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Effect of Inclusion Level and Time on Apparent Digestibility of Solvent-Extracted Soybean Meal for Common Carp (*Cyprinus carpio,* Cyprinidae)

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Abstract

Apparent digestibility of solvent-extracted soybean meal (SBM) was determined at four levels of inclusion (10, 20, 30 and 40%) and over two periods of time (days 9-17 and days 19-24) for common carp (*Cyprinus carpio*, Cyprinidae). Solvent-extracted SBM was incorporated into a casein-based reference diet, and chromium oxide (1%) was used as an external marker. Apparent digestibility of SBM was found to decrease significantly with inclusion (86.3-73.1%) but not time. The digestibility of dietary protein also decreased significantly with inclusion, and although there was a trend for SBM protein digestibility to decrease with inclusion, this was not significant. Dietary lipid and SBM crude energy digestibility were found to increase and decrease, respectively, with time. These results indicate that digestibility of solvent-extracted SBM and its constituent nutrients for common carp may be affected by both level of inclusion and time, and that these factors must be considered when determining digestibility of protein sources for use in the formulation of experimental and commercial diets for this species.

Introduction

On the basis of its high biological value, fishmeal has traditionally been the preferred source of protein in aquaculture feeds. However, the increased use of artificial diets has placed greater demands on fishmeal production, resulting in increased cost and uncertain availability of high quality fishmeal, and doubt over the ability of fishmeal production to meet future increases in demand. Thus the partial or complete replacement of fishmeal with low-cost, readily available protein sources of consistent quality has the potential to decrease feed costs, ensure feed quality and enhance aquaculture profitability.

Cost, market availability and nutritional value indicate that soybeans (*Glycine max*) are amongst the best plant protein sources for supplementing aquaculture feeds (Lim and Akiyama 1992). Soybeans have a high protein content with the best reported amino acid profile of plant protein sources for meeting the essential amino acid requirements of fish (Lim and Akiyama 1992). Soybeans also contain a high level of oil providing a source of essential fatty acids and digestible energy (Lim and Akiyama 1992). However, a range of anti-nutritional factors and other toxic substances which adversely affect growth and health of animals are found in soybeans (Lim and Akiyama 1992).

The use of soybean meal (SBM) as an alternative protein source in aquaculture feeds has been extensively studied and has provided varied results. The majority of these studies have determined fishmeal replacement in common carp (Viola et al. 1981/2, 1983; Abel et al. 1984; Viola et al. 1992), catfish (Andrews and Page 1974; Robinson and Li 1994), tilapia (Jackson et al. 1982; Shiau et al. 1987, 1990) and salmonids (Cho et al. 1974; Rumsey and Ketola 1975; Dabrowska and Wojno 1977; Reinitz 1980).

Although research into fishmeal replacement by SBM has been contradictory, it is clear that SBM will play an important role in future aquaculture diets. In order to formulate the best quality diets, a knowledge of the digestibility of nutrients present in SBM and of factors influencing that digestibility are required. These issues have not previously been reported. Therefore the aims of the present study were to determine the effects of inclusion level and time on determining the digestibility of solvent-extracted SBM for common carp (*Cyprinus carpio*, Cyprinidae).

Materials and Methods

Common carp of mean weight 45.8 g (SE 1.3 g), were netted by a professional fisher from Reedy Lake and Lake Connewarre, Victoria, Australia. Animals were stocked at 15 fish per 70-l aquaria. Each aquaria received a continuous flow recirculated freshwater (600 ml per minute) with additional aeration. Fish were held at 20±1°C on a 12 h light:12 h dark photoperiod.

Solvent-extracted SBM (Metropolitan Commodities Pty Ltd, Melbourne, Victoria, Australia) was used as the source of SBM in the present study. Prior to incorporation into the experimental diets, the SBM was ground using a Wiley mill. The proximate analysis of the SBM used is detailed in Table 1.

A casein-based reference diet was used to determine the digestibility of

Table 1. Proximate composition as determined by analysis of solvent-extracted SBM.

