Use of coffee pulp as feed ingredient for tilapia culture

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Stellingen

1. Feed ingredients containing tannins will negatively affect fish growth performance.
This thesis

2. Treatment with cellulolytic bacteria increases the nutritive value of fibrous foodstuffs for tilapia.
This thesis

3. The general public belief that the El Niño phenomenon is only causing declines in the catch of fish in the tropics, is not supported by scientific evidence.
R.D. Guerrero III, 1999. The impacts of El Niño on Philippine Fisheries. Naga, 22 (3): 14-15.

4. Destruction of mangrove areas is erroneously attributed to the rapid expansion of aquaculture in coastal areas.
Global Aquaculture Alliance, 2002. Aqua-Issues. Mangroves. http://www.gaalliance.org

5. Environmental risks of introducing tilapia culture into marine environments are much less than claimed by environmentalists.
K. Fitzsimmons, 2001. Environmental and Conservation Issues. http://ag.arizona.edu/azaqua/ista/Malaysia/Environmental%20impacts.doc

6. A smile is the lighting system of the face, the cooling system of the head and the heating system of the heart.

7. "The trouble with being punctual is that nobody's there to appreciate it" (Franklin P Jones).

Stellingen belonging to the thesis
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Abstract

This research aimed to analyze the feasibility of using coffee pulp (CoP) in diets for tilapia (*Oreochromis aureus*), thereby focusing on the specific conditions prevailing in Costa Rica. Limitations of CoP as an animal foodstuff (several ANF's and high fibre contents) and different ways to upgrade the nutritional value of CoP are mentioned and discussed. For the specific situation in Costa Rica, an overview was made of the most important agricultural activities. The annual production volumes of different plant and animal residues (by-products and wastes) were assessed, together with an analysis of the various treatments applied to the wastes and how this and other factors (such as seasonal changes) affected the chemical composition of the residues and the risk that they turn into a significant pollution factor. The most relevant of these residues, e.g., CoP and green banana, were studied into detail, their chemical composition analyzed and the presence of ANF's determined. CoP was selected for further use in the present project because of its large volume available and because of the potential risk it represents. Changes in the chemical composition of CoP during the harvesting season, thereby applying two drying methods (sun-drying and oven-drying) were examined. Both season and drying method proved to affect this composition. The growth response of tilapia receiving diets which contained CoP diets depended on the level of CoP in the diet and also on the type of production system in which the tested tilapia were reared. When natural food was present in the rearing system, the negative effect on growth of dietary CoP was diminished. A dietary inclusion level of 130 g kg^{-1} of CoP did not affect growth and feed efficiency when compared with the control diet. The limitations to use CoP as a foodstuff for fish diets were associated with the levels of tannins, caffeine and fibre in the diets. Based on growth and feed efficiency (digestibility), the following dietary critical levels were found for fingerlings of *O. aureus*: 4.4 g kg^{-1} of tannins and 106 g kg^{-1} of fibre. For caffeine it was difficult to assess clear critical levels but dietary levels between 2.4 g kg^{-1} to 4.6 g kg^{-1} tended to reduce fish growth, feed intake and nutrient digestibility of *O. aureus*. In a next step, an attempt was made to reduce the content of caffeine, total phenols (polyphenols), tannins and fibre in CoP by applying various chemical (NaOH, the combined HCl-NaOH and combined NaOH-ensilage) and biological treatments (ensilage, natural microbial decomposition and bacterial inoculation (five species of *Bacillus ssp.*)). The results showed that biological treatments may also increase CoP protein and fat contents. The best result was found with the bacterial treatment. This type of treated CoP was then included in diets for tilapia and tested. Fish fed the diet containing 60 g kg^{-1} of CoP gave similar responses as those receiving the control diet. The potential of using CoP in diets for tilapia cultured in extensive or semi-intensive systems and the problems of upscaling the bacterial treatment are discussed. These problems are mainly concerned with technological and engineering aspects.

Dedicated to my wife and children

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Chapter 1

General introduction

Introduction

In Costa Rica, agriculture provides a large proportion of the national income. At the same time, it produces a large amount of residues (by-products and wastes). In the 1993-'94 production season, the total amount of residues reached 3.5-3.8 million MT, of which a significant portion (1.8-2.0 million MT) was disposed off (wastes). Another part was used for animal feeds (≈400,000 MT), for fertilization (≈122,000 MT), or for other uses (1,215,000 MT) such as substrates in greenhouses and poultry enclosures, as fuel or for human consumption. From the wastes portion, an unknown fraction is burried in holes and in municipality dumps. Efforts to reutilize crop wastes are being made but vary in different regions of the country and, often do not keep pace with the crop productions.

In the 1993-'94 production season, the greater amount of wastes was generated by the banana, coffee and sugarcane industries: 388-575,000 MT, 436-452,000 MT and 298,000-313,000, respectively. The coffee pulp (CoP) represents 350-365,000 MT of total coffee wastes. More recent production data indicated that banana, coffee and sugarcane wastes reached 477,700-654,500 MT, 406,000-412,800 MT and 383,100-388,200 MT, respectively (1998-1999 production season). Up to 26% of the wet mass of CoP (pulp + mucilage) is lost during transportation or, when immersed in water, about half of its weight is lost within five minutes (Vázquez 1997). Therefore, it is considered that CoP constitutes a bigger risk for pollution than banana wastes, which do not disintegrate that easy. It has been estimated that 294-307,000 MT of the total CoP production is dumped into nature where it gives rise to serious pollution. Therefore, the re-utilization of coffee residues merits serious attention. Coffee wastes have a potential to be used as ingredients in animal feeds and/or as organic fertilizer, or as soil conditioner. Further, a large volume of this waste is available over a relatively long period (4-8 months). The CoP obtained from the coffee processing plants (wet depulping method) consists of the berry skin (exocarp), the pulp itself (pericarp), the mucilage (mesocarp), and also a variable amount of pedicel, impaired grains, hulls and pieces of leaves and stacks. This CoP accounts for about 40 % of the wet weight of the coffee berry (Montero 1992). Due to this variability in the physical composition of CoP, its chemical composition is also variable (Elías 1979, Abate 1988, Ramírez 1988, Ramírez & Clifford 1989).

For economic and environmental reasons, in Costa Rica as well as in other coffee producing countries attempts have been made to utilize CoP as feed for cattle, swine, poultry and fish. However, the presence of antinutritional factors (ANF's) in CoP limits its success as animal feed ingredient. These ANF's (caffeine, polyphenols or total phenols, tannins) reduce animal growth and feed utilization. Therefore, CoP is recommended only at levels (as fed basis) between 200-300 g kg^{-1} for cattle rations (Cabezas *et al.* 1979), and at levels not higher than 150 g kg^{-1} for sheep rations (Vitto *et al.* 1999). For monogastric animals, the maximum recommended dietary inclusion levels (as fed basis) vary from 160 g kg^{-1} for swine (Jarquín

1979), to 100 g kg^{-1} for chicken (Romero *et al.* 1999) or 80 g kg^{-1} for growing/fattening rabbits (Bautista *et al.* 1999a). Higher ensiled CoP levels resulted usually in low diet digestibility and lower weight gain and in some animal species induced a high mortality. For tilapia and Cachamay (*Colossoma* x *Piaractus*), reared in stagnant ponds, CoP can be included at 130 g kg^{-1} and 200 g kg^{-1} (as fed) in the diet, respectively, without resulting in any growth difference compared to control diets (Bautista *et al.* 1999b). Processing of CoP with the purpose to reduce its ANF's content may enable higher inclusion levels in animal's feeds. For this reason, the critical levels of these ANF's for the species of concern must be known.

The CoP contains variable amounts of caffeine, polyphenols, tannins and high levels of potassium and fibrous components. They are supposed to have an ANF-action, but the main agent(s) that induce the reduction in growth and its mechanism of action have never been clearly identified. Polyphenols from CoP are known to bind protein and iron, and this may reduce their bioavailability to the animal (De Rozo *et al.* 1985, Vélez *et al.* 1985).

Several treatments applied to CoP to reduce or destroy its ANF's have shown promising results. Chemical treatments proved to be rather effective to reduce some of the ANF's (Murillo *et al.* 1977, Gómez *et al.* 1988). To date, biological treatments (fungi solid-state fermentation, the inoculation of CoP with specific micro-organisms, the use of insect larvae) yielded the best results in reducing ANF's (e.g., polyphenols, tannins and caffeine), lignin and cellulose in CoP (Peñazola *et al.* 1985, Tauk 1986, López & Pabón 1986, Rolz *et al.* 1988, Lardé 1990). In addition, the increase in protein content in the final product is an extra benefit obtained from the biological treatments. None of the above-mentioned methods, however, resulted in complete reduction of the ANF's and fibres in CoP.

In Costa Rica, tilapia represents about 98% of total fish culture production (11800 MT for 2000). The most important tilapia species cultured in the country are *Oreochromis niloticus*, *O. aureus* and a red hybrid, which have similar food habits. Both *O. niloticus* and *O. aureus* grow at a similar rate under similar culture conditions. Tilapia species also readily accept diets with a high proportion of vegetable ingredients. For these reasons, *O. aureus* was chosen to study the feasibility of using CoP as an ingredient in tilapia diets.

Aim of this research

The present study focused on the feasibility of using CoP in diets for tilapia (*Oreochromis aureus*) reared in ponds. The first step was to identify the chemical composition of the CoP and to elucidate its limitations to use as a foodstuff for fish. CoP contains several ANF's such as caffeine, polyphenols and tannins and, in addition, high levels of fibrous components. Therefore, in a second step, the study focused on these ANF's and fibre, their critical dietary levels for *O. aureus* and the ways to upgrade the nutritional value of CoP to be used as a feed ingredient for fish.

The outline and objectives of the thesis

This thesis focused on different aspects of CoP related to fish feeding and contains six more chapters after this general introduction. The selection of *O. aureus* was based on the role it plays in the aquaculture industry in Costa Rica and on its omnivorous feeding behaviour. The first step was to select a waste that would be tested in tilapia diets. The selection of CoP as possible foodstuff ingredient was based on a detailed inventory of the main agricultural residues produced in Costa Rica during one production season (1994-1995), in which, the main wastes were identified in terms of volume and pollution risk. As a result of the variability in the chemical and the physical composition of CoP, its chemical composition was followed and analized during one harvesting season (1999-2000) (chapter 2). Literature data on growth found with tilapia fed diets containing CoP are discussed and then a comparative study under aquaria and pond conditions was done using *O. aureus* receiving feeds with untreated CoP to determine the possible causes of differences in growth performance (chapter 3). The effect of different ANF's (tannins and caffeine) and fibre contained in the CoP on the performance of *Oreochromis aureus* fingerlings was studied and their critical levels in the diets were investigated (chapter 4). The effect of several processing techniques (chemical and biological treatments) to upgrade the nutritional value of CoP were tested and results are presented in chapter 5. Finally, the processing technique that gave the best results (bacteria treatment) was applied to CoP used in diets for *O. aureus* to study its growth performance (chapter 6). In chapter 7 a general discussion on the results of this thesis is presented, which also includes several recommendations for the upscaling of the upgrading methods applied to CoP and for the use of CoP in diets for tilapia.

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Chapter 2

Agricultural residues in Costa Rica and the seasonal variability in the coffee pulp composition

Chapter 2.1

Tropical agricultural wastes and by-products, their potential uses in fish culture: the Costa Rican situation

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Abstract

In Costa Rica, the problem of disposal of agricultural wastes is widely recognized but the efforts to find solutions are not equal for the different sectors. This study discusses the situation of the agricultural residues in Costa Rica, identifying the activities that produce the higher amounts of residues and their use (if any). It also addresses the potential use of the residues. In Costa Rica, during the 1993-94 production season, the major agricultural sectors (crop, animal production and fisheries and aquaculture) generated a total amount of 3.51-3.78 million MT of residues. The agricultural residues are classified in by-products (used residues) and wastes (not used residues). Some residues are treated to turn them into valuable items or to diminish their polluting effects (e.g., the so-called by-products). About 1.76 million MT of by-products were used for: fertilization (≈122,000 MT), animal feeding (≈400,000 MT) and others purposes (1,215,000 MT, e.g., as fuel, as substrates in greenhouses and for human consumption). However, the remainder (1.82-1.99 million MT) was discharged into terrestrial and aquatic ecosystems causing environmental pollution. This part of the residues constitutes the real wastes. About 1.21-1.52 million MT wastes came from the major crop systems (banana 388,000-575,000 MT, coffee 436,000-452,000 MT, sugarcane 298,000-313,000 MT and oil palm 89,300 MT). In animal production, the main wastes are pig manure (≈116,900 MT), an unknown volume of dairy production wastes, slaughtering wastes (38,570-40,170 MT) and 0.35-0.68 million liter of blood. Wastes from aquaculture and fisheries reached 13-14,000 MT of which 64-69% were used in animal feeding. More recently, during the 1998-1999 production season, the banana, coffee and sugarcane industries generated 477,700-654,500 MT, 406,000-412,800 MT and 383,100-388,200 MT of wastes, respectively. Unfortunately, most of the studied wastes contain high levels of moisture and low levels of protein. Also most of these wastes contain variable amounts of antinutritional factors (e.g., polyphenols or total phenols, tannins, caffeine and threobromine), high fibre levels and some toxic substances and pesticides. All these reasons may limit the use of these agricultural wastes for animal feeding, especially in fish feeds. Some of these wastes may be recycled and used as animal foodstuffs instead of being disposed off. From a nutritional point of view, the most interesting wastes are green banana, coffee pulp and oil palm wastes. They also constitute important wastes from an environmental point of view. In aquaculture, agricultural wastes have been used as feed ingredients, whole feeds, and supplementary feeds or as fertilizers in different production systems. Fishponds may turn these wastes into productive assets (fish, fertile water and mud) reducing the waste disposal into natural ecosystems. The potential use of vegetable and animal residues in fish feeding is discussed based on their nutritional composition, on their amount available over the year and on their pollution risks.

Keywords: agricultural wastes, fish culture, Costa Rica, proximate composition.

Introduction

Agriculture generates residues, which can be used (by-products) or disposed off (wastes). Crop and animal wastes are a recognized source of environmental pollution and, therefore, efforts are made to use or recycle them. In Costa Rica, these efforts vary in the different regions and do often not keep pace with production.

One of the most logic outlets for (re-)using agricultural wastes and/or by-products is in animal feed production. Especially in areas where low market prices for animal products force farmers to use inexpensive feeds, the use of so-called low value crop wastes might become interesting.

According to their nutritional composition, volume and pollution risk, some wastes like green banana, coffee pulp and mucilage, sugarcane and fruit processing wastes may have a potential for use in animal feeds, including fish feeds. In many regions of Asia and Africa, wastes from crops, agro-industry, animal production and humans are commonly used in aquaculture. They are used as feed ingredients, as supplementary feeds or as fertilizers (Ravishankar & Keshavanath 1986, Wohlfarth & Hulata 1987, Subosa 1992, Sehgal & Sharma 1993, Tacon 1993).

However, the use of agricultural wastes may be restricted by nutritional aspects (e.g., high fibre and low protein levels) and presence of antinutritional factors (ANF's) (e.g., polyphenols or total phenols, tannins, caffeine), which may limit their inclusion at high levels in animal feeds. Fortunately, the ANF's can be destroyed, thereby increasing the nutritional value of the wastes. Other negative aspects of using agricultural wastes in animal feeds are health risks to farm workers, public rejection, and toxic substances and pesticides, which, in the case of aquaculture, may accumulate in pond sediments and fish (Ogbondeminu & Okoye 1992, Pullin 1993).

To evaluate the potential of the most important animal and crop residues from Costa Rica as foodstuffs for fish diets, an inventory was made to collect information on their volume, their availability over time and their present use. In addition, the proximate composition of various wastes was determined.

The aim of this study was to determine the amounts of residues generated by the most important agricultural industries (crop and animal production) in Costa Rica and to identify the sectors with the highest production of wastes and their pollution risks. At the same time, the potential use of these wastes in animal feeding, especially in fish feeding, was evaluated based on their nutritional composition and on the presence of ANF's.

Materials and Methods

The major residues in Costa Rica were identified from an inventory realized during 1995 by Ulloa (1997). The collected data referred to the 1993-94 production season. The inventory included type and amount of residues, quantities used or disposed off (on/off-farm) and seasonality. The residues came from (a) crop cultures: banana, coffee, sugar cane, palm oil, rice, fruits (specially pineapple), (b) livestock production: cattle (dairy and beef), pigs and poultry (eggs and meat) and (c) fisheries and aquaculture. The amount of wastes generated by the major crop activities (banana, coffee and sugarcane) in the 1998-1999 season is presented only for comparative purposes.

All types of residues were collected directly from their respective processing plants. The residues were dried at 100 °C, ground at 1-mm particle size and stored in desiccators for further analysis. The proximate composition (crude protein, crude fat, crude fibre, crude ash and moisture) of various residues was determined by standard methods (AOAC 1990). The NFE was calculated by difference between the sum of the above parameters and total wet weight. The "total utilizable carbohydrate" (starch and sugars) was measured by the Anthrone method and expressed as glucose (g kg^{-1}) (Osborne & Voogt 1986). In some cases, the proximate composition of residues was obtained from their producers and this was indicated in the corresponding tables. All chemical determinations were done in triplicate and expressed as g kg^{-1} of dry matter (DM).

To select the most relevant residues from the inventory (Table 1), the following criteria were considered: (a) their total amounts produced and amounts disposed off, (b) their use in animal feeding, (c) their nutritional composition and (d) their environmental risks. The wastes with a proven potential to use in animal feeds were more specifically evaluated.

