Effects of dietary fat levels and of antibiotics (Flumequine + Gentamycin) on nutrient digestibility in rainbow trout, Oncorhynchus mykiss (Walbaum)

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Effects of dietary fat levels and of antibiotics (Flumequine + Gentamycin) on nutrient digestibility in rainbow trout, *Oncorhynchus mykiss* (Walbaum)

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Abstract

Rainbow trout were fed two experimental diets differed in lipid content by replacement of crude starch (lipid-poor diet : 4.2% lipid or lipid or lipid-rich diet : 14.8% lipid) supplemented or not with antibiotics (a combination Flumequine + Gentamycin, 1:4). The apparent digestibility of dry matter, crude protein, lipid, carbohydrate and total energy were measured. Addition of lipid by replacement of crude starch in the fish diet in the absence of antibiotics enhanced significantly ($p<0.01$) the apparent digestibility of dry matter, crude protein, carbohydrate and total energy, but did not influence those of lipid. Antibiotics supplementation of the lipid-poor diet (consequently with a high level of carbohydrate) enhanced significantly ($p<0.01$) the apparent digestibility of dry matter, crude protein, lipid, and total energy, but not those of carbohydrate. On the other hand the antibiotics-treated lipid-rich diet (consequently with a low level of carbohydrate) showed a higher ($p<0.01$) digestibility for dry matter, lipid, carbohydrate and total energy, but not for crude protein. The stimulatory effect of antibiotics on nutrient digestibility is discussed.

Keywords : digestibility, lipid, antibiotic, trout

Effet des lipides alimentaires et des antibiotiques (Fluméquine + Gentamycine) sur la digestibilité des nutriments chez la truite arc-en-ciel, Oncorhynchus mykiss (Walbaum).

Résumé

Des truites arc-en-ciel ont reçu deux régimes alimentaires, pauvre (4,2% de lipides) ou riche en lipides (14,8% de lipides), supplémentés ou non en antibiotiques (une combinaison de Fluméquine et de Gentamycine, 1:4). La digestibilité apparente de la matière sèche de l'aliment, ainsi que celle des protéines, des lipides, des glucides et de l'énergie, a été mesurée. L'addition de lipides à l'aliment pour poissons, par substitution à l'amidon cru et en absence de tout antibiotique, augmente de façon significative ($p<0,01$) la digestibilité apparente de la matière sèche de l'aliment, des protéines, des glucides et de l'énergie, mais n'a pas d'influence sur celles des lipides . La supplémentation en antibiotiques du régime pauvre en lipides (donc à forte teneur en glucides) augmente de façon significative ($p<0,01$) la digestibilité apparente de la matière sèche de l'aliment, des protéines, des lipides et de l'énergie, mais pas celle des glucides. L'utilisation d'un régime riche en lipides (donc à faible teneur en glucides) supplémenté en antibiotiques entraîne des digestibilités significativement supérieures ($p<0,01$) pour la matière sèche de l'aliment, les lipides, les glucides et l'énergie mais pas pour les protéines. L'effet de stimulation des antibiotiques sur la digestibilité est discuté.

Mots-clés : digestibilité, lipide, antibiotique, truite.

INTRODUCTION

Nutrient digestibility measurement *in vivo* is of importance in the formulation of nutritionaly adequate diets. But the availability of individual nutrient from a complete diet can be affected by a number of factors among which interactions between nutrients (Hasting, 1969). Increasing level of dietary lipid have thus been shown to have a beneficial effect on digestible energy and on protein sparing in rainbow trout (Watanabe *et al.*, 1979). Therefore, from literature data *(table 1)*, it is not clear whether an increase in dietary fat level has any significant effect on the availability of the other nutrients. The first purpose of the present study was to examine the effect of increasing levels of dietary lipid on dry matter, protein, lipid, carbohydrate and energy digestibility in rainbow trout.

Fish fed virginiamycin supplemented diets had significantly more fat in their carcass compared with control (Ahmad and Matty, 1989). In higher animals, antibiotics might influence gastrointestinal nutrient absorption of protein and energy (Eggum *et al.*, 1979), of carbohydrate (Dutta *et al.*, 1977) and of lipid (Rotenberg and Andersen, 1982). In fish, administration of chloramphenicol, oxolinic acid and oxytetracycline to rainbow trout at a level of 0.5% was found to increase the digestibility of unsaturated fatty acids (Cravedi *et al.*, 1987). A further aim of this study was to determine the effect of a dietary combination of antibiotic agents (Flumequine + Gentamycin) on the digestibility of nutrient in rainbow trout.