Component	Content
Dry matter (g kg^{-1})	916
Protein[a]	39.9
Lipid[a]	3.3
Ash[a]	7.8
Energy (MJ kg^{-1})	19.9

[a] % dry weight

graded levels of SBM (Mackie and Mitchell 1985) (Table 2). SBM was incorporated into the reference diet at 10, 20, 30 and 40% on a dry-weight basis. The formulation and proximate composition of the experimental diets are detailed in Table 2. Diets were extruded using a Hobart #12 chopper attachment (Hobart Corporation, Troy, Ohio), die hole diameter of 1/8", powered by a Model A120 Hobart Mixer (Hobart Corporation, Troy, Ohio). Diets were air dried at 50°C and stored at -18°C until use.

Table 2. Formulation (g kg^{-1}) and proximate composition determined by analyses (g kg^{-1}) of the experimental diets.

Dietary components	Diets				
	Reference	10% inclusion SBM	20% inclusion SBM	30% inclusion SBM	40% inclusion SBM
Casein	600	540	480	420	360
Soybean meal	0	100	200	300	400
Wheat gluten	50	45	40	35	30
Corn flour	100	90	80	70	60
Tuna oil	90	81	72	63	56
α-cellulose	100	90	80	70	60
Vitamin and mineral premix	50	45	40	35	30
Chromium oxide	10	10	10	10	10
Proximate composition					
Crude protein	589	550	532	518	502
Crude lipid	88	76	61	61	54
Ash	41	42	51	52	62
Energy (MJ kg^{-1})	192	195	190	187	181
Chromium oxide	10	10	10	10	10

Each experimental diet was fed to three replicate tanks at a feeding rate of 2% body weight·wet weight diet·d^{-1}. Daily rations were fed in four approximately equal feedings at 1-h intervals, ensuring all feed was ingested. Experimental diets were fed for 24 d. Feces were collected by siphoning over a 2-h period each morning. Feces were not collected over the initial 8 d to allow for adaptation to the experimental diet; subsequently feces were collected daily from day 9 to 24, with the exception of days 11 and 18 during which insufficient feces were voided during the collection period. Analyses were performed on feces pooled over days 9-17 and days 19-24 for each experimental replicate.

Experimental diets and feces were dried to a constant weight at 50°C and ground prior to analysis. Total lipids were determined gravimetrically following methanol-chloroform extraction, energy by bomb calorimetry using a Parr Semimicro Calorimeter (Parr Instrument Co., Moline, Illinois, USA) and ash by combustion to constant weight at 550°C. Total crude protein was determined from Kjeldahl nitrogen (x 6.25) measured colorimetrically using the method of Baethgen and Alley (1989). Chromium oxide (Cr_20_3) was measured spectrophotometrically using the method of Furakawa and Tsukahara (1966).

Apparent digestibility coeffieicnts (ADCs) were calculated using the ratio of $Cr_2 0_3$ in feed and feces:

$$\text{ADC diet } (\%) = 100\text{-}100 \left(\frac{CR_2 0_3 \text{ diet}}{CR_2 0_3 \text{ feces}}\right)$$

$$\text{ADC ingredient } (\%) = \left(\frac{100}{X}\right)\left(\text{ADC test diet } - \frac{100\text{-}X}{100} \text{ ADCreference diet}\right)$$

$$\text{ADC nutrient } (\%) = 100\text{-}100 \left(\frac{\% \ CR_2 0_3 \text{ diet}}{\% \ CR_2 0_3 \text{ feces}}\right)\left(\frac{\% \text{ nutrient feces}}{\% \text{ nutrient diet}}\right)$$

ADC nutrient ingredients (%) =

$$\left(\frac{100}{X}\right)\left(\text{ADC nutrient test diet } - \frac{100\text{-}x}{100} \text{ ADC nutrient reference diet}\right)$$

where X refers to the level of inclusion of the test ingredient in the experimental diets (%).

Data were initially analyzed using repeated measures analysis of variance (Zar 1984). If an effect of only inclusion or time was found, and where no significant interaction between inclusion and time occurred, data were pooled over the non-significant variable, and analyzed using one-way analysis of variance. Differences among means were considered significant at $P<0.05$. *Post hoc* analyses were performed using Scheffé's test ($P<0.05$). Analyses were performed using StatView 512$^{\pm TM}$ statistical analysis software (Brain Power Inc., California, USA).