Results

The major agricultural residues in Costa Rica

From an economic point of view, banana, coffee, and milk and beef industries represent a large proportion of the total Costa Rican export income. In the 1993-94 season, residues from the most important agricultural and livestock sectors reached 3.5 to 3.8 million MT (Ulloa 1997). About 1.8-2.0 million MT of wastes were disposed into the environment. From this amount, crops contributed 1.3-1.6 million MT.

The major amounts of wastes were generated by the banana, coffee, sugarcane and oil palm industries, with annual production volumes of 388-575,000 MT, 436-452,000 MT, 298-313,000 MT and 89,300 MT, respectively. Livestock generated about 159-162 thousands MT of wastes (Table 1). In the 1998-1999 season, the major crop activities (banana, coffee and sugarcane) presented a moderate increment in the amount of wastes generated, except for

coffee: 477,700-654,500 MT for banana, 406,000-412,200 MT for coffee and 383,100-388,200 MT for sugarcane.

Table 1. Residues (wastes and by-products) from the most relevant agricultural activities in Costa Rica during the season 1993-94.

Residues [1]	Amount (x 1000 MT)	Used-on farm (%)	Used-off Farm (%)	Disposed on-farm (%)	Disposed off-farm (%)
Plant origin					
Banana	907-973	0	41-55	43-59	Few
Oil palm	147-149	23	17.0	61	0
Coffee	532-547	16-18	0	55-57	26-27
Sugarcane	1,444-1,520	<72.0	>11	≈21	0
Fruits	44-46	0	0.5	88	<12
Cocoa	36-37	Few	≈ 2.0	94	> 4.5
Rice	86-87	0	41-44	0	56-59
Brewery	[2]	0	100	0	0
Subtotal:	3,196-3,360	35.5-36.4	17.8-18.5	36-40.8	3.9-6.3
Animal origin					
Aquaculture	2	0	≈96	Few	> 4
Fisheries	11-12	60.0	5	0	34-37
Cattle	53-55	35-36	≈1.5	51-52	12-13
Piggery:					
Residues	46-47	90	Few	9	0.5
Manure	117-118	0.5	0	Few	<99
Poultry:					
Residues	26-30	60-69	29-38	0	1.5-2.0
Manure	36-40	36	64	0	0
Subtotal:	291-304	32.9-34.5	11.7-13.4	10.9-11.1	42.5-43.5
TOTAL:	3,487-3,664	35.4-36.1	17.5-18	33.8-38.3	7.2-9.3

[1] *Banana*: green banana, peels, seeds and bunches. *Oil Palm*: kernel meal, kernel spill, hulls, mesocarp and bunches. *Coffee*: pulp, seed husks and mucilage. *Sugarcane*: harvesting residues (variable amounts of leaves, stalk pieces, flowers sticks), bagasse, molasses, mudpress and boiler ash. *Fruit Processing*: pulp, seeds and peels from orange, pineapple, mango, papaya. *Cocoa*: seed shells, pods and fermented liquid. *Rice*: hulls, bran and broken rice. *Brewery*: a mixture of yeast and fermented grains of wheat and malt, it did not include the sludge. *Aquaculture*: shrimp and tilapia processing residues. *Fisheries*: fish and shrimp processing residues. *Cattle, piggery and poultry residues*: e.g., blood, bones, hoofs, digestive tract and content, rumen content, fat, feathers. *Pig manure*: solid fraction of the manure of pigs receiving a diet based on a mixture of maize/soybean. 5.8-9.6% of 5.2-6.5 million liter cattle blood and 9-10% of 0.55 million liter pig blood were not used.

[2] Data were not available.

Residue treatments and use

Apart from the use of some residues (by-products) as animal foodstuffs and feeds, fertilizers and/or soil conditioners (Table 2), wastes are also often buried in holes or in municipality dumps. The amounts of the latter types of disposal are unknown. The use of residues as animal foodstuffs and/or whole feeds represented about 10.7-11% of total residues (≈400,000 MT), whereas the use of residues for fertilizers and/or soil conditioners only

reached 5-5.1% (≈122,000 MT). Residues are also used as energy sources in the same processing plants, as substrates in green houses and in poultry enclosures, as fillers in food technology and/or for human consumption (1,215,000 MT, 32.4-33.6% of total residues). The remaining amounts (about 51%) are usually spread on open fields (e.g., oil palm, coffee pulp, sugarcane and banana wastes). In some cases, treatments of wastes in bioreactors, oxidation lagoons, etc. are done before disposal.

Despite large amounts of residues are used as fertilizers (≈ 122,000 MT), for animal feeds (≈ 400,000 MT) and for others purposes (1,215,000 MT), about 50% (1.82-1.99 million MT) was not used and disposed off (Ulloa 1997) (Table 1 and 2).

Table 2. Major individual agricultural residues produced (MT x 1000) in Costa Rica during 1993-94 production season and their proportional use (%).

Residues [1]	Total amount	Animal feeding	Fertilization	Other uses
Vegetable origin				
Coffee pulp	350-365	0	15-17	<1
Coffee mucilage	142	0	15-17	0
Coffee hulls	40	0	0	100
Green banana	585-612	≈ 2-5	0	39-50
Banana peels	32	≈ 30	0	0
Oil palm kernel/hulls	30	50	0	50
Oil palm mesocarp	28	0	<2	>98
Sugarcane mudpress	99	0	Unknown	0
Sugarcane molasses	120	100	0	0
Sugarcane Bagasse	995	0-5	0	<100
Orange/pineapple	39	0	<1	1
Cocoa pods/seed shells	35	1.5	<1	0
Rice hulls	54	0	0	5-10
Rice bran/broken rice	32	100	0	0
Brewery residue	[2]	100	0	0
Subtotal:	2,266-2,291	8.3-11.2	3.3-3.8	57-60.3
Animal origin				
Cattle slaughtering	53-55	34-36	0	<1
Cattle/pig blood	5.7-7 million L	5.3-6.4 million L	0	0
Poultry residues	18.5	100	0	0
Poultry manure	36-40	<3	95	0
Piggery residues	5	11	0	0
Piggery manure	117	<1	0	0
Fish/shrimp wastes	13-14	64-69	0	0
Subtotal:	242.5-249.5	19.6-20.4	10-15.2	≈ 0.2
Total:	2,508-2,540	9.4-12.1	4.4-4.9	51.3-54.4

[1] Residues without any use were not included.

[2] Data were not available.

Nutrient composition of agricultural residues

Proximate composition, energy content and presence/absence of ANF's are given in

table 3 and 4. Most of the animal and crop residues have a high level of moisture (above 700 g kg^{-1}) as they are obtained *"in situ"*.

From the residues of animal origin, the slaughtering wastes were characterized by high ash and protein contents. In contrast to most residues, poultry manure was relatively dry with a moisture content of only 160 g kg^{-1}. Manures and the slaughtering sludge had high ash (except for pig manure) and fibre levels but the sludge also contained a relatively high protein and fat contents (Table 3). The highest gross energy content was found in the tilapia residues (24.2 kJ g^{-1}) and in the blood (23.2 kJ g^{-1}) and the lowest gross energy content was determined in the poultry manure (12.9 kJ g^{-1}). The potential digestible energy for fish also presented the same trend as gross energy.

Table 3. Proximate composition (g kg^{-1}, DM) and energy content (kJ g^{-1}, DM) of several animal residues.

Component / Residues	Gross energy [1]	Energy for fish [2]	Crude ash	Crude fibre	Crude fat	NFE	Crude protein	Moisture (g kg^{-1})
Shrimp residue	18.2	7.9	330	157	29	29	456	730
Tilapia residue	≈24.2	13.5	259	≈10	150	20-30	560	700
Cattle-piggery blood [3]	≈23.2	14.2	29	≈5	≈5	≈5	956	796
Slaughtering sludge	13.5-19	9-11.9	130-230	160-270	110-190	170-410 [4]	70-250	810-920
Poultry manure [3]	14.6	6.7	193	282	20	323	183	160
Pig manure	17.8	7.4	59	399	52	375 [4]	115	759

(1) Gross energy (kJ g^{-1}): protein (g kg^{-1}) x 23.87 + lipid (g kg^{-1}) x 39.78 + NFE (g kg^{-1}) x 16.87 (DM basis).

(2) Potential digestible energy for fish, calculated according to digestible energy coefficients for *Ictalurus punctatus*, 14.6 kJ g^{-1} protein, 33.9 kJ g^{-1} fat and 10.5 kJ g^{-1} crude carbohydrate (N.R.C. 1977).

(3) Proximate composition provided by residue producers.

(4) Measured as "total utilisable carbohydrate" by the Anthrone method of Clegg and expressed as glucose (Osborne & Voogt 1986).

Most of the crop residues contain several ANF's (e.g., phenols, tannins, caffeine, and threobromine) and high fibre levels. The fruit residues such as banana, orange, and pineapple are mainly characterized by a high level of "carbohydrate" (as NFE), a low level of protein (except for papaya) and by an intermediate fibre content (except for some banana residues) (Table 4). Coffee, cocoa and sugarcane wastes contained high fibre levels and low protein and energy contents. The brewery residues showed high protein, fibre and NFE levels. Papaya residues had the highest gross energy content; followed by the oilpalm kernel meal, green

banana meal, brewery and some fruit residues (17.8 to 21.7 kJ kg^{-1}). Theoretically, the digestible energy for fish is also higher in these residues and accounts for more than 52% of the gross energy whereas for the other wastes this is less than 50%.

Table 4. Proximate composition (g kg^{-1}, DM), energy content (kJ g^{-1}, DM) and the presence of ANF's in several crop residues.

Components / Residues	Gross energy [1]	Energy for fish [2]	Crude ash	Crude fibre	Crude fat	NFE	Crude protein	ANF's	Moisture (g kg^{-1})
Oilpalm kernel meal [3]	19.6	12.4	30	60	100	677	133	No	61
Oilpalm hulls [3]	≈ 17.7	6.2	24	497	40-50	390-410	33	Yes	124
Coffee Pulp	16.9	4.8	89	571	29	189 [4]	122	Yes	850
Sugarcane: [3]									
Bagasse	16.6	7.6	30-40	430	≈ 10	≈ 500	20-30	Yes	450-500
Molasses (wet basis)	≈ 12.8	7.3	92	60	5	500-600	96	No	160
Harvesting residues	16.5	6.7	59	345	10	532	54	Yes	700
Rice hulls	≈14.7	3.6	179	537	10-20	194 [4]	75	Yes	109
Pineapple	17.5	9.6	37	150	39	724	50	Yes	840
Papaya	21.7	13.4	86	182	205	295 [4]	232	Yes	>800
Green banana	17.8	11.4	49	16	58	818	59	Yes	810
Other fruits	14.5-17.9	7.5-9.7	28-42	188-389	52-54	438-687	45-77	Yes	810-840
Cocoa pods [3]	16.1	7.5	86	243	8	598	66	Yes	850
Cocoa seed hulls [3]	17.8	8.5	121	245	53	422	159	Yes	117
Brewery residue [3]	19.5	10.2	37	232	60-70	418	248	Yes	> 750

[1], [2], [3], [4] As in table 3.

Discussion

Fishponds can be an alternative for using agricultural residues. Recycling wastes through culturing species like tilapia and carp may be one of the environmentally least harmful disposal methods (Pullin 1993). However, optimizing the operation of a fishpond for maximal wastes recycling will not always coincide with the measures needed for maximum fish production. Several results indicate that crop wastes can be included at high levels in diets for omnivorous fish when they are reared in extensive or semi-intensive systems (earthen ponds or cages located in ponds) (Bayne *et al.* 1976, Bautista *et al.* 1999). Also our results showed that *Oreochromis aureus* raised in pens (maintained in earthen ponds) with

diets containing 130 g kg^{-1} CoP meal grew similar to fish fed a control diet (unpublished data).

However, there are several aspects that have to be considered prior to use agricultural wastes in fish feeds. Nutritional constraints such as presence of ANF's and high contents of fibre and ash in some of these wastes can limit their use in fish feeds. Other factors that can also restrict their use are public health risks, religious and sociological constraints and toxicological features (Nash & Brown 1980, Ogbondeminu & Okoye 1992, Pullin 1993, Edwards 1993). In addition, the high moisture content of most of these wastes may increase the costs of transportation and drying, thereby reducing the economic feasibility to recycle these wastes.

Residues from animal origin

Animal residues are used as protein sources to partially substitute fish meal in fish feeds and have been recommended at levels of 200-500 g kg^{-1} in diets (Hasan *et al.* 1989, Dean *et al.* 1992, Moon & Gatlin 1994). To be suitable as protein sources in fish feeds, residues must have a large availability, a low cost, and a high nutritional quality (Dean *et al.* 1992). Despite some of the animal residues (e.g., fish and shrimp wastes, blood) found in Costa Rica did meet these requirements, part of them are still disposed off. They will be good alternatives to fish meal in fish feeds. Tilapia residues and blood may even have a particular high potential because their gross energy levels are high (24.2 and 23.2 kJ g^{-1}) and more than 55% may be digestible to fish.

Dry slaughtering sludge may also have a potential as an ingredient in fish diets. It has acceptable levels of protein, fat and NFE, resulting in a gross energy content of above 13.5 kJ g^{-1}, of which more than 62% may be digestible to fish. However, its inclusion level may be limited up to 200-300 g kg^{-1} due to high fibre and ash contents, the presence of ANF's and toxins. In some cases, also its high fat content may limit inclusion in fish diets. Also sludge from sewage has been included in carp diets at levels up to 400 g kg^{-1} (O'Grady & Spillett 1985). In Costa Rica, the sludge from slaughtering is not used and it is all disposed off in burying holes.

The use of processed manures in fish diets or as supplemental fish feeds may have potential (Watson 1985, Wohlfarth & Hulata 1987, Sehgal & Sharma 1991). Processing the raw manures can modify or alter some of their characteristics and improve their nutritional value. In Costa Rica, poultry and pig manures are treated physically (sieving, drying), thereby increasing their foodstuff value for cattle feeding. However, further consideration must be given to the physico-chemical characteristics of these wastes in relation to the nutrient requirements and digestion of fish. Depending on the type of diet given to pigs and poultry, these manures can contain ANF's, which limits their inclusion in fish diets, as does the low potential digestible energy to fish (less than 44%).

Residues of plant origin

Many crop residues contain ANF's, high levels of lignin and cellular wall components (e.g., cellulose, hemi-cellulose, pectines). In animals, the main negative effects of these ANF's are 1) tannins and polyphenols bind proteins and minerals (iron) forming indigestible complexes, 2) the caffeine produces a diuretic effect and increases the nitrogen discharge, 3) tannins and caffeine are responsible for a bitter taste of the diet and astringency of the animal, 4) high fibre levels reduce gut nutrient absorption, 5) high K levels impair the absorption of other nutrients and 6) lignified protein reduces the protein availability. When foodstuffs containing some of these ANF's are included at high levels in animal diets, they can induce intoxication and low feed palatability and utilization (Tacon 1993).

Upgrading of crop wastes by biological, chemical or physical treatments (or their combinations) may be necessary before being used in fish feeds. Several physical (heating, drying, soaking in water, lyophilization, cooking), biological (fermentation, ensilage, solid-state fermentation, fungi) and chemical (alkali and acids) processing techniques have been used to improve the nutritional value of these wastes. Some of these treatments were effective in destroying or reducing the ANF's levels in wastes like coffee pulp, poultry and fruit processing wastes (López & Pabón 1986, El Boushy & van der Poel 1994).

Vegetable proteins were successfully used to substitute fish meal in fish diets, e.g. oilseed cakes or meals (linseed, rapeseed, mustard, sesame, cotton seed, soybean, peanut) and protein concentrates (Jackson *et al.* 1982, Tacon & Jackson 1985, Tacon 1993). Their main constraint is usually a high level of ANF's (Krogdahl 1989, Hossain & Jauncey 1989, Tacon 1994). Processing and pretreatment to reduce or destroy their ANF's have reduced significantly their negative effects on fish growth.

Papaya and brewery residues may have a potential as protein/energy sources because of their protein content (232 and 248 g kg^{-1}) and their amounts of potential digestible energy for fish (13.3 and 10.2 kJ g^{-1}). Also other vegetable wastes can be used as energy sources, since their gross energy content is high and comparable to that of animal wastes. However, the potential digestible energy for fish from crop wastes is often highly reduced because a large proportion of their gross energy comes from fibre (over 340 g kg^{-1}). Only oilpalm kernel, papaya, green banana and brewery residues have both high gross energy levels and high potential digestible energy for fish due to lower fibre levels.

In the next paragraphs, some of the major crop wastes found in the country are studied into more detail because of their volume available, their high pollution risk, their potential to be used as foodstuffs and their facility to be collected. In addition, some of these wastes have been already tested in experimental diets for fish and these results that have been obtained with different fish species and culture systems are presented and discussed. According to the mentioned criteria, coffee pulp (CoP), cocoa wastes, brewery and fruit processing residues

and banana wastes were selected for further analyses.

