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Evaluation of Yeast Biomass (*Candida utilis*) in a Practical Diet for Rainbow Trout (*Oncorhynchus mykiss*)

J.S. Goddard*[1], A.M. Martin[2] and G. Deveau[1]

[1] *School of Fisheries, Fisheries and Marine Institute, Memorial University of Newfoundland, St. John's Newfoundland, Canada A1C 5R3*
[2] *Department of Biochemistry, Memorial University of Newfoundland, St. John's Newfoundland, Canada A1B 3X9*

تقييم الكتلة الحيوية لخميرة كانديدا يوتيليس (*Candida utilis*) المستخدمة في عليقة العملية لأسماك التراوت القزحية (*Oncorhynchus mykiss*)

المخلص: استخدمت الخميرة كانديدا يوتيليس (*Candida utilis*) المستزرعة في وسط يتكون من الطحالب المتخمرة وبقايا الأسماك المصنعة لتحل محل مسحوق الأسماك المستخدمة في عليقة أسماك التراوت القزحية (*Oncorhynchus mykiss*). كونت العليقة من مواد نيتروجينية متجانسة (٤٠ % بروتين خام) ومواد ذات طاقة متجانسة (٢٠ كيلوجول/جرام من المادة الجافة). أثبتت التجارب والتي استمرت لمدة ٥٠ يوما تضاعف وزن الأسماك ثلاث مرات مع عدم وجود اختلافات معنوية في معدل أوزان الأسماك النهائية التي تغذت على علائق تحتوي على صفر و٢٥% و ٣٥% من الخميرة. تبين أن العليقة التي احتوت على الخميرة كانت مستساغة لدى الأسماك، واتضح ذلك من الكميات المستهلكة، كما أنها كانت سهلة الهضم. دلت التحاليل الخاصة بالجسم الحي على أن الأسماك التي تغذت على الخميرة احتوت على نسبة أعلى من البروتين الخام الرماد ونسبة أقل من الدهون عند مقارنتها بالتجربة الحاكمة. كما تم رصد الانخفاض المعنوي في معدل كفاءة تحول الغذاء مع ازدياد نسبة الخميرة في العليقة. أوضحت الدراسة إمكانية استبدال ما نسبته ٢٥ – ٣٠% من كسب السمك في عليقة أسماك التراوت بالخميرة.

ABSTRACT: A yeast, *Candida utilis*, cultured on a substrate derived from a mixture of peat moss and fish processing waste, was substituted for fish meal in a practical diet for rainbow trout, *Oncorhynchus mykiss*. The formulated diets were isonitrogenous (40% crude protein) and isocaloric (gross energy 20 kJ per g dry matter). During a 50-day feeding trial fish tripled in weight, and there were no significant differences in the mean final weights of groups of fish fed diets in which 0%, 25% and 35% of fishmeal had been replaced by yeast biomass. Diets containing yeast were palatable, as determined by food intake, and were highly digestible for protein. Carcass analysis revealed that the fish fed with yeast biomass had slightly higher crude protein and ash contents, and lower lipid levels than those of the control group. Significant reductions were recorded in food conversion efficiency as the yeast content of the diets increased. The results indicate the potential for partial replacement of fish meal (between 25-35%) by *Candida utilis* biomass in feeds formulated for rainbow trout.

High quality fish meals supply the major portion of protein (40-60% by weight) in commercial diets for salmonids (Goddard, 1996). In 1996, an estimated 750,000 tonnes of fish meal were used in the global farm production of trout and salmon (Tacon, 1998). In view of the increasing cost of fish meal, and instability in long term supply, a range of alternative sources of protein have been evaluated. The use of single cell proteins (S.C.P.), a term applied to a wide range of algae, fungi (including yeasts) and bacteria, has attracted considerable interest. These micro-organisms, produced by fermentation processes, offer a number of advantages as protein producers. The majority are highly proteinaceous with an amino-acid content comparable with that of fish meal (Spinelli *et al.*, 1979; Table 1). They have short generation times (e.g., 1-3 hour for yeasts) and their production can be based on raw carbon substrates, such as petroleum or natural

*Corresponding Author.
Department of Fisheries Science and Technology
College of Agriculture, Sultan Qaboos University
P.O. Box 34, Al Khod 123 - Oman

gas, or on organic waste products (Tacon and Jackson, 1985).