Results

Apparent digestibility of the experimental diets and dietary nutrients, and SBM and its constituent nutrients determined from feces pooled over days 9-17 and days 19-24 are detailed in Tables 3 and 4, respectively.

Dry matter digestibility of the experimental diets was significantly affected by level of inclusion of SBM but not time. As the interaction between inclusion and time was not significant, data were pooled over time (Table 3). Whole diet digestibility was found to decrease significantly with increase in inclusion of SBM.

A significant effect of inclusion level but not time on digestibility of SBM was also found. Again, data were pooled over time (Table 4). The digestibility of SBM decreased significantly with increase in SBM inclusion.

Digestibility of crude protein for the experimental diets was high, being greater than 98% for all experimental diets (Table 3). Although differences were small, a significant effect of both inclusion and time on dietary protein digestibility was found. Dietary protein digestibility was significantly

Table 3. Apparent digestibility (%) of dry matter and nutrients for the experimental diets as determined for feces pooled over days 9-17 and days 19-24. Data were pooled if no significant effect of time was found. Values are mean ± SD (n = 3 unpooled and n = 6 pooled data). Values for the same time point in the same column having the same superscript are not significantly different at P<0.05. * indicates a significant effect of time (P<0.05) as determined by analysis of values pooled over inclusion.

Diet type	Feces	Apparent digestibility			
	(d)	Dry matter	Protein	Lipid*	Energy
Reference	9-17	81.9±1.6	99.3±0.9^{ab}	65.5±7.7	85.4±2.1
	19-24	83.3±0.7	99.6±1.0^{x}	77.4±0.5	86.7±0.5
	Pooled data	82.6±1.3^{a}			86.0±1.5
10% inclusion	9-17	82.4±0.2	99.1±0.2^{a}	66.1±1.6	86.1±0.2
SBM	19-24	83.6±1.0	99.5±0.2^{x}	77.2±0.3	86.8±1.7
	Pooled data	83.0±1.6^{a}			86.5±1.3
20% inclusion	9-17	82.3±0.3	98.8±0.3^{b}	69.4±3.1	86.8±1.0
SBM	19-24	80.6±1.4	99.1±0.2^{xy}	71.9±3.8	83.6±1.9
	Pooled data	81.5±1.3^{ab}			85.2±2.2
30% inclusion	9-17	80.0±1.4	98.6±0.0^{bc}	70.8±5.9	85.4±5.0
SBM	19-24	80.2±1.0	98.7±0.3^{yz}	73.5±1.4	83.2±2.0
	Pooled data	80.1±1.2^{bc}			84.3±2.9
40% inclusion	9-17	78.8±0.3	98.2±0.5^{bc}	62.5±1.2	83.7±8.1
SBM	19-24	78.8±0.3	98.1±0.3^{z}	68.3±1.0	80.3±0.3
	Pooled data	78.8±0.2^{c}			82.0±4.3

reduced at higher levels of inclusion of SBM (Table 3). Although digestibility of protein from the experimental diets was found to be affected by time, *post hoc* analysis failed to show a significant difference in digestibility of dietary protein from the respective diets measured at each time point.

Digestibility of protein from SBM was high at all inclusion levels, being greater than 95% (Table 4). The digestibility of protein from SBM tended to decrease with level of SBM inclusion, however, no sigificant effect of inclusion level nor time on digestibility of protein from SBM was found.

Dietary lipid digestibility was found to be variable (Table 3). Although there was no significant effect of inclusion level on dietary lipid digestibility, a significant effect of time was found. As the interaction between inclusion and time was not significant, the data were pooled over inclusion level. Lipid digestibility (mean±SE) was significantly higher over days 19-24 (73.1±1.7%) than over days 9-17 (67.7±1.4%).

The digestibility of lipid from SBM was also highly variable, particularly at lower levels of inclusion (Table 4). Although SBM lipid digestibility tended to decrease at the 40% inclusion level, there was no significant effect of either inclusion level nor time on digestibility of lipid from SBM.