Coffee pulp (CoP)

CoP has been included in diets for tilapia, Cachamay (*Colossoma* x *Piaractus*), common carp and African catfish reared in different systems. The growth responses found with these fish have been variable. Fish reared in earthen ponds and receiving diets containing 300 g kg^{-1} of CoP (tilapia, Bayne *et al.* 1976) or up to 200 g kg^{-1} of CoP (Cachamay, *Colossoma* x *Piaractus*, Bautista *et al.* 1999) showed a similar growth as fish fed control or commercial diets. However, common carp (300 g kg^{-1} CoP diet) and African catfish (10-300 g kg^{-1} CoP diets) reared in aquaria and concrete tanks, respectively, showed a reduced growth and feed utilization (Christensen 1981, Fagbenro & Arowosoge 1991). These differences in growth and feed utilization may be related to fish species and culture management practices. Therefore, more research is needed to elucidate the feasibility of using CoP in fish diets, especially in diets for omnivorous species, including treatments to reduce its ANF's and fibre content. CoP also showed a low level of potential digestible energy for fish (28 % of gross energy) and that could limit its use in fish feeding. Therefore, treatments to increase this digestible energy to fish, especially focusing on the reduction of the fibre content in CoP are most advisable.

CoP has also shown great variability in its chemical composition (Bressani *et al.* 1972, Elías 1979, Ramírez 1987, Ramírez 1988, Clifford & Ramírez 1991, Clifford *et al.* 1991, González *et al.* 1994). Therefore, these sources of variation must be considered when studies involving CoP are carried out or when comparisons between studies are made.

Cocoa wastes

Cocoa-pod husk, its meal and cocoa cakes have a potential value as supplemental feed or diet ingredient in the culture of tilapia, catfish and common carp (Fagbenro 1988a,b, Fagbenro 1992). Levels of these wastes lower than 250 g kg^{-1} in the diet are recommended because higher levels caused a reduced nutrient digestibility due to high fibre (250 g kg^{-1}) and threobromine contents. As a result of their high fibre content, the potential digestible energy to fish from these wastes is lower than 47% of gross energy. To improve the nutritional quality of these wastes, by reducing their ANF's and increasing their digestible energy to fish, the application of specific biological treatments are most advisable.

Brewery and fruit processing residues

Brewery residues (mixture of yeast, malt and wheat) are considered a viable alternative to fish meal or soybean meal in fish diets (Vries *et al.* 1988, Toledo & Gónzalez 1988, Pouomogne *et al.* 1992, Webster *et al.* 1993). In Costa Rica, these residues are often used in pig feeding. They have an acceptable protein content (≈250 g kg^{-1}), high levels of

NFE (≈ 420 g kg^{-1}) and gross energy (≈ 19.5 kJ g^{-1}) and low ash levels (≈ 40 g kg^{-1}). Unfortunately, their high fibre levels (≈ 230 g kg^{-1}) may limit their inclusion in fish diets to levels not higher than 200-250 g kg^{-1} and may reduce their potential digestible energy for fish to about 52% of gross energy. Also these residues must be pre-treated with biological treatments to reduce their contents of fibre. Sludge from breweries was successfully added in carp and catfish diets at levels up to 400 g kg^{-1} (Anwar *et al.* 1982, Vries *et al.* 1988). However, in Costa Rica the sludge from breweries is mainly used in pig feeding but much of the sludge from fruit processing is not used and is disposed off in burying holes. The main limitation to use the sludge from fruits in fish feeds is its high ash content and, in some cases, also its high fat content. For this reason, inclusion levels of fruit sludge should be no more than 300 g kg^{-1}. The sludge may potentially have some toxic substances like heavy metals but this has to be proven case by case. Residues from fruit processing (pulp, seeds and skins) may have potential for fish diets because of their high levels of gross energy but most important, because of their high proportion of potential digestible energy for fish (51-55% of gross energy). Because they usually also contain a variable amount of ANF's (mainly polyphenols and tannins), is advisable to apply treatments to reduce these ANF's.

Banana wastes

Green banana (GB) meal contains a high carbohydrate level (NFE ≈820 g kg^{-1}) and low levels of ash and fibre and it can be considered as good potential energy source (≈17.8 kJ g^{-1}) for fish diets, especially in diets for omnivorous species. In addition, a high proportion of its gross energy (64%, 11.4 kJ g^{-1}) may potentially be digestible for fish. The content of tannins in GB may vary depending on the banana strains, plantation management and grade of ripeness. Therefore, in some cases, GB must be processed or pretreated to reduce it tannins content before using it at high levels in fish feeds.

Conclusions

In Costa Rica, GB and CoP constitute the most relevant residues. Any attempt to use them will reduce the amount disposed to the environment and, in return, produce extra benefits. Based on their chemical composition, both wastes have potential as foodstuff, but CoP presents more "bottlenecks" (e.g., presence of polyphenols, tannins and caffeine) and, high fibre and K contents than GB (Tables 5 and 6).

Table 5. Chemical composition of green banana and coffee pulp meals (g kg^{-1}, DM). Sources: Elías (1979), Giorgetti & Ponzetta (1987), Bayne *et al.* (1976), Suntharalingam & Ravindran (1993) and own analysis.

Component	Green banana meal	Coffee pulp meal
Moisture (g kg^{-1})	808-881	767-850
Crude protein	31-59	80-148
Crude fat	14-58	12-49
Crude fibre	16-22	128-276
Crude ash	33-53	50-105
NFE	812-826	158-374
Cell wall constituents	(1)	314-556
C:N ratio	21.3	19.8-27.7

(1) Not determined.

Table 6. Polysaccharides and ANF's characterization of coffee pulp and green banana meals (g kg^{-1}, DM). Sources: Murillo *et al.* (1976), Elías (1979), Giorgetti & Ponzetta (1987), Suntharalingam & Ravindran (1993), González *et al.* (1994), Pulgarin *et al.* (1991).

Components	Green banana meal	Coffee pulp meal
Crude fibre	16	128-276
NDF	85-106	340-368
ADF	38-72	305-345
Cellulose	29-39	165-322
Hemicellulose	47-54	10-116
Pectines	(1)	60-65
Lignified protein	(1)	30
Lignin	9-10	122-205
Caffeine	0	5-20
Polyphenols	(2)	(2) (3)
Caffeic acid	(1)	5-16
K	20-40	14-37

(1) Not determined.
(2) Tannins in banana meal (≈ 10 g kg^{-1}) and in CoP meal (14-86 g kg^{-1}).
(3) Chlorogenic acid: 26-34 g kg^{-1}.

Despite its lower nutritional quality, CoP has already been studied for its use in fish culture, as previously discussed. Because of the physico-chemical differences between CoP and GB, CoP poses a higher pollution risk than GB. The CoP can loose about 260 g kg^{-1} of its wet weight (by leaching) during transportation (Vázquez 1997) and when it is immersed in aquatic environments it can easily loose about 50 % of its weight in five minutes (fine and loose particles and soluble components). In addition to its higher pollution risk, CoP contains higher levels of ANF's and cell wall components (cellulose, hemicellulose, neutral and acid detergent fibre: NDF and ADF) than GB (Tables 5 and 6). Lignified protein (lignin binding-protein complexes), lignin and pectines are also largely present in CoP and may interfere with its use in fish feeding, since these components reduce the digestibility of feeds. Consequently,

utilization of CoP as a feed ingredient in fish diets asks for more research than GB.

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Chapter 2.2

Influence of the season and drying method on chemical composition of coffee pulp

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Abstract

Coffee pulp (CoP) was analysed and checked for monthly composition changes during the 1999-2000 harvesting season in Costa Rica. Two drying methods were compared: sun-drying and oven-drying. Samples of fresh CoP were taken from the processing plant last week of every month. For drying methods, samples were taken from the same batch of CoP. The sun-dried CoP showed higher crude and true protein, ash, neutral and acid detergent fibre contents than oven-dried CoP ($P<0.05$). The sun-dried CoP also showed lower cellulose, total phenols, tannins and caffeine contents than oven-dried CoP ($P<0.05$). Differences between both drying methods appear to be due to the drying time, which was mainly affected by the rainy season. The latter prolongated the sun-drying method for six days. This allowed some degradation by micro-organisms and also some leaking of substances which could produce changes in the chemical composition of CoP. The moisture and fibre contents of the monthly CoP samples decreased along harvesting season but protein contents remained similar. Carbohydrate, total phenols and tannin contents increased from November to January. The latter differences are related to changes in the depulping process and the start of the dry season because from November onwards the depulping process was done without any use of water. Consequently, water soluble components, part of the mucilage and loose particles were not washed out from the coffee pulp.

Keywords: coffee pulp, drying methods, chemical composition, seasonal variability.

Introduction

Coffee pulp (CoP) is the first residue obtained during processing of coffee berries, and it represents about 40% of the whole berry weight (on weight wet basis) (Montero 1992). The physical composition of CoP as it is obtained from the processing units is variable. Apart from differences in the proportion of ripe / unripe berries, it also may contain impurities picked up with the berries such as pieces of leaves, fruit pedicels, stack pieces and some grasses. Depending on the adjustment of depulping devices, the pulp may also contain variable proportions of coffee hulls with and without silver skin, and impaired coffee grains and grain pieces.

Changes in the chemical composition of CoP are related to differences in processing methods, in coffee varieties, in culture practices, in harvesting time, in coffee storage time at the processing unit and, in extraction and analytical methods (Elías 1979, Abate 1988, Ramírez 1988, Ramírez & Clifford 1989, Clifford & Ramírez 1991a,b). Also the method to dehydrate CoP differs from place to place (Bressani *et al.* 1975). In studies on the use of CoP, these sources of variation in composition have not been identified nor been taking into account in feeding trials in animals. Therefore, in this study, monthly CoP samples were analysed for chemical composition changes during part of the 1999-2000 harvesting season in Costa Rica. Moreover, two drying methods were compared viz. sun-drying and oven-drying.

Materials and Methods

Monthly coffee pulp sampling

Triplicate random samples of fresh CoP were taken the last week of every month from the same processing plant. Samples were taken from a common outlet channel (same batch). After one hour of transportation, they were immediately dried at 60 °C for 24 h in an oven with recirculating air, and then samples were milled at 1-mm particle size and stored in a desiccator for further analysis. The CoP sampling lasted from August 1999 till January 2000.

Drying methods

Two common drying methods were applied in triplicate to monthly samples: sun-drying and oven-drying. For sun-drying, CoP was spread equally on flat containers and exposed to the sun until it got dried (about 12 g kg^{-1} moisture). Oven-drying of CoP was performed at 60 °C for 24 h in an oven with recirculating air. Both sun-dried and oven-dried CoP was milled at 1-mm and stored for further analysis.

Chemical analyses of CoP samples were done in triplicate. To determine the true protein levels in CoP, the samples were boiled in water to dissolve the amides present. After boiling, the protein was precipitated in the presence of copper (II) hydroxide and

subsequently, separated from the dissolved amides by filtration (Stutzer & Barstein 1900). The N content of the filtrate was determined according to the Kjedahl method. Caffeine was measured according to Morris (1973), polyphenols (total phenols) and tannins were determined using the methods of Slinkard & Singleton (1977) and Field (1991), carbohydrate content was determined as "Total Utilisable Carbohydrate" by the Anthrone method of Clegg and expressed as glucose (g kg^{-1}) (Osborne & Voogt 1986). The other chemical components (neutral and acid detergent fibre (NDF and ADF), cellulose, crude protein, crude fat, crude ash) were determined using AOAC (1990) procedures.

Data analysis

Data from the drying methods and from the CoP samples taken at various harvesting time were analyzed separately by a one-way ANOVA. Treatment means were compared using the LSD test with 95% confidence intervals. The statistical analysis was done using the software STATGRAPHICS 7.1 PLUS.

Results and Discussion

Differences in the drying methods of CoP

The sun-dried CoP showed higher crude and true protein, ash, NDF and ADF contents than oven-dried CoP ($P<0.05$). Contrary to this, the sun-dried CoP showed lower cellulose, total phenols, tannins and caffeine contents than oven-dried CoP ($P<0.05$) (Table 1). True protein values were around 60-66% of the crude protein content, which agrees with López & Pabón (1986). The amount of amides can be estimated to be around 36 and 41 g kg^{-1} for both oven-dried and sun-dried CoP. Differences between both drying methods appear to be due to the drying time. During the rainy season the sun-drying of CoP lasted six days whereas during the dry season it could last two days or less. During this period of six days some microbial growth could happen and biodegradation (aerobic decomposition) of CoP could take place, possibly resulting in higher protein content and lower contents of antinutritional factors (ANF's), such as cellulose, total phenols, tannins and caffeine. Similar results were found by Rolz *et al.* (1982) and Tauk (1986). During the sun-drying process, a drainage liquid was produced which most probably contained some water soluble components such as caffeine, some total phenols and sugars. This leaching may have contributed to the reduced levels of the mentioned compounds found in sun-dried CoP.

Proximate composition and cell wall components of oven-dried CoP samples showed values (on dry matter basis) similar to those found by Elías (1979), Pulgarin *et al.* (1991), Gathuo *et al.* (1991). From the cell wall polysaccharides of oven-dried CoP, ADF and NDF remained between normal values. However, cellulose gave a higher value than usually found in literature (Elías, 1979) (Table 1). Caffeine content in oven-dried CoP was higher but total phenols (polyphenols) and apparent tannins were much lower than those reported by Molina

et al. (1974), Murillo *et al.* (1976), Elías (1979), Bressani & Braham (1980), Zuluaga & Tabachi (1980), Clifford & Ramírez (1991a,b), Clifford *et al.* (1991). García *et al.* (1985) and Ramírez (1988) found that polyphenols contents varied between 6.5 g kg^{-1} to 29 g kg^{-1} and 6 g kg^{-1} to 20 g kg^{-1} in 12 coffee cultivars, respectively. Ramírez (1987) also showed somewhat higher polyphenols values (24.5-31.8 g kg^{-1}) in eight coffee cultivars.

Table 1. Chemical composition and content of ANF's of oven-dried and sun-dried CoP. Data expressed as g kg^{-1} dry matter basis (means ± standard error).

Components [1] \ Drying Method	Oven-dried CoP	Sun-dried CoP
Carbohydrate [2]	186 ± 0.32 [a]	133 ± 0.53 [b]
True protein [3]	80 ± 0.12 [a]	119 ± 0.32 [b]
Crude protein	121 ± 0.82 [a]	155 ± 0.35 [b]
Crude fat	29 ± 0.21 [a]	30 ± 0.09 [a]
Crude fibre	165 ± 1.12 [a]	207 ± 0.81 [b]
Crude ash	89 ± 0.22 [a]	115 ± 0.12 [b]
NFE	596 ± 2.51 [a]	493 ± 1.76 [b]
NDF	390 ± 0.05 [a]	481 ± 4.12 [b]
ADF	384 ± 0.60 [a]	468 ± 2.91 [b]
Cellulose	286 ± 1.0 [a]	177 ± 1.68 [b]
Total phenols	20 ± 0.0 [a]	10 ± 0.07 [b]
Tannins	7.4 ± 0.08 [a]	3.5 ± 0.01 [b]
Caffeine	18.0 ± 0.06 [a]	13.1± 0.04 [b]

(1) Means in the same line with different letters are significantly different ($P<0.05$).

(2) Carbohydrate refers to starch and sugars expressed as glucose.

(3) True protein method discriminates amides from the crude protein method.

In the present study, the content of NFE was similar to that found in literature (493 g kg^{-1} for sun-dried and 596 g kg^{-1} for oven-dried CoP). However, a detailed review of carbohydrate components of CoP (starch and non-starch polysaccharides) reveals that the measured crude fibre fraction was too low or lower than the sum of its different components (cellulose, lignin and part of hemicellulose). It must be considered that in general CoP contains lignin levels between 122-205 g kg^{-1} of DM (Murillo *et al.* 1976, Elías 1979, Zuluaga & Tabachi 1980). This discrepancy is believed to be due to errors associated with the traditional crude fibre method. According to Spiller (1988) and Bach Knudsen (2001), this method is not precise enough for fibre rich residues and, because of the solubilisation of structural polysaccharides and lignin; this method only measures a small and variable fraction of fibre components. A more detailed appraisal of the carbohydrate structure in foodstuffs with expected high cell wall content is, therefore, preferable.

Monthly variation in CoP composition

Moisture content of CoP samples decreased along harvesting season. It can be related to the changes into the depulping process introduced in November, e.g., improvement of the machine and no use of water, thereby reducing the water content of the pulp.

Protein contents remained similar during the sampling period but lipid levels showed two peaks viz. in September and November. The increment in the "carbohydrate" values from November may also be related to changes in the depulping process and the start of the dry season. Due to these changes the soluble sugars were not completely washed out from the CoP. These changes also reduced the amount of dissolved and suspended solids in the residual water of the entire process (Vázquez 1997). Another factor that may contribute to the increment in "carbohydrate" contents is the fact that CoP from the first two months contained a higher proportion of unripe pulp than the CoP from later months and, as a consequence, less sugar were present in the CoP (Table 2).

Table 2. Variation in chemical composition and in content of some ANF's in oven-dried CoP during the 1999-2000 harvesting season. Data expressed as g kg^{-1} dry matter basis. (Means ± standard error).

