected by the diet; the high values found in sturgeon skin were due to an anatomical feature of this fish in that it is larger than other fish. Similar to productive traits, in a successive step of statistical elaboration, the data were merged on the basis of the presence or absence of PM in the diet (Table 3b). According to this grouping, it is interesting to notice that fish fed with PM diets showed a lower content of fat in all the considered tissues, except the skin. In particular, considering the liver, strong differences in fat storage appeared between the two groups.

### Discussion

#### Performances

This experimentation shows that in the diets of hybrid sturgeon 'AL' CG protein can substitute FM up to 85% of total protein, without affecting productive traits. This is interesting, considering that CG and wheat gluten are largely present in the modern aqua-feed industry in other species nutrition (Robaina *et al.* 1997). The high substitution utilized provides a clear indication of CG in comparison with other plant proteins as PM. These results with PM are in agree-

**Table 2b** Productive traits (mean  $\pm$  SD)

	Pea meal	No pea meal
SGR	0.21 $\pm$ 0.07 <sup>a</sup>	0.62 $\pm$ 0.16 <sup>b</sup>
FCR	5.35 $\pm$ 1.71 <sup>a</sup>	1.98 $\pm$ 0.29 <sup>b</sup>
PER	0.50 $\pm$ 0.15 <sup>a</sup>	1.20 $\pm$ 0.16 <sup>b</sup>

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

FCR, feed conversion ratio; FCR, feed conversion ratio; PER, protein efficiency ratio.

**Table 3a** Tissues proximate composition and gross energy (MJ kg<sup>-1</sup> wet weight) content on a wet weight basis (mean  $\pm$  SD,  $n = 12$ )

	FM	CG55	CG55PM25	CG45PM25
<b>Muscle</b>				
Crude protein	13.9 $\pm$ 1.3	15.4 $\pm$ 0.6	15.4 $\pm$ 1.0	14.2 $\pm$ 3.8
Ether extract	3.1 $\pm$ 1.0	2.5 $\pm$ 1.2	1.5 $\pm$ 0.5	1.9 $\pm$ 0.7
Ash	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.9 $\pm$ 0.3	0.8 $\pm$ 0.3
Gross energy	4.9 $\pm$ 0.2 <sup>a</sup>	4.6 $\pm$ 0.3 <sup>a</sup>	4.5 $\pm$ 0.1 <sup>a</sup>	4.3 $\pm$ 0.1 <sup>b</sup>
<b>Liver</b>				
Crude protein	8.3 $\pm$ 1.5	8.5 $\pm$ 0.9	10.3 $\pm$ 3.1	12.3 $\pm$ 5.4
Ether extract	16.8 $\pm$ 6.2	19.7 $\pm$ 2.2	9.9 $\pm$ 5.2	8.7 $\pm$ 5.2
Ash	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.9 $\pm$ 0.3	1.0 $\pm$ 0.5
Gross energy	11.1 $\pm$ 1.4 <sup>a</sup>	11.5 $\pm$ 0.4 <sup>a</sup>	7.7 $\pm$ 1.1 <sup>b</sup>	7.4 $\pm$ 1.0 <sup>b</sup>
<b>Viscera</b>				
Crude protein	19.0 $\pm$ 5.4 <sup>a</sup>	16.9 $\pm$ 1.8 <sup>b</sup>	17.2 $\pm$ 0.8 <sup>b</sup>	16.3 $\pm$ 0.8 <sup>b</sup>
Ether extract	1.3 $\pm$ 0.3	2.6 $\pm$ 0.6	1.3 $\pm$ 0.3	1.0 $\pm$ 0.2
Ash	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1
Gross energy	5.0 $\pm$ 0.1 <sup>a</sup>	5.0 $\pm$ 0.1 <sup>a</sup>	4.5 $\pm$ 0.1 <sup>b</sup>	4.3 $\pm$ 0.1 <sup>b</sup>
<b>Skin</b>				
Crude protein	20.9 $\pm$ 1.6	19.3 $\pm$ 1.1	20.4 $\pm$ 2.8	17.1 $\pm$ 3.0
Ether extract	3.8 $\pm$ 2.0	4.8 $\pm$ 1.8	4.4 $\pm$ 2.2	4.5 $\pm$ 1.5
Ash	4.9 $\pm$ 0.8	4.3 $\pm$ 1.1	4.8 $\pm$ 1.0	5.1 $\pm$ 1.0
Gross energy	6.6 $\pm$ 0.6	6.6 $\pm$ 0.5	6.7 $\pm$ 0.5	6.3 $\pm$ 0.4