Diets containing yeasts, partially substituted for fish meal, have been tested most extensively in the salmonids, particularly trout (Iida *et al.*, 1970; Andruetto *et al.*, 1973; Shima and Nakada, 1974a,b; Gropp *et al.*, 1976; Shimma *et al.*, 1976; Matty and Smith 1978; Beck *et al.*, 1979; Mahnken *et al.*, 1980; Higuera *et al.*, 1981; Murray and Marchant, 1986; Rumsey and Hughes 1990; Goddard and Martin, 1993). Diets containing single cell proteins fed to tilapia (Appler and Jauncey, 1983), yeast and bacterial proteins fed to common carp (Atack *et al.*, 1979) and yeast protein fed to Atlantic salmon (Bergstrom, 1979).

The majority of experiments conducted with yeasts as replacement ingredients for fish meal in practical diets for various fish species have centered on the use of the alkane/petrochemical yeast, *Candida lipolytica* (Tacon and Jackson, 1985). Inclusion of yeasts in salmonid diets has given satisfactory results at levels between 15%-30% of the total diet, replacing between 25%-50% of the fish meal component (Beck *et al.*, 1979; Spinelli *et al.*, 1979; Mahnken *et al.*, 1980; Ramsey and Hughes, 1990). Attempts to use yeasts as the sole protein source in salmonid diets have resulted in both reduced weight gain and feed conversion efficiency, compared to fish meal protein (Nose, 1973a,b,; Arai *et al.*, 1975; Matty and Smith, 1978; Beck *et al.*, 1979; Murray and Marchant, 1986). Poor performance has been attributed to deficiencies in certain amino acids and the presence of high levels of nucleic acids (5-12% by weight for yeasts), which appear to be of limited nutritional value in monogastric animals, including fish (Schultz and Oslage, 1976); Tacon and Jackson, 1985).

The purpose of this study was to determine the potential of a *Candida utilis* yeast biomass, grown on a low-cost substrate derived from a fish processing waste and peat mixture, as an ingredient in a practical diet for rainbow trout. Fish processing waste is an abundant resource, accounting for an estimated 64% of the landed weight of fish used in the preparation of filleted and canned products (Nair and Gopakumar, 1982). *Candida utilis* was chosen because of its favourable biomass composition and its ability to metabolize a wide range of monosaccharides (Shay and Wegner, 1985).

TABLE 1

Comparison of the amino acid profile of Candida utilis biomass with herring meal and the essential amino acid (EAA) requirements of rainbow trout, Oncorhynchus mykiss

Amino Acid	Candida utilis[1] (g/100g protein)	Herring meal[2] (g/100g protein)	EAA Requirements of Rainbow trout[3] (g/100g protein)
Arginine	5.1	4.5	3.5
Histidine	2.4	1.6	1.2.
Isoleucine	4.5	3.1	2.4
Leucine	5.3	5.2	4.4
Lysine	6.7	5.6	5.3
Methionine	1.1	2.1	1.8
Phenylalanine	3.8	2.7	3.1
Threonine	4.6	2.9	3.4
Tryptophan	ND	0.8	0.5
Valine	5.8	4.3	3.0

[1] Martin *et al.* (1993)
[2] NRC (1993)
[3] Ogino (1980)
ND - Not determined

Materials and Methods

PRODUCTION OF YEAST BIOMASS: *Candida utilis* (Henneberg), ATCC 9950, obtained from the American Type Culture Collection, Rockville, MD, USA, was incubated, stored and grown as described by Martin *et. al.* (1993). The yeast biomass was produced in a 14 lt fermenter in 9 lt of nutrient-supplemented fish offal-peat compost extract to which inoculant culture was added. The fish offal-peat compost was hydrolyzed and an extract prepared following the procedure reported by Martin and Chintalapati (1989).