Months / Components[1]	August	September	October	November	December	January
Depulping system	With water	With water	With water	No water	No water	No water
Moisture (g kg^{-1})	854 ±0.02 ^{a}	857 ±0.22 ^{a}	844 ±0.27 ^{ab}	849 ±0.04 ^{ab}	829 ±0.44 ^{b}	805 ±0.38 ^{c}
Crude protein	79 ±0.14 ^{a}	82 ±1.09 ^{a}	75 ±0.13 ^{a}	75 ±0.47 ^{a}	83 ±1.11 ^{a}	81±0.08 ^{a}
Crude fat	89 ±0.36 ^{ab}	139 ±0.80 ^{b}	51 ±0.31 ^{a}	137 ±2.59 ^{b}	60 ±0.36 ^{a}	70 ±0.72 ^{a}
Crude ash	54 ±0.06 ^{a}	63 ±0.38 ^{a}	91 ±0.02 ^{b}	88 ±0.26 ^{bc}	82 ±0.25 ^{bc}	78 ±0.04 ^{c}
NFE [2]	778 ±0.56 ^{a}	716 ±0.98 ^{a}	783±2.07 ^{a}	700±1.09 ^{b}	775 ±2.11 ^{a}	771 ±1.35 ^{a}
Carbohydrate [3]	345 ±0.33 ^{a}	329 ±1.22 ^{a}	216 ±0.50 ^{b}	396 ±1.25 ^{c}	486 ±0.65 ^{d}	530 ±0.22 ^{e}
Non-starch polysaccharides	433 ±0.50 ^{a}	387 ±0.55 ^{a}	567 ±0.09 ^{b}	304 ±2.11 ^{c}	289 ±0.35 ^{c}	241 ±0.99 ^{d}
Cellulose	170 ±0.43 ^{a}	169 ±0.31 ^{a}	101 ±0.13 ^{b}	114 ±0.16 ^{bc}	99 ±0.41 ^{cd}	91 ±0.09 ^{d}
Total phenols	14 ±0.04 ^{a}	7.0 ±0.03 ^{b}	27±0.06 ^{c}	33 ±0.13 ^{d}	30 ±0.05 ^{cd}	25 ±0.31 ^{c}
Tannins	6.4 ±0.03 ^{a}	3.4 ±0.11 ^{b}	12±0.08 ^{cd}	13 ±0.07 ^{c}	11 ±0.03 ^{cd}	10 ±0.02 ^{d}
Caffeine	4.45 ±0.01 ^{ab}	4.4 ±0.02 ^{b}	4.6±0.01 ^{c}	4.4 ±0.0 ^{b}	4.55±0.01 ^{ac}	4.55±0.01 ^{ac}

(1) Means in the same line with different letters are significantly different ($P<0.05$).

(2) NFE refers to starch, sugars and non-starch polysaccharides.

(3) Carbohydrate refers to starch and sugar expressed as glucose.

Also the reduction in the fibre content may be related to changes in the depulping process. Because less soluble organic materials were washed out from the CoP during depulping (without water in the later months), the relative proportions of fibre and cellulose were reduced in the CoP. Similarly, the increase of total phenols and tannin contents in the CoP can be explained by the changes in the depulping process because they were not washed out from the pulp. Differences in the ash content of the pulp may be related with differences

in soil condition, which determine the type of minerals available to the plant which may vary according to period of the year (dry or rainy season) and plantation management.

Most of the differences found in the chemical composition of CoP may only confirm that the composition of CoP varies due to location (soil type, altitude), coffee strains, impurities and systems of production (Elías 1979). Most of the time, the processing plants received a mixture of different types of strains, and variations in this mixture may have contributed to the differences found in the chemical composition. For caffeine, polyphenols or total phenols and tannin contents, the extraction and analytical methods are other relevant factors explaining variation in concentrations (García *et al.* 1985, Ramírez 1987, Ramírez 1988, Clifford *et al.* 1991). For all these reasons, in studies involving CoP, these factors should be standardised or should be considered when differences are found in CoP chemical composition data. Actually, best is to analyse the chemical composition of commercial CoP and perform digestibility studies prior to use CoP in animal feeding.

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Chapter 3

Growth of *Oreochromis aureus* fed with coffee pulp diets and reared in two culture systems

Growth of *Oreochromis aureus* fed with coffee pulp diets and reared in two culture systems

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Abstract

A study was done to compare growth and feed utilization of *Oreochromis aureus* that were fed graded levels of coffee pulp (CoP) and reared in aquaria or in pens. Diets contained 0, 130, 260 and 390 g kg^{-1} of oven-dried CoP. In aquaria, fish receiving increasing dietary CoP levels (from 0 to 390 g kg^{-1}) showed a progressive reduction in final body weight, growth rate and protein efficiency ratio (PER) ($P<0.05$). The feed conversion ratio (FCR) was higher and different at 390 g kg^{-1} dietary CoP. Dietary CoP reduced the digestibility of dietary dry matter and carbohydrate. Also in pens, CoP reduced final weight, growth rate and PER but to a much smaller extend than in aquaria. Fish fed the diet with 130 g kg^{-1} CoP had similar growth and feed utilization than those fed the control diet ($P>0.05$). The high dietary fibre levels together with the presence of antinutritional factors (ANF's) in CoP diets may explain why tilapia grew less and had a lower feed utilization. The natural productivity of the pond in which pens were allocated could explain why results in pens were better than in aquaria. Results showed that the inclusion of CoP in tilapia diets might be limited up to 130 g kg^{-1} when fish are raised in earthen ponds and natural food is available.

Keywords: tilapia, coffee pulp, aquaria, pens, fish nutrition, *Oreochromis*.

Introduction

Coffee pulp (CoP) is the first residue obtained during processing of coffee berries, and it represents about 40% of the whole berry (on wet basis) (Montero 1992). The CoP has been used in animal feeds with variable results (Braham & Bressani 1979, Bressani & Braham 1980). In fish, there are few records where the inclusion of CoP was tested in a comprehensive and systematic way. Most studies used one dietary level of CoP (e.g., 300 g kg^{-1} for carp, Christensen 1981) or did a laboratory study only (e.g. graded levels from 0 to 300 g kg^{-1} for African catfish, Fagbenro & Arowosoge 1991). In both studies, a reduced growth and feed digestibility was found. The authors suggested that the antinutritional factors (ANF's) in CoP were responsible for the reduced growth of the fish. However, this suggestion was not corroborated by the results of Ulloa & van Weerd (1997) who found also reduced growth and feed digestibility in tilapia receiving graded levels of CoP (0, 130, 260 and 390 g kg^{-1}) which had been pretreated with NaOH and contained strongly reduced levels of ANF's (caffeine, total phenols and tannins). Also the latter study was performed in aquaria. However, when CoP diets were fed to fish reared in ponds, results differed from aquaria or tanks. For instance, 300 g kg^{-1} of CoP in supplementary foods did not affect the growth of tilapia reared in extensive pond culture (García & Bayne 1974, Bayne *et al.* 1976). Similar results were obtained when three CoP levels (0, 100 and 200 g kg^{-1}) were fed to Cachamay (*Colossoma* x *Piaractus*) reared in cages (located in earthen ponds) (Bautista *et al.* 1999). A possible explanation for this difference between culture systems is that in fishponds the nutritionally negative effects of CoP may be masked by the available natural food. For this reason, a comparative experiment was designed to study this topic in more detail and, graded levels of CoP were fed to tilapia grown either in aquaria or in ponds.

Materials and Methods

Two feeding experiments were conducted: one in an experimental recirculation system (aquaria) and another in pens located in a fishpond. Fresh CoP was first oven-dried for 24 h at 50 °C, milled through a 1-mm diameter die and then analyzed for chemical composition (Table 1). In both experiments, four diets were tested which contained 0, 130, 260 and 390 g kg^{-1}, respectively, of oven-dried CoP. The diets were formulated to be approximately isoproteinous (Table 2). All dry ingredients were mixed during 15 minutes before adding the lipids. Subsequently, mixing continued for another 15 minutes. Next, water was added gradually until a desirable paste-like consistency was reached. This paste was forced through a 1-mm die using a meat grinder. Pellets were then dried during 16 h at 50 °C. Finally, the feed could be easily manually crumbled to pellet size. The feeds used in the aquaria study were supplemented with 1 g kg^{-1} Cr_2O_3 for determination of apparent digestibility coefficients (ADC).

Table 1. Chemical composition and content of ANF's of oven-dried CoP. Data expressed as g kg^{-1} (dry matter basis, DM).

Components	(g kg^{-1})	Components	(g kg^{-1})
True protein [1]	110	NDF	585
Crude protein	129	ADF	546
Crude fat	20	Cellulose	183
Crude ash	62	Total phenols	20
NFE [2]	789	Tannins	7.4
Carbohydrate [3]	168	Caffeine	18

(1) True protein method discriminates the amides from the crude protein method.

(2) NFE refers to non-starch polysaccharides, starch and sugars.

(3) Carbohydrate refers to starch and sugars expressed as glucose.

Table 2. Ingredient (g kg^{-1} as feed basis) and nutrient composition (g kg^{-1}, DM) of the diets with coffee pulp (CoP) used in the feeding trials (aquaria and pens) with fingerlings of *O. aureus*.

Diets (g kg^{-1}) / Ingredients	D1(0 CoP)	D2(130 CoP)	D3(260 CoP)	D4(390 CoP)
Fish meal	420	420	430	450
Blood meal	50	50	60	60
Soybean meal	170	120	70	0
Coffee pulp	0	130	260	390
Cassava starch	310	220	130	30
Soybean oil	20	20	20	20
Vitamins [1]	20	20	20	20
Sodium alginate	0	0	0	20
Chromium oxide	10	10	10	10
Nutrients				
Moisture (g kg^{-1})	47	67	60	47
Crude fat	96	86	98	98
Crude ash	116	126	136	155
Crude protein	393	371	369	348
NFE [2]	395	416	397	399
Carbohydrate [3]	356	306	230	121
DE (kJ g^{-1}) [4]	12.7	11.6	11.1	9.7

(1) (amount /kg premix): 800000 IU vitamin A, 200000 IU vitamin D, 10 g vitamin E, 1 g vitamin K, 2 g Thiamin, 3 g Riboflavin, 15 g Pantothenate, 2 g Pyridoxine, 2 mg B_{12}, 20 g Niacinamide, 0.5 g Biotin, 200 g Ascorbic acid, 1 g Folic acid, 100 g Choline.

(2) NFE refers to non-starch polysaccharides, starch and sugars.

(3) Carbohydrate refers to starch and sugars expressed as glucose.

(4) Digestible energy: calculated according to nutrient digestible energy coefficients for *Ictalurus punctatus*, 14.6 kJ g^{-1} protein, 33.9 kJ g^{-1} fat and 10.5 kJ g^{-1} crude carbohydrate (N.R.C. 1977).

In the aquaria experiment, each aquarium (30 x 50 x 30 cm) was stocked with 12 *Oreochromis aureus* fingerlings weighing between 5 to 8 g. Fish were fed "*ad libitum*" three

times daily (9 h, 13 h and 17 h) for 8 weeks. At the beginning and at the end of the experiment, samples of fish were taken for fish body composition. Water quality was checked at the outflow of each system. Water temperature and dissolved oxygen were measured twice daily (8:00 and 17:00 h). The pH, nitrite and total ammonium levels were measured every week. By adjusting the water flow, nitrite and total ammonia levels were maintained below 0.15 and 2.5 ppm, respectively, and oxygen level above 3.5 ppm. The photoperiod was kept at 13 h day^{-1} (from 6 h to 19 h).

In the pond experiment, each pen was stocked with 20 *Oreochromis aureus* fingerlings (1.5 fish m^{-3}) weighing between 10 to 11 g. Fish were fed following the feeding rate table of Berzak (1992) two times daily (9 h, 14 h) for 22 weeks. For the present study, it meant that daily feeding levels roughly varied from 10 to 2% of body weight during the course of the experiment. A "no feeding" treatment was included in the study to determine the relevance of the contribution of natural food to the fish. Every two weeks all fish were weighed to adjust the feeding table. In both experiments, each treatment had four replicates.

Growth (relative growth rate of metabolic weight: RGR_m, daily weight gain: DWG), feed utilization parameters (feed intake: FI, feed conversion ratio: FCR, protein efficiency ratio: PER) and survival rate (%) were calculated.

Chemical analysis of CoP, diets and fish were done by standard procedures: caffeine was determined according to Morris (1973), total phenols (polyphenols) and tannins were measured according to the procedure of Slinkard & Singleton (1977) and Field (1991), carbohydrate or "total utilizable carbohydrate" (starch and sugar) was measured by the anthrone method of Clegg and expressed as (g kg^{-1}) glucose (Osborne & Voogt 1986). To determine the true protein levels in CoP, the samples were boiled in water to dissolve the amides present. After boiling, the protein was precipitated in the presence of copper (II) hydroxide and subsequently, separated from the dissolved amides by filtration (Stutzer & Barstein 1900). The N content of the filtrate was determined according to the Kjedahl method.The other chemical components (neutral and acid detergent fibre (NDF and ADF), cellulose, crude protein, crude fat, crude ash) were analyzed according to AOAC (1990).

Data from the aquaria trial were analyzed by a covariance analysis with initial weight as a covariant and dietary treatment as class variable because the initial sizes of the fish at stocking were significantly different. Data from the pen trial were analyzed by a one-way ANOVA. Treatment means were compared by the LSD test with 95% confidence intervals. The statistical analysis was done using the software STATGRAPHICS 7.1 PLUS.

Results and Discussion

In the aquaria trial, final body weight, growth rate and PER decreased with increasing dietary CoP levels (from 0 to 390 g kg^{-1}) ($P<0.05$). The FCR was significantly higher only at 390 g kg^{-1} of dietary CoP (Table 3). The high survival rate (above 92%) found with CoP diets

suggests that *Oreochromis aureus* fingerlings can tolerate relative high levels of CoP substances (caffeine, total phenols, tannins and high potassium levels), which are suspected to cause high mortality in some domestic animals (poultry, rats and turkeys) (Bressani *et al.* 1973, Gómez *et al.* 1985, Donkoh *et al.* 1988). The drastic reduction in feed intake at higher dietary CoP levels may be associated with polyphenols and tannins which are known to produce a bitter taste in diets for domestics animals (Bressani *et al.* 1975, Bressani & Braham 1980, de Rozo *et al.* 1985). During the feeding period, it was observed that the fish often rejected the pellets from diets with higher CoP levels which may indicate that the taste of these feeds affected feed intake negatively. Another possible explanation for this reduced feed intake might be the increased level of indigestible fibres in those diets.

The high dietary fibre levels together with the presence of ANF's (high K level, total phenols, tannins, caffeine) may explain why tilapia grew less and had a lower feed utilization with CoP diets. These factors could interfere with nutrient availability and digestion by fish but also could induce toxicity (high K level) and low feed acceptability (Bressani & Braham 1980, de Rozo *et al.* 1985, Vélez *et al.* 1985, Mehansho *et al.* 1987). Protein digestibility was not affected by the inclusion of CoP in diets but dietary dry matter and carbohydrate digestibility of tilapia were significantly reduced in fish fed diets containing CoP (Table 3). These negative effects could possibly be attributed to a reduced availability of carbohydrate and/or to the increased levels of fibres in diets. Tannins can also interact with polysaccharides making them unavailable to the fish (Mueller-Harvey & McAllan 1992). Obviously, if higher CoP levels are used in fish feeds, these factors should be reduced or destroyed.

Table 3. Average values of body weight, RGR_m, FCR, PER, FI and, diet and nutrient digestibility of fingerlings *O. aureus* fed different dietary CoP levels and reared in an aquaria system. (Means ± standard error).

Diets (g kg^{-1}) / Variable [1]	0 CoP	130 CoP	260 CoP	390 CoP
Initial body weight (g)	7.2 ±0.05 ^{a}	5.4 ±0.04 ^{b}	6.2 ±0.03 ^{b}	7.7 ±0.05 ^{a}
Final body weight (g)	45.0 ±0.65 ^{a}	29.4 ±0.95 ^{b}	15.5 ±1.05 ^{c}	7.9 ±0.87 ^{d}
RGR_m (g/$kg^{0.8}$/day)	17.2 ±0.12 ^{a}	12.6 ±0.29 ^{b}	5.8 ±0.61 ^{c}	1.6 ±0.51 ^{d}
FCR	1.2 ±0.05 ^{a}	1.3 ±0.29 ^{a}	2.0 ±0.35 ^{a}	4.3 ±0.15 ^{b}
PER	2.1 ±0.07 ^{a}	2.0 ±0.15 ^{a}	1.4 ±0.11 ^{b}	0.7 ±0.03 ^{c}
FI (g/fish/d)	0.83 ±0.14 ^{a}	0.54 ±0.05 ^{b}	0.28 ±0.05 ^{c}	0.1±0.01 ^{d}
Digestibility (g kg^{-1}, DM)				
Dry matter	740 ±0.11 ^{a}	682 ±1.05 ^{ab}	621 ±1.15 ^{b}	624 ±0.56 ^{b}
Protein	827 ±0.25 ^{a}	822 ±0.89 ^{a}	834 ±1.15 ^{a}	841 ±1.28 ^{a}
Carbohydrate [2]	874 ±0.98 ^{a}	882 ±0.935 ^{a}	632 ±0.67 ^{c}	610 ±0.45 ^{c}

[1] Means in the same line with no letters in common differ statistically ($P<0.05$).

[2] Carbohydrate refers to starch and sugar expressed as glucose.

In pens, the reduction in final weight, growth rate and PER was much lower than in aquaria. Fish fed the diet with 130 g kg^{-1} CoP had a similar growth and feed utilization than those fed the control diet (P>0.05) (Table 4, Figure 1). The results found in pens concur with findings of Bayne *et al.* (1976) with tilapia and of Bautista *et al.* (1999) with Cachamay (*Colossoma* x *Piaractus*), who found similar growth in fish fed CoP and control diets. Also the amount of feed that could be fed to the fish decreased with increasing levels of CoP in the diet (260 and 390 g kg^{-1}). This is in accordance with the results from the aquaria and suggests indirectly that fibrous components in the CoP diets were a main factor limiting the growth of fish.

Table 4. Average values of body weight, RGR_m, FCR and feed given of fingerlings *O. aureus* fed with different dietary CoP levels and reared in pens mantained in an earthen pond. (Means ± standard error).