Apparent digestibility of crude energy from the experimental diets showed no significant effect of inclusion level or time (Table 3).

Digestibility of crude energy for SBM showed a significant effect of time but not inclusion level (Table 4). The interaction between inclusion level and time was not significant, and the data were therefore pooled over inclusion level. Digestibility of crude energy from SBM (mean±SE) was significantly higher over days 9-17 (88.4±3.2%) than days 19-24 (77.1±3.9%).

Table 4. Apparent digestibility (%) of SBM and its nutrients as determined for feces pooled over days 9-17 and days 19-24. Data were pooled if no significant effect of time was found. Values are mean ± SD (n = 3 unpooled and n = 6 pooled data). Values for the same time point in the same column having the same superscript are not significantly different at P<0.05. * indicates a significant effect of time (P<0.05) as determined by analysis of values pooled over inclusion.

Inclusion SBM (%)	Feces (d)	Apparent digestibility			
		Dry matter	Protein	Lipid	Energy*
10	9-17	86.1±2.2	97.5±1.6	71.7±15.9	92.3±1.2
	19-24	86.5±10.4	98.4±2.6	75.1±26.0	88.6±17.3
	Pooled data	86.3±6.7^{a}	97.9±2.0	73.4±19.4	
20	9-17	83.8±1.4	96.7±1.7	85.1±15.5	92.6±5.0
	19-24	70.0±7.1	97.0±1.0	50.0±19.2	71.2±9.4
	Pooled data	76.9±8.9^{ab}	96.9±1.3	67.4± 24.9	
30	9-17	75.3±4.8	97.1±0.3	83.4± 9.7	85.4±16.8
	19-24	73.6±3.1	96.7±1.4	64.3± 4.7	75.2±6.8
	Pooled data	74.4±4.3^{b}	96.9±0.7	73.8±14.6	84.3± 2.9
40	9-17	74.1±0.9	96.6±1.2	58.1± 3.3	8.1±20.6
	19-24	72.2±0.7	95.9±0.7	54.6± 3.1	70.7±0.7
	Pooled data	73.1±1.2^{b}	96.2±0.8	56.3± 2.8	

Discussion

The dry matter digestibility of SBM reported in the present study is higher than previously reported for carp (Chu et al. 1991) and chinook salmon (Hajen et al. 1993). In view of the omnivorous nature of common carp, it is not unexpected that dry matter digestibility would be in the range observed. SBM digestibility was found to be significantly reduced at greater than 20% inclusion. The decrease in digestibility as SBM inclusion increased above 20% may have resulted from anti-nutritional factors, in particular, protease inhibitors. The action of protease inhibitors would suggest a linear decrease in digestibility with the incorporation of SBM. Although decrease in digestibility was not linear with the incorporation of SBM in this study, it is possible that increased release of intestinal proteases may have masked the effect of anti-nutritional factors at levels below 20% inclusion. Such an effect is also reflected in the digestibility of protein from the experimental diets and SBM. The ability of fish to compensate for low levels of protease inhibitor has been demonstrated previously (Robinson et al. 1981; Viola et al. 1983; Wilson and Poe 1985; Krogdahl et al. 1994). The increase in amount of insoluble carbohydrate in the experimental diet with inclusion of SBM may also have adversely affected SBM digestibility (De Silva and Anderson 1995).

Crude protein from the experimental diets was greater than 98% digestible by common carp in all cases. High digestibility of protein from casein-based diets has previously been reported (Kitamikado et al. 1964). Although a significant effect of inclusion and time was found on digestibility of dietary protein, the change in digestibility was slight and would not be of physiological significance. The digestibility of protein from SBM was also high, greater than 95% at all levels of inclusion. Wilson and Poe (1985) reported apparent digestibility of SBM protein to be 95% and 97% for channel catfish from extruded and pelleted diets, respectively, although reported ADCs for SBM protein have generally been much lower (Smith et al. 1980; Ferraris et al. 1986; Lorico-Querijero and Chiu 1989; Anderson et al. 1992; Hossain et al. 1992; Hajen et al. 1993). As feces were collected by siphoning in the present study, it would be expected that protein digestibility would be overestimated due to the leaching of water-soluble protein (Windell et al. 1978; Smith et al. 1980; Lied et al. 1982). Such an effect can be large and may explain the high digestibility of protein reported in the present study.