During fermentation, mechanical agitation and sparging with air were used and sterile technique was observed throughout. The fermentation was ended after 24 hours and the yeast cells separated from the medium by filtration using continuous centrifugation. The yeast paste from this operation was dried and powdered, and stored in a freezer until use (Martin *et al.*, 1993). The amino acid content of the biomass is shown in Table 1.

DIETS: Three diets containing 40% crude protein (N x 6.25) were formulated in which the yeast biomass replaced fish meal at 0%, 25% and 35% of the total dietary nitrogen. The diet formulations are listed in Table 2, and are based on formula C202 (Ontario Ministry of Natural Resources, Canada). Chromic oxide (1%) was added to sub-samples of each dietary mix for the determination of apparent protein digestibility after the method of Austreng and Refstie (1979). The diets were blended using a Hobart bench food mixer, pelleted by wet extrusion through a 4 mm die, then dried and stored at -20°C.

ANALYTICAL TECHNIQUES : Crude protein (N x 6.25), total lipid, moisture, fibre and ash were determined in the feed ingredients, and in the formulated feeds, according to standard AOAC methods (AOAC, 1980). Amino acid composition analysis was performed on the yeast biomass in a Beckman 121 MB amino acid analyzer using Benson D-XB.25 resin and a single

TABLE 2

Diet formulation and composition (g/100g diet)

	% Yeast		
	0	25	35
Ingredient			
Herring meal	30.0	22.5	19.5
Candida utilis	0.0	14.3	20.0
Soybean meal	17.0	17.0	17.0
Corn gluten	13.9	13.0	13.0
Wheat middlings	24.0	16.5	13.3
Vitamin mix[1]	2.0	2.0	2.0
Mineral mix[2]	1.0	1.0	1.0
Guar gum	1.0	1.0	1.0
Herring oil	12.0	12.8	13.3
	% (dry matter basis)		
Composition			
Protein	40.7	40.2	40.5
Lipid	16.9	17.6	17.1
Moisture	12.9	11.0	11.3
Fibre	2.5	2.4	2.2
Ash	5.2	6.9	7.6
Energy (kJ/g)	20.04	20.24	19.97

[1] Vitamin premix supplying mg/kg (or IU/kg) dry diet: Vitamin A 2500 IU; Vitamin D 1600 IU; Vitamin E 30; Vitamin K 10; Vitamin B_{12} 1.0; Biotin 1.5; Folic acid 10; Pantothenic acid 60; Pyridoxine 16; Niacin 200; Riboflavin 20; Thiamine 8; Ascorbic acid 200; Choline 3000.

[2] Mineral premix supplying mg/kg dry diet: Iron 60; Copper 5; Zinc 30; Manganese 30; Iodine 0.5; Cobalt 3; Calcium 3000; Phosphorous 6000; Magnesium 500.

column, three buffer method (Blackburn, 1968; Mondino *et al.*, 1972; Ohara and Ariyoshi, 1979). The gross energy values were determined using an adiabatic bomb calorimeter (Model 1241, Parr Instrument Co., Moline, IL, USA). The chromic oxide content of diets and faeces was determined by the wet oxidation method of Furukawa and Tsukahara (1966).

EXPERIMENTAL PROCEDURES: The test diets were each fed to triplicate groups of 10 juvenile trout (initial mean weight 18.4 g ± 0.55) for 50 days. The trout were obtained from a local hatchery and held in the laboratory for 4 weeks prior to the start of the feeding trial. The trout were held in 125 lt rectangular, glass fronted tanks, supplied with water from a partial recycle system. Each tank was individually aerated, with a water flow rate of 5-6 lt min^{-1}. Recycled water was settled, passed through a physical filter of 3-5 mm quartzite and through a biological filter containing plastic ring bio-filter medium. The water then passed through a further tank, which contained a cooling/aeration unit prior to returning, *via* a header tank to the tank supply. The water temperature was maintained at 10.0 ± 0.5°C, pH 7.4-7.5, and dissolved oxygen at or near full saturation. The system was also supplied with fresh water from an artesian well. The trout were fed to satiation twice a day and records kept of total food consumed for each tank. They were individually weighed and their length measured at the beginning and end of the feeding trial. On the first day of the experiment, 10 fish, representative of the stock were selected at random, starved for 48 hours, killed and frozen at -20°C for subsequent gross carcass analysis. At the end of the experiment, 5 fish from each treatment were similarly sampled. Prior to analysis, the whole fish samples pooled from each treatment were thawed and minced through a 4 mm die. Standard methods were used to determine the initial and final whole body composition (AOAC, 1980).