Diets (g kg^{-1}) / Variables [1]	0 CoP	130 CoP	260 CoP	390 CoP	No feed
Initial body weight (g)	10.0 ±0.21 ^{a}	10.5 ±0.34 ^{a}	10.0 ±0.23 ^{a}	10.8 ±0.59 ^{a}	10.0 ±0.55 ^{a}
Final body weight (g)	174.2 ±1.45 ^{a}	176.3 ±2.01 ^{a}	144.2 ±1.44 ^{b}	98.0 ±0.56 ^{c}	24.9 ±0.65 ^{d}
RGR_m (g/$kg^{0.8}$/day)	15.9 ±0.12 ^{a}	15.9 ±0.25 ^{a}	14.4 ±0.55 ^{ab}	10.7 ±0.67 ^{b}	3.2 ±0.11 ^{c}
FCR	1.7 ±0.08 ^{a}	1.8 ±0.08 ^{a}	1.8 ±0.05 ^{a}	2.4 ±0.09 ^{b}	-
PER	1.5 ±0.15 ^{a}	1.4 ±0.06 ^{a}	1.5 ±0.21 ^{a}	1.1 ±0.1 ^{b}	-
Feed given (g/fish/d)	2.1 ±0.22 ^{a}	2.3 ±0.13 ^{a}	1.8 ±0.08 ^{b}	1.6 ±0.17 ^{b}	-

[1] means in the same line with no letters in common differ statistically (P<0.05).

Contrary to the results in aquaria, in pens tilapia growth was not altered by dietary CoP levels between 0 and 130 g kg^{-1}. The natural productivity of the pond could explain why the results in pens differed from those in aquaria. In the "no feeding" treatment, natural food supported fish growth for two weeks and after this period, when fish reached about 25 g, the available natural food only was enough to maintain fish weight for the remaining experimental period. Assuming that the same amount of natural food was available to the fish in the other treatments, the higher food conversion values found in pens could result from overfeeding. Our results have shown that the potential inclusion of CoP in tilapia diets may be limited up to 130 g kg^{-1} when fish are raised in earthen ponds and natural food is available.

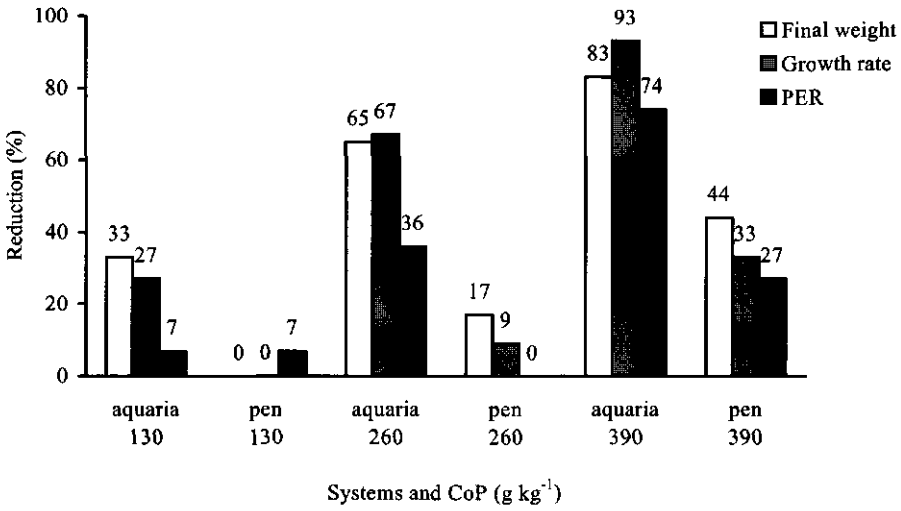

Figure 1. Reduction in growth and feed utilization of *O. aureus* reared in aquaria and pens.

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Chapter 4

Dietary fibre, caffeine and tannins affect growth and feed digestibility in tilapia fingerlings (*Oreochromis aureus* Steindachner)

Dietary fibre, caffeine and tannins affect growth and feed digestibility in tilapia fingerlings (*Oreochromis aureus* Steindachner)

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Abstract

Four different experiments were conducted to evaluate different dietary levels of fibre (paper pulp), caffeine and tannins (tannic acid) on growth and feed utilisation of tilapia (*Oreochromis aureus*) fingerlings. Diets for the fibre experiment contained 21 (control), 48, 63, 80 and 106.5 g kg^{-1} of fibre. Diets for the caffeine study contained 0, 2.4, 3.1 and 4.6 g kg^{-1} of caffeine. Diets for the two tannin experiments contained 0, 0.8, 1.1 and 1.7 g kg^{-1}, and 0, 3.2, 4.4 and 6.2 g kg^{-1} tannic acid, respectively. All diets were pelleted and fed to "apparent satiation" in triplicate. The highest dietary fibre level (106.5 g kg^{-1} fibre) showed the worst feed conversion ratio (FCR) and growth of tilapia ($P<0.05$). The results from other tested fibre levels did not differ significantly from the control level. The feed intake (FI) tended to be lower with fish fed the highest dietary fibre level ($P=0.08$). None of the fibre levels tested significantly affected the digestibility of dry matter (522 – 604 g kg^{-1}) and of carbohydrate (627 – 696 g kg^{-1}). Growth of *O. aureus* tended to be lower with diets containing caffeine ($P=0.09$), and the FI was significantly lower at the highest dietary caffeine level (4.6 g kg^{-1}). Dietary caffeine levels of 2.4 g kg^{-1} or above reduced dry matter and protein digestibility but increased carbohydrate digestibility ($P<0.05$). Dietary tannin levels of 4.4 g kg^{-1} or higher depressed significantly growth, FI, FCR and protein efficiency ratio of tilapia. Dietary tannin levels of 1.7 g kg^{-1} or above reduced dry matter and protein digestibility ($P<0.05$). Fish survival was not affected by treatments in any particular experiment ($P>0.05$) and it was above 90% in each of four experiments. Our results indicated that fibre and tannins could be the main CoP compounds responsible for the negative effects of dietary CoP in the performance of tilapia. The dietary critical levels of fibre and tannins for *O. aureus* fingerlings appear to be closed to 106 g kg^{-1} and 4.4 g kg^{-1}, respectively. However, in diets including CoP, these levels can be lower because these components might work synergistically or counteract each other or with other CoP compounds.

Keywords: tannins, fibre, caffeine, antinutritional factors, tilapia.

Introduction

Many of the agricultural residues from vegetable origin contain variable amounts of antinutritional factors (ANF's) such as polyphenols or total phenols, tannins, protein inhibitors, and others such as caffeine (present in coffee wastes) or threobromine (present in cocoa wastes). These residues also contain high levels of fibrous components. These ANF's and fibrous components may affect the animal's growth, especially in monogastric. Coffee pulp (CoP) is the main residue from the coffee processing industry and widely available in many tropical countries. In 1996 its world production reached about 22 million MT (estimation based on the production data from FAO 1997). In coffee producing countries, the vast amounts of CoP constitute an environmental risk and therefore, ways are searched to recycle CoP. One of the target industries, which might use CoP, is fish farming.

CoP contains variable levels of polyphenols, tannins, caffeine, K and cell wall components (e.g., cellulose, hemicellulose, lignin, pectines and lignified protein) all of which may interfere with the use of CoP in fish feeding. Indeed, dietary tannins and caffeine are deleterious to animal growth (Bresanni & Braham 1980, Dale *et al.* 1980). In chickens, levels of 5 g kg^{-1} or higher of dietary tannic acid reduced weight gain and increased feed conversion ratio. However, at 5 g kg^{-1} tannin level, negative effects could be eliminated by supplying additionally methionine, choline and arginine to the chicken diets (Fuller *et al.* 1967, Dale *et al.* 1980). The critical levels of tannins and/or caffeine vary with species. For example, calves fed diets containing 7.5 g kg^{-1} tannic acid showed similar growth responses as the animals receiving the control diet (Bresanni & Braham 1980). When caffeine was added to these tannin-containing diets growth rate was further reduced. In the absence of tannins, caffeine alone also reduced daily weight gain of calves when included at levels above 1.2 g kg^{-1}. For fish, e.g., common carp (*Cyprinus carpio* L), Becker & Makkar (1999) found that 2 g kg^{-1} tannic acid in the diet depressed feed intake and growth.

In previous studies, it was found that inclusion of CoP in diets for tilapia (Ulloa & van Weerd 1997, Ulloa & Verreth, in review) or for common carp (Christensen 1981) and for African catfish (Fagbenro & Arowosoge 1991) reduced the growth, feed utilisation and nutrient digestibility in these three species. Results obtained with tilapia (Bayne *et al.* 1976, own unpublished data) and with Cachamay (*Colossoma* x *Piaractus*) (Bautista *et al.* 1999) indicated that negative effects of CoP containing diets are more pronounced in fish reared in aquaria than in fish reared in ponds (Ulloa & Verreth, in review). We believe that the explanation for these negative effects of CoP must be related to either its ANF's content or to its high fibre content. Therefore, the inclusion levels of CoP in animal feeds will be limited by the concentration of ANF's and fibre in the pulp. For the same reason, CoP inclusion levels should not exceed the toxic concentration of those ANF's and/or for fibre. As these concentrations are species specific, the maximum allowable inclusion levels have to be determined for each particular species. The present study was designed to elucidate the

critical levels for each of these compounds for tilapia. Different levels of tannic acid, caffeine and fibre (mostly cellulose) were fed to tilapia (*Oreochromis aureus*) fingerlings. Tannin and caffeine were supplied into diets in purified form, representing concentrations closed to those found when 0, 130, 260 and 390 g kg^{-1} of CoP would be included in diets. Fibre levels were supplied by paper pulp. The information obtained from this study will be further used to investigate the feasibility of including CoP in tilapia diets.

Materials and Methods

Experimental Design

The study was geared to test the critical levels of tannins, caffeine and fibre for tilapia fingerlings. All together, four different experiments were carried out in recirculation units consisting of 16 aquaria (45 L), a bio-filter of volcanic stone and a sedimentation tank. Each experiment addressed one of the mentioned compounds. In the first experiment, different fibre levels were tested. Next, both caffeine and a limited range of tannins were tested in parallel experiments. In the fourth experiment, the tannin experiment was repeated, applying thereby a wider range of tannin concentrations.

1) Fibre experiment: The test diets contained 21 (the control), 48, 63, 80 and 106.5 g kg^{-1} fibre. Actually, concentrations were slightly different from the pre-set ones: (40: control, 65, 90, 115 and 140 g kg^{-1}). Paper pulp, composed of 970 g kg^{-1} fibre (mostly cellulose), 10 g kg^{-1} ash and 20 g kg^{-1} moisture, was used as a fibre source replacing the cassava starch. Diets were fed to tilapia for 61 days and were assigned randomly to the aquaria. The experiment was run with three replicates per treatment (N=15).

2) Caffeine experiment: To find critical levels of caffeine, diets containing 0, 2.4, 3.1 and 4.6 g kg^{-1} of caffeine were fed to the fish. These concentrations were also slightly different from the pre-set ones (0, 2, 3.5 and 4.5 g kg^{-1}), equivalent to CoP inclusion levels of respectively, 0, 130, 260 and 390 g kg^{-1}. Diets were fed to fish for 61 days and were assigned randomly to the aquaria system, using three replicates (N=12).

3) Tannin experiments: To find critical levels of tannins, two experiments were carried out. In the first of these experiments (the third experiment) diets contained 0, 0.8, 1.1 and 1.7 g kg^{-1} tannic acid. These concentrations were slightly different from the pre-set ones: 0, 0.6, 0.12 and 1.8 g kg^{-1}, equivalent to CoP inclusion levels of respectively, 0, 130, 260 and 390 g kg^{-1}. Diets were assigned randomly to the aquaria using three replicates per treatment (N=12). Also this experiment lasted 61 days. Because no clear effects were found, possibly due to the relative small range of tested dosages, a wider range of tannin levels was tested in a subsequent experiment (the fourth). This experiment lasted 28 days

because in all previous experiments, tilapia increased initial weight by more than five-fold within a period of four weeks, which should be sufficient for nutritional studies, as recommended by FAO (1994). In this fourth experiment, tested tannin levels were 0, 3.2, 4.4 and 6.2 g kg^{-1} tannic acid, respectively, which again differed slightly from the pre-set ones (0, 3.0, 4.5 and 6.0 g kg^{-1}). The experiment was run with four replicates per treatment (N=16).

For the sake of time efficiency the caffeine and the first tannin experiments were carried out simultaneously in independent recirculation units (each of the units served for one particular experiment).

Experimental Procedures

For all four experiments, fish were taken from an all-male sex-reversed (17-α-methyltestosterone) population and acclimatised in the experimental units for one week. Fish were fed during this period with the respective control diet (Table 1). At the beginning of each experiment each aquarium was stocked with 10 fish. Fish weights at stocking varied between 3.2 and 4.2 g, except for the fibre experiment, where slightly larger fish (7 to 10.4 g) were used to stock the aquaria. In all experiments fish were fed by hand to "apparent satiation" twice daily (10: 00 and 15: 00 h).

In all four experiments, water quality was checked in the outflow of each system: Water temperature and dissolved oxygen were measured twice daily (8:00 and 17:00 h) and pH, nitrite and ammonium levels were measured weekly. By adjusting water inflow, pH, nitrite and total ammonia levels were maintained between 6.0-7.5 and, below 0.18 and 0.05 ppm, respectively. Temperature and dissolved oxygen were kept above 28.5 °C and 3.5 ppm, respectively. Photoperiod was kept at 12 h light per day (6: 00 to18: 00 h).

Diet preparation and analysis

For each experiment, diets were formulated to be approximately isoproteinous and isoenergetics. The realised concentrations were slightly different from the pre-set fibre/tannins/caffeine levels (see above). The composition by ingredient of control diets was similar for all four experiments, except for the fibre experiment, which had 10 g kg^{-1} less fish meal and 10 g kg^{-1} more cassava starch (Table 1 and 2). However, the chemical composition of control diets of the 2nd tannin and the fibre experiments slightly differed from that of control diets of the 1st tannin and the caffeine experiments. These changes are attributable to differences in the chemical composition of the ingredients used because they originated from different sources (batches). Dietary amino acid, minerals and essential fatty acid contents, in general, followed the requirements for tilapia (Tacon 1990, Ulloa 1995).

Table 1. Ingredient composition (g kg^{-1}, as feed basis) of diets used (fibre experiment, caffeine experiment, 1st and 2nd tannin experiments) in different feeding trials with fingerlings of *O. aureus*.

Ingredients [1] / Diets (g kg^{-1})	Fish meal	Soy meal	Blood meal	Cassava starch	Soy oil	Vitamin [2]	Paper pulp	Cr_2O_3
Fibre experiment								
Control (21F)	420	170	50	310	20	20	0	10
(48F)	420	170	50	274	30	20	26	10
(63F)	420	170	50	244	35	20	51	10
(80F)	420	170	50	215	40	20	75	10
(106.5F)	420	170	50	179	50	20	101	10
Caffeine experiment								
Control (0 C/T) [3]	430	170	50	300	20	20	0	10
(2.4C)	430	170	50	298	20	20	0	10
(3.1C)	430	170	50	296.5	20	20	0	10
(4.6C)	430	170	50	295.5	20	20	0	10
1st tannin experiment								
(0.8T)	430	170	50	299.4	20	20	0	10
(1.1T)	430	170	50	298.8	20	20	0	10
(1.7T)	430	170	50	298.2	20	20	0	10
2nd tannin experiment								
(3.2T)	430	170	50	297.0	20	20	0	10
(4.4T)	430	170	50	295.5	20	20	0	10
(6.2T)	430	170	50	294.0	20	20	0	10

(1) Ingredients: fish meal (tuna meal mixed, crude protein (CP): 590 g kg^{-1}, fat: 103 g kg^{-1}), blood meal (spray dried, CP: 815 g kg^{-1}), cassava starch (flour), soybean meal (dehulled solvent extracted, CP: 460 g kg^{-1}, fat: 29 g kg^{-1}).

(2) (Amount/kg diet): 16000 IU vitamin A, 4000 IU vitamin D, 0.2 g vitamin E, 0.02 g vitamin K, 0.4 g Thiamine, 0.06 g Riboflavin, 0.3 g Pantothenate, 0.04 g Pyridoxine, 0.04 mg B_{12}, 0.4 g Niacinamide, 0.01 g Biotin, 4 g Ascorbic acid, 0.02 g Folic acid, 2 g Choline.

(3) Ingredient composition of control diets for caffeine and tannin experiments.

All dry ingredients were thoroughly mixed for 15 minutes before lipids (soy oil) were added. Mixing continued for another 15 minutes. Next, water was added gradually until a paste-like consistency was reached. This paste was forced through a 1-mm mesh die using a meat grinder. The spaghetti-like feed was dried for 16 h at 50 °C, and then feed was manually crumbled to pellet size. Feeds were supplemented with 1 g kg^{-1} Cr_2O_3 for digestibility determinations.

Table 2. Proximate composition (g kg^{-1}, DM basis) of diets containing different tannic acid, caffeine and fibre levels used in feeding trials with fingerlings *O. aureus*.