Although not significant, digestibility of protein from SBM tended to decrease with inclusion level of SBM. As the decrease in SBM protein digestibility coincided with decreased dietary protein digestibility, this suggests the presence of low levels of protease inhibitor. SBM protease inhibitors have been previously shown to reduce protein digestibility in fish (Krogdahl et al. 1994; Olli et al. 1994). As the reference diet contained a highly digestible protein source in casein, and as the loss of soluble protein from the feces prior to collection also overestimated protein digestibility, it is possible that significant effects of protease inhibitors may have been masked by the high protein digestibility observed.

Digestibility of crude lipid from the experimental diets ranged from 62.5% to 77.4%, with digestibility of dietary lipid being significantly greater over days 19-24 than days 8-17. Dietary lipid digestibility was lower than that generally reported for fish oil-based diets (Austreng et al. 1979; Takeuchi et al. 1979; Ellis and Smith 1984; Storebakken 1985; Chu et al. 1991; Médale et al. 1991; Hossain et al. 1992) and for a mixture of fish oil and SBM oil (5:2) by rainbow trout (Ellis and Smith 1984). However, ADCs for dietary lipid reported in the present study are consistent with values determined for tuna oil digestibility by common carp (Appleford and Anderson 1997). The increase in dietary lipid digestibility with time would appear to reflect an adaptation of the digestive physiology of the animals to the experimental diets. A similar increase in tuna oil digestibility with time has previously been reported (Appleford and Anderson 1997).

Apparent digestibility of SBM oil by common carp in the present study was highly variable, reflecting the low level of crude lipid in solvent-extracted SBM (3.3%). Soybean oil digestibility was 58-85% for days 8-17 and 47-75% for days 19-24, although there was no significant effect of either inclusion level or time on digestibility. The digestibility of SBM oil was low compared with values previously reported for grass carp and Mozambique tilapia, 99% and 93%, respectively (Law 1986; Hossain et al. 1992).

The low level of lipid digestibility, both from the experimental diets and from SBM, may be due to the high levels of complex carbohydrate in the diets. The reference diets contained 10% α-cellulose, while inclusion of SBM into the diets would further introduce indigestible complex carbohydrate into the experimental diets (Lim and Akiyama 1992). It has previously been reported that high levels of complex carbohydrate may adversely affect lipid digestibility by fish (Steffens 1989; Médale et al. 1991; Fagbenro 1992). A decrease in lipid digestibility for common carp with an increase in α-cellulose content of the diet has previously been observed (Appleford and Anderson, unpubl. observ.).

Whole diet crude energy digestibility was not affected by level of inclusion of SBM or time, although SBM crude energy digestibility was found to decrease with time, being significantly lower over days 19-24 than over days 8-17. A consistent digestibility of SBM energy up to 40% inclusion supports the findings of Anderson et al. (1991) who found the digestibility of crude energy from SBM by tilapia, *Oreochromis niloticus*, was not affected by inclusion level up to 60%, although 100% substitution resulted in a significant decrease in energy digestibility. Anderson et al. (1991) also found an effect of time on digestibility of crude energy from SBM in tilapia, although in that study, crude energy digestibility increased with increase in the adaptation period to the diet (1 week versus 15 weeks). Although the change in digestibility of SBM energy in the present study was not consistent with the findings of Anderson et al. (1991), it is apparent that digestibility varies significantly with time.

If SBM is to be used as a replacement for fishmeal in artificial diets for aquaculture, it is necessary to accurately determine its digestibility in order to maintain the required digestible protein level and amino acid ratios. The present study has shown an effect of both inclusion and time on digestibility. The decrease in digestibility of SBM with level of inclusion emphasizes the need to determine the digestibility of a dietary ingredient over the range of levels at which it is to be included during diet formulation. The changes in digestibility of dietary lipid and SBM crude energy indicate the need to also allow for this factor when determining the digestibility of ingredients for use in the formulation of experimental or commercial diets.

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