Diets containing chromic oxide were fed to each treatment group for 5 days prior to the collection of faecal samples. After feeding on the sixth day the tanks were siphoned clean of all food and faeces. Faeces were then siphoned from each tank at 15 minute intervals. The faeces were collected on a fine mesh, transferred to petri dishes, and oven dried at 60°C for 24 hours. The faeces from each treatment were pooled, then finely ground and stored at -20°C for subsequent analysis.

CALCULATIONS AND STATISTICS: Apparent protein digestibility (APD) was calculated as follows: APD (%) = 100 x (a - b)/ a, where a = protein in feed/chromic oxide in feed, and b = protein in faeces/chromic oxide in faeces (Austreng and Refstie, 1979) . Food conversion efficiency (FCE) and specific growth rate (SGR) were calculated as follows: FCE = total wet weight gain x 100/total diet fed. SGR = [(ln w_t - ln w_i)/T] x 100, where w_t is the weight of the fish after time T, w_i is the initial weight and T is the feeding period in days. Condition factor (K) was calculated using the formula: K = 100 x weight (g)/total length^3 (cm) (Goddard, 1996).

Data were subjected to analysis of variance (SAS Institute Inc. 1985) to determine if responses varied significantly ($P < 0.05$). Duncan's multiple range test (Duncan 1955) was then used to identify where significant differences occurred among means.

Results

YEAST BIOMASS: The yeast biomass produced was a light brown powder with a sweet odour. It had the following proximate composition (%): protein (N x 6.25) 50.12 ± 2.46; lipid 1.31% ± 0.23; ash 18.17 ± 2.09; moisture 8.29 ± 0.02 (n=3, ± SD) (Martin

et al., 1993). The profile of amino acids for *Candida utilis* compared favourably with that of herring meal. With the exception of methionine, it met the essential amino acid requirements as determined for rainbow trout (Table 1).

GROWTH STUDY: Over a 50-day feeding trial the fish tripled in weight (Table 3). There were no significant differences between the mean final weights of the control groups and those fed diets containing yeast biomass. Specific growth rate (SGR) exceeded 2% body weight day^{-1} in all groups, although a decreased SGR was evident for the groups fed diets containing yeast, compared with that of the control group (Table 3). Condition factors increased for each group during the course of the experiment. The final condition factors did not differ significantly however between the treatment groups (Table 4).

FEED UTILIZATION: Food conversion efficiency was calculated on a dry food to wet flesh weight basis. Decreased food conversion efficiency was evident in the groups fed diets containing yeast compared with the controls (Table 3).

APPARENT PROTEIN DIGESTIBILITY: Apparent protein digestibility was determined from a single experiment in which faecal samples for each treatment group were pooled. Apparent protein digestibility values were high for both control and treated groups (Table 3). The highest apparent protein digestibility (88.3%) was recorded from the group fed a diet in which 35% of the fish meal component of the diet was replaced by yeast biomass.

TABLE 3

Growth and food utilization by rainbow trout following a 50-day feeding trial[1].

	% Yeast		
	0	25	35
Mean initial weight (g)	17.15	19.74	18.34
Mean final weight (g)	52.70	55.54	50.54
Survival (%)	100.00	100.00	100.00
Food intake (g/day)	0.77	0.83	0.80
Specific growth rate	2.25^{a}	2.07^{b}	2.03^{b}
Food conversion efficiency	92.57^{a}	86.16^{b}	80.60^{c}
Apparent protein digestibility (%)[2]	82.60	83.20	88.30

[1] Values within a row with different superscripts are significantly different ($P < 0.05$).