Nutrients / Diets (g kg^{-1})	Moisture (g kg^{-1})	Crude fat	Crude fibre	Crude ash	Crude Protein	Carbohydrate	DE (kJ g^{-1}) [1]
Fibre experiment							
Control (21F)	76	76	21	124	386	393	12.35
(48F)	51	107	48	110	384	350	12.94
(63F)	61	107	63	118	377	336	12.64
(80F)	59	124	80	124	371	301	12.77
(106.5F)	91	128	106.5	122	382	262	12.68
Caffeine experiment							
Control (0 C/T) [2]	55	77	24	137	443	315	12.47
(2.4T)	54	87	22	130	443	317	12.77
(3.1T)	47	79	13	134	445	328	12.64
(4.6T)	53	101	22	126	427	324	13.06
1st tannin experiment							
(0.8T)	62	83	12	148	438	318	12.56
(1.1T)	52	81	23	136	443	316	12.56
(1.7T)	54	88	09	134	447	323	12.89
2nd tannin experiment							
Control (0T) [2]	70	82	24	139	420	338	12.47
(3.2T)	43	74	16	131	416	363	12.39
(4.4T)	53	70	20	132	421	357	12.26
(6.2T)	86	76	20	139	421	344	12.35

[1] Digestible energy: calculated according to nutrient digestible energy coefficients for *Ictalurus punctatus*, 14.65 kJ g^{-1} protein, 33.91 kJ g^{-1} fat and 10.46 kJ g^{-1} crude carbohydrate (N.R.C. 1977).

[2] Chemical composition of control diets for caffeine and 1st tannin experiments. Chemical composition of control diet for 2nd tannin experiment was slightly different because ingredients used originated from different sources.

Measurements

At the end of each of the four experiments, the fish mean body weight of each aquarium replicate was determined by weighing all the individual fish and averaging these data. Faeces collection started after two weeks by pipetting faeces from the aquarium bottom when voided by fish. Faeces from each replicate were collected twice daily (starting one hour after feeding) for two or three weeks, dried at 50 °C for 24 h. Daily faeces samples from each replicate were pooled until the desired amount of faeces (2.5 – 3.0 g) was obtained. Dried faeces samples were stored in plastic containers in a dessicator for further nutrient and indicator analysis.

Diets were analysed for moisture, crude protein, crude fat, crude fibre and crude ash by standard methods (AOAC 1990). Carbohydrate content (sugars and starch) was

determined as "Total Utilisable Carbohydrate" by the Anthrone method of Clegg and expressed as glucose (Osborne & Voogt 1986). Tannins were extracted from diets with a methanol/water solution (70% v/v) by reflux for two hours at boiling temperature and their determination was done using the Folin-Ciocalteu reagent according to Slinkard & Singleton (1977) and Field (1991). For caffeine measurements, diet samples (2 g, DM) were boiled in a solution of water and 0.1 N sulphuric acid and filtered. The filtrate was mixed with zinc acetate, potassium ferricyanide and heavy magnesium oxide in different consecutive steps. After filtration, the filtrate was read spectrophotometrically at 272 nm (Morris 1973). Levels of chromic oxide were measured according to the dry ash method of Mink *et al.* (1969). All determinations were done in triplicate.

Parameters measured and data analysis

Growth and feed utilisation were calculated by: Relative metabolic growth rate (RGR_m, g/kg $^{0.8}$/d), feed conversion ratio (FCR), feed intake (FI, g/fish/d), protein efficiency ratio (PER), the apparent net protein utilisation (NPU_a, %) and survival rate (S, %). Apparent digestibility coefficients (ADC) for dry matter and nutrients were calculated using formulas described by Mukhopadhyay & Ray (1997).

Results of the four independent experiments were analysed separately. Statistical analysis of data for all experiments was done by a one-way ANOVA, except for data of the fibre experiment. Because the initial sizes of the fish at stocking in the fibre experiment were significantly different, these data were analysed by covariance analysis with initial mean weight as covariant and dietary treatment as class variable. Treatment means of all experiments were compared by the LSD test with 95% confidence intervals. Variables were tested for homogeneity of variances using the test of Bartlett. Statistical analysis was done using the software STATGRAPHICS PLUS STATISTICAL GRAPHICS SYSTEM 7.0 (Manugistics, Inc. and Statistical Graphics Corporation, 1993).

Results

In the present study, four different experiments were designed to respectively test the effect of different dosages of dietary fibre, caffeine and tannins on the growth and feed utilisation of tilapia fingerlings.

1) Fibre experiment

In the fibre experiment, fish receiving the highest fibre level (106.5 g kg^{-1}) had the worst growth (final body weight: 43.2 g, RGR_m: 13.8 g/kg$^{0.8}$/d), FCR (1.44) and PER (1.82) ($P<0.05$). Also feed intake and protein utilisation (NPU_a) tended to be lower in this group of fish ($P= 0.08$, Table 3). Digestibility measurements produced interesting results. Dry matter (522 to 608 g kg^{-1}) and carbohydrate (627 to 696 g kg^{-1}) digestibility coefficients were not affected by fibre dietary

levels (P>0.05). However, the protein digestibility was lower in the control diet than in all diets with higher fibre levels (P<0.05). Survival was not affected by dietary fibre levels, ranging from 91.1 to 96.7% (P<0.05).

Table 3 Average values of RGR_m, FCR, PER, NPU_a, FI and diet and nutrient digestibility of *O. aureus* fingerlings fed diets with different fibre levels (fibre experiment). (Means ± standard error).

Diets (g kg^{-1} fibre) / Variables [1]	Control (21F)	(48F)	(63F)	(80F)	(106.5F)
Initial body weight (g)	7.0 ±0.15 ^{b}	7.7 ±0.25 ^{ab}	8.4 ±0.33 ^{ab}	7.9 ±0.16 ^{ab}	10.4 ±0.11 ^{a}
Final body weight (g)	57.0 ±0.46 ^{b}	52.3 ±0.54 ^{ab}	53.2 ±0.65 ^{ab}	53.9 ±0.89 ^{ab}	43.2 ±0.29 ^{a}
RGR_m (g/$kg^{0.8}$/day)	17.5 ±0.39 ^{b}	16.2 ±0.16 ^{ab}	16.2 ±0.25 ^{ab}	16.3 ±0.28 ^{ab}	13.8 ±0.11 ^{a}
FCR	1.25 ±0.1 ^{ab}	1.24 ±0.09 ^{a}	1.24 ±0.16 ^{a}	1.3 ±0.12 ^{ab}	1.44 ±0.1 ^{b}
PER	2.07 ±0.15 ^{ab}	2.11±0.22 ^{b}	2.15 ±0.05 ^{b}	2.09 ±0.13 ^{ab}	1.82 ±0.19 ^{a}
NPU_a (%)	38.0 ±0.84 ^{a}	38.7 ±0.34 ^{a}	36.2 ±0.55 ^{a}	36.4 ±0.89 ^{a}	34.8 ±0.87 ^{a}
FI (g/fish/d)	1.0 ±0.08 ^{a}	0.89 ±0.15 ^{a}	0.91±0.11 ^{a}	0.95 ±0.12^{a}	0.83 ±0.21 ^{a}
Digestibility (g kg^{-1})					
Dry matter	554 ±2.15 ^{a}	522 ±0.90 ^{a}	590 ±2.56 ^{a}	608 ±1.01 ^{a}	604 ±1.44 ^{a}
Protein	777 ±2.51 ^{a}	816 ±2.67 ^{ab}	841 ±1.23 ^{b}	832 ±1.01 ^{ab}	854 ±2.49 ^{b}
Carbohydrate [3]	635 ±0.95 ^{b}	[2]	627 ±0.89 ^{b}	696 ±0.88 ^{b}	656 ±1.65 ^{b}

[1] Means in the same line with no letters in common differ statistically (P<0.05).

[2] Missing value.

[3] Carbohydrate refers to starch and sugar expressed as glucose.

2) Caffeine experiment

Dietary caffeine levels tended to reduce the growth (final weight and RGR_m) of *O. aureus* (P=0.09). However, the FI was significantly reduced at the highest dietary caffeine level (4.6 g kg^{-1}). Feed digestibility was strongly affected by dietary caffeine levels. Fish fed the diet without caffeine (control diet) showed significantly higher dry matter and protein digestibility than fish fed other treatments (caffeine levels) (660 and 856 g kg^{-1}, respectively). In contrast to this, carbohydrate digestibility was higher in all fish groups fed diets containing caffeine (P<0.05) (Table 4).

Table 4 Average values of body weight, RGR_m, FI, FCR, PER and diet and nutrient digestibility of *O. aureus* fingerlings fed diets with graded caffeine levels (caffeine experiment). (Means ± standard error).

Diets (g kg^{-1}) / Variables [1]	Control (0 caffeine)	(2.4 caffeine)	(3.1 caffeine)	(4.6 caffeine)
Initial Weight (g)	3.4 ±0.06 [a]	3.5 ±0.04 [a]	3.2 ±0.03 [a]	3.4 ±0.07 [a]
Final Weight (g)	54.8 ±0.84 [a]	52.0 ±0.45 [a]	51.1 ±0.12 [a]	49.8 ±1.06 [a]
RGR_m	26.1 ±0.68 [a]	24.8 ±0.53 [a]	25.6 ±0.16 [a]	24.5 ±0.98 [a]
FI (g/fish/d)	0.84 ±0.08 [ab]	0.9 ±0.04 [a]	0.8 ±0.06 [ab]	0.78 ±0.04 [b]
FCR	0.99 ±0.05 [a]	1.13 ±0.04 [b]	1.0 ±0.03 [a]	1.0 ±0.06 [a]
PER	2.29±0.07 [a]	2.0 ±0.02 [b]	2.19±0.09 [a]	2.28±0.08 [a]
Digestibility (g kg^{-1})				
Dry matter	660±0.19 [a]	566 ±0.23 [b]	534 ±0.16 [b]	565 ±0.24 [b]
Protein	856 ±0.09 [a]	795 ±0.19 [b]	766 ±0.25 [b]	762 ±0.56 [b]
Carbohydrate [2]	729 ±0.12 [a]	764 ±0.85 [b]	760 ±1.05 [b]	811 ±1.46 [b]

[1] Means in the same line with no letters in common differ statistically ($P<0.05$).

[2] Carbohydrate refers to starch and sugar expressed as glucose.

3) Tannin experiments

Tannic acid levels similar or lower than 3.2 g kg^{-1} did not affect fish growth as indicated by final weight and RGR_m, FI, FCR and PER of *O. aureus* fingerlings ($P>0.05$). However, at higher dietary tannic acid levels (4.4 and 6.2 g kg^{-1}), all these variables were significantly reduced compared to the control level. Also protein and dry matter digestibility was significantly lower at tannin levels of 1.7 g kg^{-1} or above than in the control diet. Carbohydrate digestibility increased with dietary tannin levels up to 1.7 g kg^{-1} (Table 5).

Table 5 Average values of body weight, RGR_m, FI, FCR, PER and diet and nutrient digestibility of *O. aureus* fingerlings fed diets with graded tannins levels (1^{st} and 2^{nd} tannin experiments). (Means ± standard error).

		Diets	1^{st} tannin experiment			Diets	2^{nd} tannin experiment	
Diets (g kg^{-1}) / Variables [1]	(0T)	(0.8T)	(1.1T)	(1.7T)	(0T)	(3.2T)	(4.4T)	(6.2T)
Initial Weight (g)	3.4±0.10 [a]	3.4±0.12 [a]	3.4±0.05 [a]	3.4±0.06 [a]	4.2±0.16 [a]	4.2±0.17 [a]	4.0±0.1 [a]	4.2±0.11 [a]
Final Weight (g)	55.4±1.33 [a]	52.9±1.15 [a]	61.2±1.44 [a]	56.4±1.67 [a]	24.3±0.33 [a]	22.9±0.35 [a]	18.7±0.11 [b]	18.4±0.12 [b]
RGR_m	26.2±0.35 [a]	25.4±0.45 [a]	28.0±0.34 [a]	26.8±0.14 [a]	28.4±0.23 [a]	26.9±0.08 [a]	23.2±0.16 [b]	22.4±0.14 [b]
FI (g/fish/d)	0.82±0.08 [a]	0.88±0.06 [a]	0.96±0.18 [b]	0.86±0.08 [a]	0.75±0.09 [a]	0.68±0.10 [b]	0.58±0.04 [c]	0.56±0.06 [c]
FCR	0.97±0.05 [a]	1.1±0.08 [b]	1.02±0.09 [ab]	1.0±0.04 [ab]	1.05±0.06 [ab]	1.02±0.01 [a]	1.12±0.09 [b]	1.11±0.08 [b]
PER	2.31±0.41 [a]	2.08±0.07 [b]	2.22±0.26 [a]	2.27±0.11 [a]	2.27±0.08 [ab]	2.36±0.09 [a]	2.12±0.11 [b]	2.13±0.12 [b]
Digestibility (g kg^{-1})								
Dry matter	640±0.32 [a]	646±0.56 [a]	642±0.67 [a]	568±0.76 [b]	751±0.12 [a]	684±0.16 [b]	716±0.79 [b]	710±0.59 [b]
Protein	844±0.24 [a]	840±0.78 [a]	835±0.19 [a]	768±0.47 [b]	888±0.65 [a]	864±0.41 [b]	862±0.29 [b]	858±0.37 [b]
Carbohydrate	710±0.18 [a]	776±0.21 [b]	766±0.32 [b]	859±0.35 [c]				

[1] Means in the same line with no letters in common differ statistically ($P<0.05$).

Survival was not affected by either caffeine or tannic acid treatments, ranging from 91% to 100%, except for the diet with 2.4 g kg^{-1} caffeine (88%). This lower survival was attributed to the aggressive behaviour of fish observed in two of the three replicate aquaria.

Discussion

1) Dietary fibre

Growth, feed and protein utilisation of *O. aureus* was reduced at the highest dietary fibre level (106.5 g kg^{-1}). This result corroborates with those found for other tilapia species (Anderson *et al.* 1984, Shiau *et al.* 1989, Dioundick & Stom 1990). This high fibre level (106.5 g kg^{-1}) is above the maximum value (80-90 g kg^{-1}) recommended for several fish species (Tacon 1990, De Silva & Anderson 1995). For fingerlings of *Oreochromis niloticus* (L.), Al-Ogaily (1996) found the best growth, feed and protein efficiency values with 90 and 120 g kg^{-1} dietary fibre levels. Also common carp (*Cyprinus carpio* L.) have performed well at these high or even at higher fibre levels (Pereira *et al.* 1994). These contradictory results may be explained by differences in the feeding behaviour of the fish species.

Diets with high fibre content contain also the largest amount of indigestible components (e.g., cellulose). This may cause a lower availability of dietary energy (Buddington 1979, Shiau *et al.* 1988). Fish fed diets with fibre levels below 106.5 g kg^{-1} had similar growth rates and feed utilisation indices, which suggests that addition of moderate amounts of fibre into diets is not harmful to fish. This effect is associated with an effective passage of digesta through the gut. Due to its long digestive tract and its associated microflora, tilapia may also be capable to partially digest cellulose and other higher polysaccharides (Viola & Arieli 1982, Dioundick & Stom 1990, Al-Ogaily 1996).

The results on nutrient digestibility (dry matter and carbohydrate were not affected and protein was increased) did not help in giving a plausible explanation for the mentioned negative effect of high dietary fibre levels on performance of tilapia. Another mechanism that could explain the lower growth rate found with tilapia fed the highest dietary fibre level is a reduced feed intake. The observed low feed palatability in those fish receiving the highest fibre dietary level supports the possible mechanism through the feed intake was reduced. These observations suggest that feed intake indeed might have played a role in our results. This reduction in the feed intake could be caused by a deleterious effect of the excess dietary fibre (Tacon 1990, De Silva & Anderson 1995). According to Shiau *et al.* (1988), high dietary fibre levels reduced gut passage time in tilapia, and this reduction could induce a lower nutrient intake by the gut absorptive epithelium. We assume that this mechanism also occurred in the present study with tilapia fed the highest dietary fibre level. However, this hypothesis has still to be proven because in our study we did not measure the digesta gut passage time or the nutrient intake rates by the gut. In summary, more studies are needed to find a more clear explanation for the reduced performance of tilapia when fed elevated dietary levels of fibre.

2) Dietary caffeine

In the present study, increasing dietary caffeine levels tended to reduce the growth of *O. aureus* fingerlings. In ruminants, dietary caffeine levels, equal or higher than 2.4 g kg^{-1} reduced growth, FI, FCR and protein retention (Bressani *et al.* 1975, Bressani & Braham 1980). In sheep, lower critical caffeine levels (contained in coffee pulp) were found. Levels equal or above 1.1 g kg^{-1} of caffeine already reduced feed intake and growth (Demeke 1991). This critical caffeine level found in the study on sheep is lower than that found in the other studies on ruminants (see above). This result may be explained by possible additive effects of polyphenols and other ANF's present in coffee pulp. Apart from the diuretic effect and the bitter taste that caffeine can produce, it is not clear which others adverse effects on digestion and absorption of nutrients may be associated with caffeine. Its stimulatory effect and its high nitrogen content (≈ 30 g kg^{-1}) are factors which may also affect negatively the performance of animals (Bressani 1979). To our knowledge, no data are available showing a possible diuretic effect of caffeine in fish.

Dietary caffeine levels similar or higher than 2.4 g kg^{-1} reduced dry matter and protein digestibility, whereas carbohydrate digestibility increased. Bressani (1979) indicated that caffeine interferes with proper utilisation of nutrients. This lower availability of protein and dry matter concurs with the tendency for lower growth found in this study. Apparently, the increase in carbohydrate digestibility did not compensate for the reduction in dry matter digestibility. The differences between the reduction of protein digestibility (6.1 to 9%) and the increase in carbohydrate digestibility (3.1 to 8.2%) seem too small to explain the overall reduction in dry matter digestibility. Hence, the latter might also be caused by a lower digestibility of fat and fibre.