MATERIALS AND METHODS

Twelve rainbow trout families, *Oncorhynchus mykiss* (Walbaum, 1792) genetically selected to have from 45 ± 4 (mean ± S.D.) to 89 ± 9 caeca (Bergot *et al.*, 1976), were distributed into groups of 20 fish each. The fish (150 ± 20g) were kept in 60 l cylindroconical tanks each with water flow of 4 $l.min^{-1}$. Temperature was 14.9 ± 0.9°C, and photoperiod was regulated to 12h light and 12h dark. Fish were held in the tanks for 15 days prior to the beginning of the experiment and were adapted to diets for 2 days (de la Noüe *et al.*, 1980). Fish were fed 1.5% of their live weight once a day.

Two experimental diets differed in lipid content by replacement of crude starch (lipid-poor : 4.2% lipid ; lipid-rich : 14.8% lipid) as reported in *table 2*. Each diet either contained antibiotics (a combination of 0.8 g/kg Flumequine and 3.45 g/kg Gentamycin sulfate) or did not. Diets were wet pelleted and dried overnight at 32°C.

Table 2. –

a) Formulation of the experimental diets (% dry matter).

Ingredients	L-R (1)	L-P (2)
Herring fish meal	50	50
Crude corn starch	24	35
Cellulose	5	5
Fish oil (3)	16	5
Vitamin premix (EIFAC, 1971)	2	2
Mineral premix (Luquet, 1971)	1	1
Sodium alginate	1	1
Chromium oxide (4)	1	1

b) Proximate analysis.

	L-R (1)	L-P (2)
Dry matter (DM %)	94.07	93.21
Crude protein (N%DM x 6.25)	43.63	43.00
Lipid (%DM)	14.78	4.22
Carbohydrate (%DM)	26.04	36.87
Total energy(kj/g)	20.26	18.21

(1) Lipid-rich diet
(2) Lipid-poor diet
(3) Fatty acids composition : 14:0 = 2.25% ; 16:0 = 23.47% ; 16:1 = 5.26% ; 18:0 = 13.39% ; 18:1 = 47.99% ; 18:2 = 5.02% ; unidentified = 2.62%
(4) included as an inert digestion tracer.

Table 1. – *Influence of an increase of lipid level in fish feed on the nutrient digestibility (+ increase ; - decrease ; = no effect).*

Fish species	Digestibility (1) D	P	L	C	E	References
Catfish	+				+	Andrews *et al.*, 1978.
Goldfish		=				Furukawa and Ogasawara, 1952.
Carp		=	=	=		Schwarz and Kirchgebner, 1982.
Carp		+				Ogino *et al.*, 1976.
Carp			+			Takeuchi *et al.*, 1979.
Rainbow trout		=				Kitamikado *et al.*, 1964.
Rainbow trout		+				Ogino *et al.*, 1976.
Rainbow trout		=	=	+	+	Watanabe *et al.*, 1979.
Rainbow trout		=	+			Takeuchi *et al.*, 1979.
Rainbow trout	-	=		-		Bergot and Brèque, 1983.
Rainbow trout					=	Kaushik and de Oliva Teles, 1985.
Rainbow trout	+	=		+	-	Cho, 1987.
Rainbow trout	+	=		+	=	Kim, 1989.

(1) : (D) Dry matter ; (P) Protein ; (L) lipid ; (C) Carbohydrate ; (E) Energy.

Faeces were recovered using the continuous automatic collector as described by Choubert *et al.* (1982), immediately frozen (-18°C), freeze-dried and stored at +4°C until analyzed. The analysis of dry matter (drying at 105°C until constant weight), crude protein (Kjeldahl-Foss, N x 6.25), total lipid (Folch *et al.*, 1957), carbohydrate (Dubois *et al.*, 1956), total energy (Parr adiabatic bomb) and chromic oxide (Bolin *et al.*, 1952) were performed on diets and faeces.

Apparent digestibility coefficients (ADC) were calculated according to Maynard and Loosli (1969) :

$$ADC = 100\left(1 - \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}} \times \frac{\%\ Cr_2O_3\ \text{in diet}}{\%\ Cr_2O_3\ \text{in faeces}}\right)$$

Data were analysed by multiway analysis of variance. The influence of pyloric caeca was tested by regression of mean digestibility coefficients of families on their mean number of caeca (Snedecor and Cochran, 1967).

RESULTS

Number of pyloric caeca did not influence ($p \geq 0.05$) the apparent digestibility of dry matter, crude protein, lipid, carbohydrate and total energy whatever the level of fat and antibiotics supplementation (*fig.1*). The regression of mean digestibility coefficients of families on their mean number of caeca did not provide any significant estimate of regression slope. The pooled value for lipid-poor and lipid-rich diets with or without antibiotics are reported in *table* 3. In the absence of antibiotics, addition of lipids to the diet by replacement of crude starch enhanced significantly ($p<0.01$) the apparent digestibility coefficients of dry matter, crude protein, carbohydrate and total energy, but did not influence those of lipid.