[2] Mean values determined from pooled samples

CARCASS COMPOSITION: Gross carcass composition of whole trout fingerlings at the beginning and end of the experiment is shown in Table 4. Significantly higher lipid levels were recorded from fish fed the control diet than those fed diets containing yeast biomass. Higher ash levels were recorded from fish fed diets containing yeast biomass than from fish in the control groups.

Discussion

The results show that up to 35% of the fish meal in a practical diet for rainbow trout could be replaced by *Candida utilis* without a significant reduction in overall growth. There was however some evidence of reduced feed utilisation in groups of trout fed diets containing yeast. The reductions in SGR and food conversion efficiency with increased yeast content, indicate a reduced nutritional value for yeast versus fish meal. Previous studies on rainbow trout, using different species of yeasts grown on petro-chemical substrates, have also demonstrated partial success in fish meal replacement. Replacement between 25-40% of the fish meal component (equivalent to a dietary yeast SCP inclusion of 15-25% by weight) has been reported to result in no loss of growth, or food conversion efficiency in rainbow trout (Gropp *et al.*,1976; Mahnken *et al.*,1980; Spinelli *et al.*,1979; Rumsey and Hughes, 1990). In feeding trials using yeasts at higher inclusion levels, or as sole protein sources, reduced growth and conversion efficiency have been reported (Nose,1974a,b; Beck *et al.*,1979; Murray and Marchant, 1986). Supplementation with crystalline amino acids has been examined. Supplementation of petroleum yeasts with cystine and arginine (Nose,1974a,b), methionine and arginine (Beck *et al.*,1979) and methionine (Murray and Marchant, 1986) has been shown to enhance growth in rainbow trout. In

TABLE 4

Carcass composition (wet weight basis) and condition indices of rainbow trout fed diets containing (Candida utilis). Data are means of two replicates[1].

		% Yeast		
Carcass composition	Initial	0	25	35
Moisture	73.50	71.32	72.04	71.83
Protein	14.63	14.51	14.81	14.95
Lipid	9.66^{a}	12.61^{b}	11.61^{b}	11.76^{b}
Ash	2.17^{a}	2.11^{b}	2.22^{c}	2.21^{c}
Condition Index (K)	1.24^{a}	1.40^{b}	1.41^{b}	1.38^{b}

[1]Values within a row with different superscripts are significantly different ($P<0.05$).

the present study each of the essential amino acid requirements of the trout was met by combining yeast biomass with fish meal and other protein sources.

There was no evidence from this study of reduced palatability influencing the feeding and growth experiments. The fish were fed carefully to satiation in glass-fronted tanks where their feeding response could be easily observed. Food intake did not differ significantly between the control and treated fish groups and the diets appeared equally palatable as judged by the behavioural responses of the fish to the feeds. The lower SGR and food conversion values may however have resulted from high levels of nucleic acids, nucleotides and ash present in the yeast biomass. Nucleic acids and nucleotides were not measured in the present study but have been reported to limit the nutritional value of microbial protein in animal feeds (Udall and Scrimshaw, 1986), particularly for monogastric animals such as fish (Tacon and Jackson, 1985).

The reasons for the changes in carcass composition are not clear from the present study although they closely parallel those reported by Davies and Wareham (1988) who fed diets containing single cell protein to tilapia. There is some evidence that nucleotides inhibit intestinal motility in fish (Fange and Grove, 1979). Increased retention times for digesta in the intestine may result in higher assimilation levels. This may explain the maximum apparent protein digestibility values measured in those fish fed the most yeast. This would not however account for the observed decrease in lipid content. This may reflect the fatty acid profile of the yeast biomass; a factor not examined in the present study.

In conclusion, these results demonstrate the potential for using *Candida utilis* yeast biomass as a partial replacement (25-35%) for fish meal. The need for more extensive trials is indicated in order to establish the long term effects of using yeast as a major ingredient in trout feeds. The factors that result in decreased feed utilisation need to be established. Also the potential benefits of yeast biomass as a source of vitamins, minerals and immuno-stimulants merit further study (Raa, 1990).

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