3) Dietary tannins

According to the results of the present study, dietary tannin levels equal or lower than 3.2 g kg^{-1} are not harmful to tilapia. However, dietary levels of 4.4 g kg^{-1} or higher depressed growth, FI and PER and increased FCR. Also in Indian carp fingerlings, *Labeo rohita* (Hamilton), tannin levels of 3.0 g kg^{-1} or higher (supplied by de-oiled salseed meal, *Shorea robusta*) reduced growth and feed utilisation (Mukhopadhyay & Ray 1996). Our results also agree with those of Becker & Makkar (1999) who found that 20 g kg^{-1} of dietary tannic acid depressed the performance of common carp. Unfortunately these authors did not check lower concentrations. Similarly, dietary tannic acid levels of 5.0 g kg^{-1} depressed growth in poultry (Dale *et al.* 1980). In ruminants, dietary tannin levels of 7.5 and 8.0 g kg^{-1} did not have any measurable adverse effect on animal performance (Bressani & Braham 1980, Vargas *et al.* 1982), which may indicate that these animals are more resistant to tannins than non-ruminants. Results found in literature indicated that there exist interspecies differences in the response to dietary tannins (Mueller-Harvey & McAllan 1992).

Apart from the dietary tannin concentration itself, the tannin: protein ratio in the feeds may also affect the critical levels of dietary tannins. When dietary tannin: protein ratios are reduced by increasing dietary protein, the effect of tannins on the performance of animals is reduced or in some cases even eliminated (Jarquín *et al.* 1977, Gómez *et al.* 1985, Mueller-Harvey & McAllan 1992, Jansman *et al.* 1995). According to these authors the animal response to different tannin: protein ratios is species dependent. Our results indicated that diets with low tannin levels (0 to 1.7 g kg^{-1}) and tannin: protein ratios of 0.002 to 0.004 do not affect the growth of tilapia.

In the present study, a negative effect of dietary tannins on feed intake of tilapia was found. This may be one of the causes for the reduced growth that we found at higher tannin levels. The other factor that may explain this is presumably the known effect of tannins on protein digestibility. Indeed, tannins interfere negatively with protein and dry matter digestibility by binding dietary proteins and endogenous proteases in the digestive tract (Mueller-Harvey & McAllan 1992, Vélez *et al.* 1985, Mehansho *et al.* 1987). This reduces digestion and absorption of dietary protein and increases excretion of endogenously secreted proteins.

Our results with *O. aureus* agree only partly with this general trend in animal response to dietary tannins. In the present study, the digestibility of protein and dry matter decreased at higher tannin levels, but contrary to the other studies, carbohydrate digestibility increased with increasing dietary tannin level up to 1.7 g kg^{-1}. However, this increase in carbohydrate digestibility did not compensate for the reduction in dry matter digestibility. The latter might be attributed to the reduced protein digestibility but also to the possible lower digestibility of fat and minerals. Unfortunately, in this study we did not measure fat and mineral digestibility, and therefore this hypothesis must still be confirmed. Indeed, Mukhopadhyay & Ray (1997) found reduced protein and fat digestibility at increasing dietary tannin levels (2.8 and 4.2 g kg^{-1}) in Indian major carp (*Labeo rohita*). Also in pigs, the digestibility of protein, dry matter and minerals was reduced in diets containing 5.6 and 6.2 g kg^{-1} tannins (Jansman *et al.* 1995).

In summary, apart from possible differences in the type of tannins in the feeds, other dietary protein sources or the level of dietary protein may influence the deleterious effects of tannins. This means that polyphenols (total phenols) and tannins from crop residues need to be more specifically identified and characterised in order to elucidate their nutritional effect on animals.

In the present study, survival rates were consistently high (above 91%), irrespective of the dietary tannins, caffeine or fibre level applied, with the only exception of one caffeine treatment (2.4 g kg^{-1}), which reached a survival rate of 88%. This suggests that *Oreochromis aureus* fingerlings can tolerate high dietary levels of these components. Similar results were found by Bayne *et al.* (1976), Fagbenro and Arowosoge (1991) and Ulloa & van Weerd (1997). Interestingly, results with poultry and other monogastric animals, such as rats and turkeys,

showed that high levels of dietary tannins and caffeine induced high mortality in these animals (Bressani *et al.* 1973, Gómez *et al.* 1985, Donkoh *et al.* 1988). This difference in ANF's tolerance could be explained by species differences in detoxification systems or by different adaptation responses to the ANF's (Mueller-Harvey & McAllan 1992).

Fish fed diets containing higher levels of fibre, tannic acid and caffeine initially refused to eat these feeds. The depressed feed intake agrees with results found in ruminants, swine, poultry and turkey fed CoP-diets containing these ANF's (Bressani *et al.* 1975, Jarquín *et al.* 1977, Wiseman 1983). High dietary levels of tannins, caffeine and fibre may produce this low feed palatability (Bressani & Braham 1980, Mueller-Harvey & McAllan 1992).

In order to get a better understanding of the mechanisms behind the effect of these ANF's on fish, it is advisable to perform histopathological analysis, which could include measurements of blood haemoglobin, red and white cell counts, condition of the intestinal villi, liver and kidney together with analysis of digestive enzyme activities.

The results of this study demonstrated that dietary tannins and fibre, in order of importance, had the strongest negative effect on the performance of *O. aureus* fingerlings, whereas dietary caffeine showed not clear effect on the growth of fish. Our findings also indicated that dietary tannic acid levels of 4.4 g kg^{-1} or higher and dietary fibre levels of 106 g kg^{-1} or higher reduced the growth and feed utilisation of tilapia. Also for diets containing CoP, it can be assumed that tannins and fibre are the main responsible for the reduction in growth and feed utilisation of fish, which indeed could restrict the inclusion levels of CoP in diets. However, the critical levels of fibre and tannins found in the present study were based on pure compounds and the translation in terms of allowable CoP concentrations has to be done with caution because the same ANF's in CoP might counteract each other or exert a synergistic effect. The findings with common carp, African catfish and tilapia could support this hypothesis because CoP diets, which contained much lower levels of tannins (0.8 to 1.2 g kg^{-1}), fibre and caffeine (1.7 to 2.2 g kg^{-1}) than those found in the present study, reduced growth and feed utilisation (Christensen 1981, Fagbenro & Arowosoge 1991, Ulloa & van Weerd 1997). To verify this possible synergistic effect of ANF's, similar studies as the present one have to be designed, but then using CoP as source of ANF's or by using combinations of different fibre, tannins and caffeine levels in diets.

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Chapter 5

Upgrading of Coffee pulp using chemical and biological methods

Chapter 5.1

Effect of different chemical treatments on nutritional and antinutritional properties of coffee pulp

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Abstract

Different chemical treatments were tested to improve the nutritional value of coffee pulp (CoP): 1) Alkali, NaOH solutions of 50 g kg^{-1} and 100 g kg^{-1} for 24 and 48 h. 2) A combination of acid and alkali, with first a treatment with HCl (1.5 M and 3 M for 24 and 48 h), followed by a NaOH solution of 50 g kg^{-1} for 48 h. 3) A combination of alkali and ensilage, with first a treatment with a NaOH solution of 50 g kg^{-1} for 48 h, followed by ensilage with molasses (50 and 100 g kg^{-1} of CoP) for 2 and 3 months. The CoP treated with NaOH alone or with the combination HCl-NaOH showed higher contents of ash, fat and cellulose but lower contents of total phenols (polyphenols), tannins and caffeine than oven-dried CoP (OD-CoP) ($P<0.05$). The true protein content in the CoP was not affected by the alkali treatment but was reduced in the acid-alkali treated CoP compared to OD-CoP. A combined treatment with alkali-ensilage resulted in higher true protein, fat and ash contents ($P<0.05$) and in similar contents of cellulose than in OD-CoP. Total phenols, tannins and caffeine contents were lower in treated CoP than in OD-CoP, but there was no difference in the size of the effect for caffeine between one treatment to the other. The higher ash content found in alkali-ensilaged CoP could result from the addition of the alkali. The ANF's reduction was much higher in the chemical treated CoP than in the alkali-ensilaged CoP. The alkali treatment yielded the best overall results in upgrading the nutritive value of CoP.

Keywords: coffee pulp, chemical treatments, waste upgrading, antinutritional factors, ensilage.

Introduction

The wet method of processing coffee berries generates different types of residues: pulp, mucilage, hulls and residual water. Their amounts vary depending on for example, the condition of machinery, ripeness of berries and impurities. The Costa Rican Coffee Institute (ICAFE) estimated for the 1993-94 season their production volumes at 350,000-365,000 MT of pulp, 142,250 MT of mucilage and 40,000 MT of hulls for Costa Rica alone. Worldwide coffee wastes were estimated around 22 million MT of coffee pulp (CoP), almost 8,6 million MT of mucilage and 2,4 million MT of hulls (estimation based on production data for 1996, FAO 1997). Obviously, these figures may fluctuate yearly according to variations in coffee production.

The disposal of coffee wastes represents an enormous pollution problem in the producer countries. For instance, in 1990 in Colombia one MT green coffee generated waste water, the volume of which was equivalent to the domestic sewage of 2000-2500 people (Gathuo *et al.* 1991, Pulgarin *et al.* 1991). Therefore, several attempts have been made to re-use the coffee wastes for different purposes, including animal feeding (Adams & Dougan 1981, Pulgarin *et al.* 1991). Coffee pulp has been incorporated into diets for domestic animals and fish with variable success (Bayne *et al.* 1976, Bressani & Braham 1980, Abate & Pfeffer 1986, López & Pabón 1986, Donkoh *et al.* 1988, Demeke 1991, Fagbenro & Arowosoge 1991). In pond culture, tilapia fed a diet with 300 g kg^{-1} CoP showed a similar growth than the group receiving a control diet, but in concrete ponds, similar and lower dietary levels of CoP (100-300 g kg^{-1}) reduced the growth response of catfish (Bayne *et al.* 1976, Fagbenro & Arowosoge 1991).

The use of CoP is restricted mainly through its high fraction of cell wall constituents and more particularly, through its content of antinutritional factors (ANF's) such as total phenols (polyphenols), tannins and caffeine. These ANF's may interfere with the acceptance of food and/or the absorption of nutrients (Gómez *et al.* 1985, Vélez *et al.* 1985, De Rozo *et al.* 1985, López & Pabón 1986, Mehansho *et al.* 1987). Therefore, attempts have been made to destroy or diminish the content of these ANF's in CoP which is usually achieved by treating the CoP with alkali solutions, by ensiling, by soaking in water, by pressing, etc. (Murillo *et al.* 1975, Murillo *et al.* 1976, Cabezas *et al.* 1976, López & Pabón 1986, Gómez *et al.* 1988, Rolz *et al.* 1988). Tannin contents in CoP (dry and ground) were reduced by NaOH addition (solutions of 25, 50 and 75 g kg^{-1}) for 24 h, but ash and cell wall contents were increased (Egaña *et al.* 1977, cited by Gómez 1979). The effect of longer exposure time and higher reagent concentration, however, has not been evaluated yet. Furthermore, their results may be controversial because Murillo *et al.* (1975) found that the level of acid detergent fibre, cellulose and hemicellulose decreased in coffee hulls treated with NaOH at 25, 50 and 100 g kg^{-1} for 24 h. Also in other plant residues, NaOH reduced the concentration

of cell walls (Klopfenstein 1994). Combinations of acid-alkali treatment and alkali-ensilage have been applied to precondition the wastes for subsequent treatments. The use of sulphuric acid in straw improved its dry matter digestibility (Owen *et al.* 1984). Further, Owen *et al.* (1984) suggested that acids could be successfully used to pretreat and pre-condition fibrous materials before a subsequent treatment is given. A mixture of alkali-treated and acid-treated straw was used successfully in cattle (Fahmy & Orskov 1983 and Streeter & Horn 1982, cited by Owen *et al.* 1984). Such treatments have not been tested yet with coffee pulp.

According to Klopfenstein (1978), NaOH would be most effective in improving the nutritional quality of fibrous residues. Therefore, in the current study, it was decided to test this treatment again, however using higher concentrations and longer exposure time than in previous studies. In the current study, an attempt was made to increase the nutritional value of CoP by the following methods: (a) NaOH treatment; (b) Pretreatment with HCl followed by NaOH and (c) Pretreatment with NaOH followed by ensilage. In the current study, the response of CoP to these treatments was analyzed by measuring changes in the chemical composition of treated and untreated CoP samples.

Materials and Methods

The procedures of the coffee processing units involve washing, depulping, fermentation, dehulling, selection, drying and packing. Fresh CoP was collected from the processing unit at the beginning of the harvest season. By this time, the processed coffee berries were mainly harvested from lowland (500-600 m above sea level) and the dominant species is *Coffea arabica* var. *caturra*. Fresh CoP was taken from a mixture of coffee berries maintained in water tanks for one day and from berries that arrived at the plant recently. Apart from the pulp, it also contained variable amounts of coffee leave pieces, damaged grains, berry pedicels, seed husks and stalk pieces. The amounts of these impurities depend on the quality of berry batches and the condition of the depulping machine. The fresh CoP was kept overnight at 4-6 °C and its initial pH was 4.4.

Treatments

Treatments applied to CoP consisted of 1) alkali treatment, 2) a combined acid-alkali and 3) a combined alkali-ensilage treatment. In addition, one kg untreated fresh CoP was oven-dried for 24 h at 60 °C and used as control on initial conditions. All treatments were applied in triplicate and at the end the solid fraction of treated CoP was oven-dried for 24-48 h at 60 °C and milled at 1-mm particle size for further analysis.

1) The alkali treatment: One kg fresh CoP samples were mixed with 50 and 100 g kg^{-1} NaOH solutions (1:1, CoP:NaOH, w/v) and maintained in sealed plastic containers for 24 and 48

h. After treatment, the CoP was washed five times with tap water, neutralized with HCl 0.5 M and drained.

2) The combined acid-alkali treatment: One kg samples of fresh CoP, maintained in sealed glass containers, were mixed with HCl 1.5 M and 3 M HCl (1:1, CoP:HCl, w/v) for 24 and 48 h. After HCl treatment, CoP was washed five times with tap water, neutralized with a NaOH solution of 50 g kg^{-1} and subsequently, treated with a NaOH solution of 50 g kg^{-1} (1:1, CoP:NaOH) for 48 h. Finally, CoP was neutralized with 0.5 M HCl and drained.

3) The alkali-ensilage treatment: Samples of 4.5 kg coffee pulp were treated with a NaOH solution of 50 g kg^{-1} for 24 h (1:1 w/v, CoP:NaOH) and maintained in sealed plastic containers. Subsequently, CoP was washed five times with tap water lowering its pH to 10-11 and, subsequently ensilaged with 50 and 100 g kg^{-1} of molasses (on weight basis, CoP:molasses) for two and three months, respectively. Molasses were diluted in the same amount of water for better mixing with CoP. The molasses are a by-product of sugar cane industry and consist of 92 g kg^{-1} ash, 60 g kg^{-1} fibre, 5 g kg^{-1} fat, 96 g kg^{-1} protein, 587 g kg^{-1} NFE and 160 g kg^{-1} moisture (gross energy of 12.8 kJ g^{-1}) (own analysis). They were added to the silage reservoirs because they promote bacterial growth (Murillo 1979).

Analyses

Treated and untreated CoP samples were analyzed for crude fat (920.39A), crude ash (942.05) by standard methods (AOAC 1990). The cellulose was measured gravimetrically according to Meites (1963). True protein was used instead of crude protein to correct for nitrogen from amides. To determine the true protein levels in CoP, samples were boiled in water to dissolve the amides present. After boiling, saturated aluminium potassium sulphate (2-3 ml), copper sulphate (10 ml, 20% w/v) and 0.8 M sodium hydroxide (10 ml) were added. In this condition, the protein was precipitated in the presence of copper (II) hydroxide and subsequently, separated from dissolved amides by filtration. The precipitate was washed with hot water several times, filtered again and its N content was analyzed by the Kjeldahl method (Stutzer & Barstein 1900). Total phenols (polyphenols) and tannins were extracted from CoP with a methanol/water solution (70% v/v) by reflux for two hours at boiling temperature. Their determinations were done using the Folin-Ciocalteu reagent according to Slinkard & Singleton (1977) and Field (1991). Polyphenols were expressed as total phenols (g kg^{-1}) and tannins as the difference between total phenols and phenols from Polyvinylpolypyrrolidone (PVPP) treated samples (Field 1991). Caffeine was measured spectrophotometrically according to Morris (1973). CoP samples were boiled in a solution of water and 0.1 N sulphuric acid and filtered. To the filtrate was added 1 M zinc acetate and potassium ferrocyanide (0.25 M) and after mixing well, this solution was filtered again. Next, to the filtrate was added heavy magnesium oxide and the mixture was boiled. By adding the latter

reagents to the filtrate all interfering impurities were removed. Finally, the mixture was filtered again and filtrate was read at 272 nm for caffeine determination. All determinations were done in triplicate.

The effect of different treatments on the CoP composition was analyzed by Multifactorial ANOVA according to the model $Y_{ijk} = \mu + (time)_i + (concentration)_j + (time \times concentration)_k$ + error. Afterwards, treatment comparisons with CoP initial condition (oven-dried CoP) were done by an one way ANOVA. Differences between treatment means were tested by LSD Test at 95% confidence interval. All variables were tested for homogeneity of variances using the test of Bartlett. The statistical analyses were done using the STATGRAPHIC PLUS 7.1 software.