Antibiotics enhanced the digestibility of dry matter, lipid and energy whatever the lipid and carbohydrate levels in the diet. Moreover antibiotics supplementation of the lipid-poor diet (consequently with a high level of carbohydrate) enhanced significantly ($p<0.01$) the apparent digestibility of dry matter, crude protein, lipid and total energy but not those of carbohydrate. On the other hand, the antibiotics-treated lipid-rich diet (consequently with a low level of carbohydrate) showed a higher ($p<0.01$) digestibility for dry matter, lipid, carbohydrate and total energy but not for crude protein.

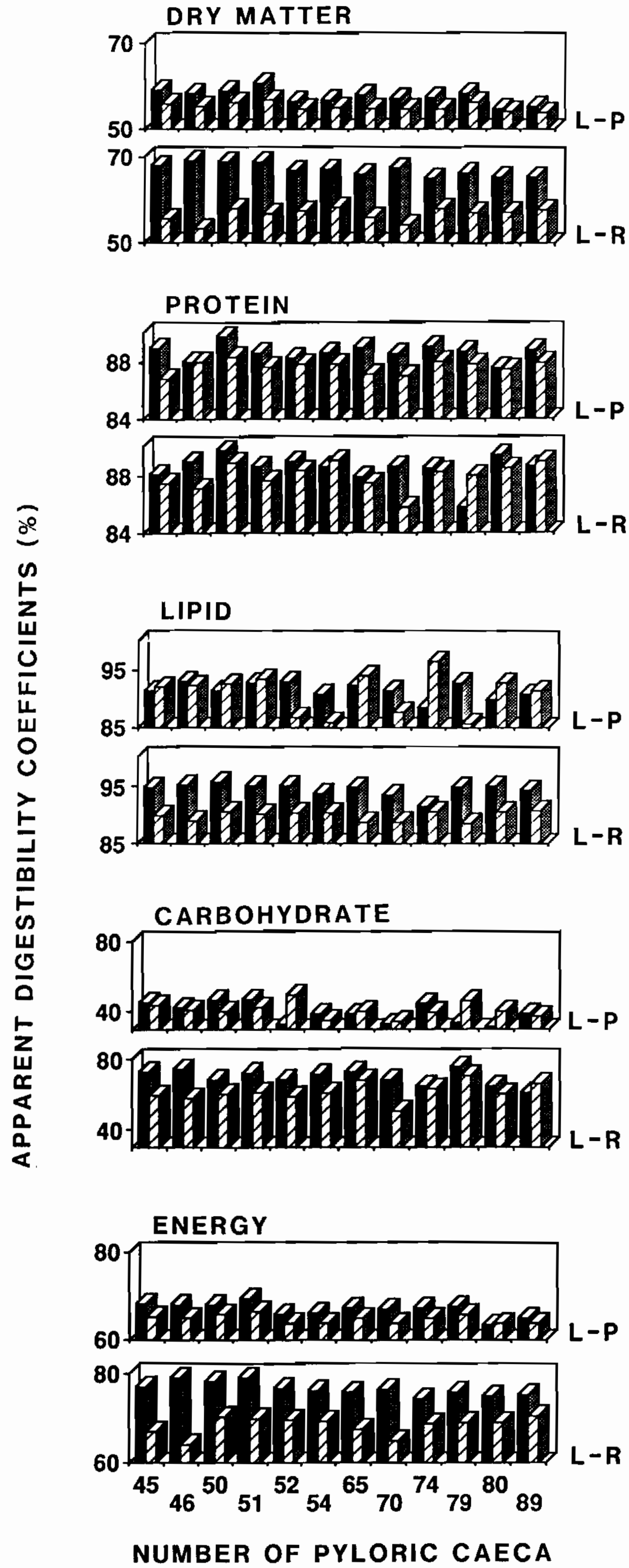

Figure 1. - Apparent digestibility coefficients of nutrient in rainbow trout as affected by the number of pyloric caeca for lipid-poor (L-P) and lipid-rich (L-R) supplemented ■ or not ▨ with antibiotics.

Table 3. – Effect of lipid level in the diet supplemented or not with antibiotics on the apparent digestibility coefficients of nutrient in rainbow trout (% ± S.D., n=24). For a given nutrient results with the same subscript are not statistically different (pz0.05).