In the row, different superscript letters indicate inter-diet statistical differences ( $P < 0.05$ ).

ment with several others obtained with turbot, rainbow trout and Australian eel (Burel, Boujard, Kaushik, Boeuf, Van Der Geyten, Mol, Kühn, Quinsac, Krouti & Ribailleur 2000; Engin & Carter 2002), but are in contrast with other experiments in tilapia and milkfish (Santiago & Lovell 1988; Borlongan, Eusebio & Welsh 2002). This result is probably related to the different physiology of sturgeon (primitive fish) compared with other farmed finfish or from different strains of pea utilized in far eastern countries. Others studies show a low utilization of CG in yellowtail and gilthead sea bream, where the maximum levels of CG inclusion were 10% and 20% (Robaina *et al.* 1997), while other works recommended gluten levels from 20–25% up to 40% in diets for rainbow trout. These

**Table 3b** Tissues proximate composition on a wet weight basis; comparison of pea meal diets vs. no pea meal diets (mean  $\pm$  SD)

	Crude protein	Ether extract	Ash
<b>Muscle</b>			
Pea meal	14.8 $\pm$ 1.0	1.7 $\pm$ 0.6 <sup>b</sup>	0.9 $\pm$ 0.1
No pea meal	14.6 $\pm$ 1.3	2.8 $\pm$ 1.1 <sup>a</sup>	1.1 $\pm$ 0.1
<b>Liver</b>			
Pea meal	11.5 $\pm$ 3.9	9.4 $\pm$ 4.8 <sup>b</sup>	0.9 $\pm$ 0.1
No pea meal	8.4 $\pm$ 1.2	18.2 $\pm$ 4.5 <sup>a</sup>	0.8 $\pm$ 0.1
<b>Viscera</b>			
Pea meal	16.7 $\pm$ 0.9	1.2 $\pm$ 0.3 <sup>b</sup>	0.8 $\pm$ 0.1
No pea meal	17.9 $\pm$ 1.8	1.9 $\pm$ 0.8 <sup>a</sup>	0.9 $\pm$ 0.1
<b>Skin</b>			
Pea meal	18.7 $\pm$ 3.2	4.5 $\pm$ 1.7	4.9 $\pm$ 0.9
No pea meal	20.1 $\pm$ 1.5	4.3 $\pm$ 1.8	4.6 $\pm$ 0.9

In the column, different superscript letters indicate inter-diet statistical differences ( $P < 0.05$ ).  
FM, fish meal.

differences in CG utilization recorded among different fish species are not clear, but they could be related to anatomical and physiological differences in the digestive system. In this experimentation, the fish feed that was CG based was more economically convenient with respect to the FM-based diet; the estimated cost was more than 20% less expensive. Significant differences were obtained between sturgeons fed with or without PM and these results confirmed the low efficiency of PM feedstuffs for sturgeon nutrition. All the indexes are approximately one-third worst in the case of PM utilization; this could be caused by the presence of pea anti-nutritional factors and/or low protein quality.

### Body composition

The proximate composition of different tissues was primarily considered in order to investigate the protein and fat deposition in principal organs and secondarily to investigate the main effects of fish nutrition on the quality of the final product in the sturgeon. The liver and skin are the more interested organs in fat storage, whereas the muscle composition did not affected by sturgeon nutrition, this is good advantage for consumers. Similar contents of liver and muscle fat were recorded in blackspot seabream (*Pagellus bogaraveo*) fed different protein/lipid levels (Figueiredo-Silva, Corraze, Borges & Valente 2010), whereas lower levels of muscle fat were recorded in meagre (*Argyrosomus regius*) juveniles fed different dietary lipid levels

(Chatzifotis, Panagiotidou, Papaioannou, Pavlidis, Nengas & Mylonas 2010).

In general, this experiment showed that CG can be used as a partial substitute of FM in artificial feeding of hybrid sturgeon 'AL', while PM is not efficaciously utilized. As expected, CG shows an inferior result compared with FM, but it is more economically feasible (the price is 30% less than a commercial diet).

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