	Digestibility	Coefficients		
Nutrient	L-R+A (1)	L-R-A (2)	L-P+A (3)	L-P-A (4)
Dry matter (DM%)	66.94 ± 1.53 [a]	56.55 ± 1.81 [b]	57.55 ± 1.94 [b]	55.15 ± 1.97 [c]
Protein (N%DM x 6.25)	88.46 ± 1.15 [a]	87.90 ± 1.02 [a]	88.60 ± 0.81 [a]	87.60 ± 0.64 [b]
Lipid (%DM)	94.35 ± 1.63 [a]	89.78 ± 0.97 [b]	91.41 ± 2.20 [c]	90.84 ± 4.68 [b]
Carbohydrate (%DM)	69.36 ± 4.44 [a]	61.26 ± 5.92 [b]	39.39 ± 6.79 [c]	40.80 ± 5.78 [c]
Energy (cal/g)	76.55 ± 1.55 [a]	68.37 ± 2.32 [b]	66.98 ± 1.88 [c]	64.77 ± 1.96 [d]

(1) Lipid-rich diet supplemented with antibiotics
(2) Lipid-rich diet without antibiotics
(3) Lipid-rich diet supplemented with antibiotics
(4) Lipid-rich diet without antibiotics

DISCUSSION

The results of the present study show that addition of lipid to trout diet along with a concomittant decrease in crude starch increased significantly the digestibility of dry matter, carbohydrate and energy. These findings support some results compiled in *table* 1. However part of the observed variabilities have to be related to the different methods of faeces collection (Choubert *et al.*, 1982), the fat content and the type of fat ingested (Takeushi *et al.*, 1979). Nevertheless as addition of lipid in the diet was done by partial replacement of crude starch, the increase in the digestibility of carbohydrate observed with the lipid-rich diet without antibiotics may also be due to the lower level of starch in the diet (Bergot, 1975).

However data presented here show no evidence for increasing the digestibility of the main nutrients in rainbow trout relative to the number of pyloric caeca. Whatever their number (45 to 90) the digestibility of dry matter, protein, lipid, carbohydrate and energy remained unaffected. This is in line with previous findings (Ulla and Gjedrem, 1985 ; de la Noüe *et al.*, 1989). It would seem that the overall physiological capacity of the intestine including pyloric caeca remain constant (Buddington and Diamond, 1987) since the diameter, lenght and surface of pyloric caeca decrease as their number increases (Bergot *et al.*, 1975). However a beneficial effect of the number of pyloric caeca have been shown on the digestibility of some amino acid (Buddington and Diamond, 1987) and lipid (Janson and Olosson, 1960).

The two antibiotics used, Flumequine and Gentamycin, have some different characteristics. Flumequine is commonly used against fish bacterial diseases (Boulanger and Collas, 1989) due to its wide antimicrobial spectrum. This drug is effectively absorbed by intestinal mucosa. On the other hand, Gentamycin has been used to eliminate bacterial flora from the digestive tract of mammals (Hazenberg *et al.*, 1983), but this wide spectrum antibiotic is not absorbed. Nevertheless, the combination of these two antibiotics is recommanded (Neuman, 1979) according to the usual bacterial components of the digestion flora in fish (Lésel, 1981).

Diets supplemented with antibiotics showed a better digestibility whatever their lipid level for dry matter, lipid and energy. This is consistant with the general acceptance of antibiotics stimulation effect on alimentary efficiency as reported in broilers (Miles *et al.*, 1984), turkey poults (Buresh *et al.*, 1984) and swine (Maxwell, 1984). A depressive effect has however been recorded in rat (Eggum *et al.*, 1979 ; Rotenberg and Andersen, 1982). Moreover in fish, chloramphenicol, oxolinic acid and oxytetracycline were found to increase the digestibility of some unsaturated fatty acids (Cravedi *et al.*, 1987). Nevertheless antibiotics supplementation did not affect the digestibility of protein with a lipid-rich diet nor those of carbohydrate with a lipid-poor diet.

The mechanism of the stimulatory effect of antibiotics is still unknown. Therefore it seems difficult to consider only their action on bacterial flora as it has been reported in broiler (Young *et al.*, 1963) or rat (Rotenberg and Andersen, 1982) since in fish living in cold or temperate water the role played by intestinal microorganisms in nutrient digestion is negligible (Sacquet *et al.*, 1979 ; Lésel, 1981 ; Bergot and Brèque, 1983). Another explanation would be an increase of the permeability of the intestinal mucosa by antibiotics (March and Briely, 1967). But this hypothesis contradicts findings of Cesano *et al.*(1972). Perhaps the most acceptable view is that of Neuman (1979) who have suggested a modification of the transit rate by antibiotics. An increase in velocity of foodstuffs passage will affect the nutrient digestibility by minimizing the time of contact between digestive enzymes and nutrient (Windell *et al.*, 1978 ; Luquet and Fauconneau, 1979 ; Cho and Slinger, 1979). A compensation phenomenom of digestive capacity in response to transit rate rise still has been reported by Hochachka (1967) and would include not only the control of synthesis and function of digestive enzymes but also the modification of the affinity between digestive enzymes and nutrients (Somero, 1969 ; Léger *et al.*, 1976).

As it is unjustified to use antibiotics just to enhance the nutrients digestibilities, the knowledge of antibiotics-nutrient interactions (Toothaker and Welling, 1980), independent from their antibiotic action, would be of zootechnical interest.

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