Results

1) NaOH treatment

The NaOH addition and exposure time increased the ash content ($P<0.05$) in the CoP samples whereas cellulose was not significantly affected, when compared to oven-dried CoP (OD-CoP). The NaOH addition and exposure time reduced significantly the content of total phenols (more than 70%), tannins (more than 65%) and caffeine (more than 85%) compared to OD-CoP. The interaction between NaOH concentration and treatment time was not significant for most variables measured, except for true protein. Fat content was affected only by exposure time (higher value at 24 h) (Table 1).

Table 1. Changes in CoP composition after the NaOH treatment (at two concentrations and two-exposure time) compared to oven-dried CoP. Data expressed as g kg^{-1} of dry matter (DM).

Time		24 h		48 h		
Treatment / Variable	Oven-dried CoP	NaOH (50 g kg^{-1})	NaOH (100 g kg^{-1})	NaOH (50 g kg^{-1})	NaOH (100 g kg^{-1})	Significant effect (1)
True protein	80	96 ±0.5	80 ±0.1	71 ±1.0	109 ±0.5	T*C
Crude fat	29	49 ±0.1	41 ±0.4	30 ±0.6	22 ±0.7	T
Crude ash	89	144 ±1.4	155 ±2.1	219 ±1.8	167 ±1.4	NS
Cellulose	286	286 ±1.1	246 ±1.8	311 ±2.1	279 ±1.6	NS
Total phenols	20	2 ±0.1	2 ±0.08	3 ±0.1	2 ±0.06	NS
Tannins	7	1 ±0.01	0.5 ±0.02	1 ±0.06	1 ±0.04	NS
Caffeine	18	2 ±0.06	2 ±0.02	3 ±0.1	2 ±0.02	NS

(1) Significant effect for the factorial analysis, T: time, C: concentration, T*C: interaction ($P<0.05$). NS: not significant.

2) HCl-NaOH treatment

The HCl/NaOH treatment reduced the true protein but increased fat and ash contents compared to OD-CoP (P<0.05). Protein tended to be lower and fat tended to be higher at higher HCl concentration (P=0.08 and 0.09). The cellulose content at 48 h was similar to OD-CoP (P>0.05) but at 24 h it was significantly higher. The HCl-NaOH addition lowered total phenols (more than 80%), tannins (more than 80%) and caffeine (more than 65%) contents (P<0.05) (Table 2). The caffeine tended to be lower at the higher HCl concentration (P=0.06). There was no interaction between reagent and time for any of the variables measured.

Table 2. Changes in CoP composition after the HCl-NaOH treatment (at two concentrations and two-exposure time) compared to oven-dried CoP. Data expressed as g kg^{-1} of DM.

Time		24 h		48 h		
Treatment / Variable	Oven-dried CoP	HCl (1.5 M)	HCl (3 M)	HCl (1.5 M)	HCl (3 M)	Significant effect [1]
pH	4.5	12.3 ±0.1	11.8 ±0.15	12.1 ±0.08	11.9 ±0.2	NS
True protein	80	56 ±0.2	50 ±0.6	66 ±0.4	55 ±0.5	C (P=0.08)
Crude fat	29	41 ±0.3	41 ±0.3	33 ±0.3	46 ±0.7	C (P=0.09)
Crude ash	89	105 ±0.8	120 ±0.06	120 ±0.8	117 ±1.4	NS
Cellulose	286	348 ±2.3	320 ±1.8	250 ±1.6	280 ±1.7	T
Total phenols	20	2 ±0.01	5 ±0.09	3 ±0.05	3 ±0.09	NS
Tannins	7	1 ±0.00	2 ±0.09	1 ±0.02	1 ±0.02	NS
Caffeine	18	6 ±0.06	2 ±0.08	6 ±0.06	1 ±0.02	C (P=0.06)

[1] Significant effect for the factorial analysis, T: time, C: concentration, T*C: interaction (P<0.05). NS: not significant.

3) NaOH-ensilage treatment

The NaOH-ensiled CoP had higher true protein, fat and ash contents than OD-CoP (P<0.05). The concentration of added molasses only affected the protein content (higher values at 50 g kg^{-1} molasses), and the ensilage time only affected the ash content (higher ash values at three months). Cellulose values were reduced at the higher molasses concentration (P<0.05). The NaOH-ensilaged CoP had lower contents of total phenols (more than 45% less), caffeine (more than 44% less) and tannins (more than 55% less) than OD-CoP (P<0.05). The total phenols and tannin contents were affected by the molasses concentration (lower values at 50 g kg^{-1} molasses). No interaction was found for any of the variables measured (Table 3).

The NaOH-ensilaged CoP with 50 g kg^{-1} molasses had higher pH (5.3-5.6) than CoP-

ensilaged with 100 g kg^{-1} molasses and the fresh CoP (4.4-4.9), however, pH of NaOH-ensilage CoP with 10 g kg^{-1} molasses was similar to pH of fresh CoP. The amount of drainage liquid measured increased with addition of molasses to the pulp (50 g kg^{-1} molasses: 0.61 l – 0.66 l vs. 100 g kg^{-1} molasses: 0.81 l -0.86 l ($P<0.05$). The presence of mud contamination only occurred as small spots in some replicates, which were removed and eliminated.

Table 3. Changes in CoP composition after the NaOH-ensilage treatment (at two concentrations and two-exposure time) compared to oven-dried CoP. Data expressed as g kg^{-1} of DM.

Time		2 months		3 months		
Treatment / Variable	Oven-dried CoP	Molasses (50 g kg^{-1})	Molasses (100 g kg^{-1})	Molasses (50 g kg^{-1})	Molasses (100 g kg^{-1})	Significant effect [1]
True protein	80	107 ±0.02	87 ±0.6	114 ±0.2	82 ±0.4	C
Crude fat	29	100 ±1.2	92 ±1.1	101 ±1.1	107 ±0.6	NS
Crude ash	89	169 ±1.7	166 ±0.9	184 ±0.2	196 ±0.3	T
Cellulose	286	294 ±1.0	247 ±1.9	277 ±1.2	251 ±0.3	C
Total phenols	20	7 ±0.3	10 ±0.1	7 ±0.1	12 ±0.06	C
Tannins	7.4	2.7 ±0.07	3 ±0.04	1.6 ±0.08	3.5 ±0.01	C
Caffeine	18	10 ±0.7	11 ±0.6	11 ±0.8	4 ±0.2	NS
pH	4.5	5.6 ±0.0	4.6 ±0.4	5.3 ±0.04	4.4 ±0.5	C

[1] Significant effect for the factorial analysis, T: time, C: concentration, T*C: interaction ($P<0.05$). NS: not significant.

Discussion

The proximate composition of the oven-dried CoP (OD-CoP) showed values (on dry matter basis) similar to those found in literature (Bressani *et al.* 1972, Elías 1979, Pulgarin *et al.* 1991, Gathuo *et al.* 1991), except cellulose, which was higher in this study than in literature reports. The true protein content found was about 60-66% of the crude protein content, which could mean that about 40% of CoP protein might be indigestible for animals. This is in good agreement with López & Pabón (1986). The caffeine content in OD-CoP was higher but total phenols (polyphenols) and apparent tannins were much lower than those reported by Bressani *et al.* (1972), Elías (1979), Clifford & Ramírez (1991a,b), Clifford *et al.* (1991). These differences may confirm not only chemical variability of the CoP due to location (soil type, altitude), coffee strains (species and varieties) and culture management (Bressani *et al.* 1972, Elías 1979) but also differences in extraction and analyzing methodology (specially for the ANF's) (García *et al.* 1985, Ramírez 1987, Ramírez 1988, Clifford *et al.* 1991). Phenols and tannins also bind with (starch/non-starch) polysaccharides or are absorbed by cellulose, which could affect the extraction and, as a result, the correct

determination of these components in CoP (Mueller-Harvey & McAllan 1992).

Chemical (NaOH and HCl-NaOH) CoP treatments

The two NaOH concentrations and/or exposure time used did not produce significant changes in the true protein, fat and cellulose contents of treated CoP. This result coincides with findings of Egaña (1977, cited by Gómez 1979). Contrary to these results for coffee pulp, in coffee hulls NaOH (solution of 25 to 100 g kg^{-1}) reduced the acid detergent fibre, cellulose, hemicellulose and to lesser extent lignin (Murillo *et al.* 1975). The alkali treatments applied to CoP did not induce the partial hydrolysis and solubilization of the cell walls as found in other fibrous materials (Jackson 1977, Klopfenstein 1994). These differences are probably related to differences in chemical and physical characteristics between the pulp and hulls (CoH) of coffee. The CoH has lower protein and fat contents, and higher ash and fibre components than CoP does; CoH has a more rigid physical structure than CoP does.

The NaOH treatments decreased the ANF's contents in CoP (total phenols, tannins and caffeine). Gómez (1979), Price *et al.* (1979), Reichert *et al.* (1980), López & Pabón (1986), Garrido *et al.* (1989) and Marquardt (1989) found similar results in legumes and CoP treated with NaOH or other alkalis. No effect of alkali treatments on caffeine content, however, had been found previously. Cleavage of ester linkages and rupture of ether-lignin linkages of straws (Jackson 1977, Klopfenstein 1994) caused by the alkali addition may result in some phenolic acids release. Dissolution of tannins in the alkali may also contribute to their reduction. Acid/alkali treatments only have been used with sugarcane bagasse and no changes in cellulose digestibility were found (Stone *et al.* 1965, cited by Owen *et al.* 1984). Samples of CoP taken after HCl treatment and before NaOH addition showed only a slight reduction in tannin content. This is contrary to findings with sorghum (Reichert *et al.* 1980). The HCl-NaOH treatment appears to induce higher leaching of soluble organic matter from CoP than the NaOH treatment, and this could produce the relative increment of the non-soluble fractions (ash and fat) and the reduction of true protein. In addition, this result also could be related to the washing/draining process applied to the CoP after HCl treatment. The application of acids (HCl, sulphuric acid and CH_3COOH) has shown also both positive and negative responses in improving the nutritive value of straws (Streeter & Horn 1982, Fahmy & Orskov 1983, cited by Owen *et al.* 1984).

The acid-alkali and NaOH treatments improved the quality of CoP by reducing the content of ANF's, but they also reduced its quality by increasing the ash content. However, the alkali alone produced fewer negative effects on CoP proximate composition than the acid-alkali treatment did.

NaOH-ensilage

True protein and fat levels increased as a consequence of this treatment. This result

might be correlated, however, with the bacterial growth during anaerobic fermentation. The higher ash contents found could be a direct effect of remaining NaOH in the CoP and of the added molasses due to its high levels of minerals (GEPLACEA 1989). Cellulose was similar to OD-CoP, which suggests that the addition of NaOH before ensiling did not make cellulose more accessible for further degradation as found in straws (Klopfenstein 1994). Differences in the chemical structure of the cellulolytic matrix of CoP, perhaps, could explain this difference. The reduction of total phenols, tannins and caffeine found in treated CoP agrees with trends observed in table 4. This reduction can be a result of the utilization of these components by the microorganisms and/or their leaching in the draining liquids (Murillo 1979). Microorganisms from CoP have been found to be able to use caffeine and tannins (Rolz *et al.* 1982, Marin & Raimbault 1992).

Table 4. Comparison [1] of results of different chemical treatments applied to CoP and coffee hulls (CoH).

Stuff	Treatment	True protein	Crude fat	Crude ash	Cellulose	Total phenols	Tannins	Caffeine	References
CoP	NaOH	0	++	++	0	--	--	--	Current study
CoP	NaOH	0 [2]		++	0		--	0	Gómez (1979)
CoH	NaOH	-- [3]			--				Murillo *et al.* (1975)
CoP	$CaOH_2$		0			-- [4]	--	0	Gómez (1979)
CoP	HCl-NaOH	--	++	++	0	--	--	--	Current study
CoP	NaOH-ensilage	++	++	++	--	--	--	--	Current study

[1] Decrease: --, Increase: ++, Similar: 0 as compared to fresh dry CoP. Empty spaces indicate that parameters were not measured.

[2] Total nitrogen.

[3] Crude protein.

[4] Chlorogenic acid.

The pH values of the NaOH-ensilage CoP with 50 g kg^{-1} molasses were outside ranges found by other authors (Murillo *et al.* 1976, Bressani & Braham 1980, Rolz *et al.* 1988). This may have been caused by the previous addition of the alkali, which gave a higher initial pH (10-11) before ensilage. However, other characteristics of silages such as colour, odour and appearance were found to be similar (Braham *et al.* 1973, cited in Murillo, 1979).

All treatments, in general, increased the ash content of CoP, specially the alkali-ensilaged ones. True protein contents were increased only by the NaOH-ensilaged treatment whereas the acid-alkali showed an opposite result. None of the three treatments applied to CoP affect the cellulose content. The reduction of ANF's in alkali-ensilage was not higher than that found in other trials (NaOH and HCl-NaOH). In summary, NaOH and NaOH-ensilage appeared the most appropriate treatments to improve the nutritive value of CoP,

especially in relation to the reduction of ANF's contents.

Finally, it appears that the composition of CoP is also affected by the extent of the relative amount of impurities (Abate 1988), which may increase cell wall contents and presence of ANF's in the CoP. This factor also explains some of the differences between treated CoP compared to previous results. The magnitude of this factor, however, is unknown and difficult to quantify. CoP treated by chemical treatments or another means must be tested "in vivo" in animals to elucidate if the beneficial effect determined in those treatments is translated to the animals.

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Chapter 5.2

Biological treatments affect the chemical composition of coffee pulp

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Abstract

Biological treatments were applied to fresh coffee pulp (CoP) to upgrade its nutritive value for monogastric animals by reducing its content of cellulose and of antinutritional factors (ANF's) such as total phenols (polyphenols), tannins and caffeine. Treatments were: 1) ensiling with 0, 50 and 100 g kg^{-1} molasses for 2 and 3 months, 2) aerobic decomposition for 0, 7, 14, 21, 28, 35 and 42 days, 3) aerobic bacteria inoculation (*Bacillus ssp.*) for 0, 7, 14, 21 and 28 days. Ensilaged CoP (E-CoP) showed significantly higher fat and ash contents than oven-dried CoP (OD-CoP). Similarly, true protein values tended to increase. The cellulose contents of E-CoP were significantly lower than OD-CoP. The E-CoP total phenols levels were significantly lower than OD-CoP, tannins tended to be lower, whereas, caffeine levels remained unaffected. Improvement in the nutritional quality of E-CoP for monogastric animals is associated with higher fat and protein contents and, reduction of cellulose, total phenols and tannins. The aerobic decomposition treatment improved the nutritional quality of CoP by increasing true protein (from 47 to 116 g kg^{-1}) and fat (from 91 to 130 g kg^{-1}) contents. In addition, total phenols (from 11 to 2 g kg^{-1}), tannins (from 7 to 1 g kg^{-1}), caffeine (from 8 to 0 g kg^{-1}) and cellulose contents (from 126 to 114 g kg^{-1}) were reduced by an increase in treatment time ($P<0.05$). Ash contents increased (from 91 g kg^{-1} at 0 days to 187 g kg^{-1} at 42 days) and carbohydrate and dry matter contents decreased with increasing decomposition period. The bacterial treatment reduced dry matter content of CoP from 362 to 184 g kg^{-1}. Bacteria treated-CoP increased the protein content up to 21 days (from 137 to 392 g kg^{-1}) and decreased it after 28 days. Cellulose, total phenols and tannins contents reduced with an increase in time of bacterial degradation, from 168 to 41 g kg^{-1}, 8.3 to 3.2 g kg^{-1} and, 7.9 to 2 g kg^{-1}, respectively. Results indicated that the bacteria treatment improved the CoP quality by increasing protein content and reducing cellulose and ANF's, especially after 21 days of treatment. Both the aerobic decomposition (after 21-28 days) and the aerobic bacterial degradation of CoP (after 21 days) appear more suitable to improve the nutritional quality of CoP for monogastric animals than the ensilage.

Keywords: coffee pulp, biological treatments, wastes upgrading, antinutritional factors.

Introduction

Coffee pulp (CoP) is the main solid residue from the wet processing of coffee berries and it constitutes about 41% of the wet weight of the coffee berry (Montero 1992). For 1996 the total world production of coffee wastes was estimated at about 22 million MT CoP, 2.4 million MT mucilage and 8.6 million MT hulls (estimation based on production data, FAO 1997). These figures fluctuate yearly according to variations in coffee production and processing technique used. The problem of disposal of coffee wastes, however, is enormous and represents a potential pollution risk in producer countries.

Efforts to recycle the CoP include activities such as composting, feeding to animals, producing organic fertilizers and biogas or single-cell protein (SCP) and others (Rolz *et al.* 1982, Adams & Dougan 1981, Pulgarin *et al.* 1991). In animal feeding, the use of CoP is restricted by the high content of fibre and by the presence of antinutritional factors (ANF's) such as polyphenols (total phenols), tannins and caffeine. They may interfere with food intake and with nutrient absorption (De Rozo *et al.* 1985, López & Pabón 1986, Mehansho *et al.* 1987). Upgrading of CoP by biological treatments involves anaerobic and aerobic processes that include the use of microorganisms such as yeast, bacteria and fungi (ensilage, aerobic decomposition, natural fermentation, solid state fermentation, SCP) (Murillo 1979, Rolz *et al.* 1982, Lopez & Pabón 1986, Tauk 1986). Direct effect of these treatments, in most cases, will be the increase in the protein content and the reduction of fibre or cellulose and some ANF's (caffeine, total phenols and tannins) in the final product (Tauk 1986, López & Pabón 1986, Rolz *et al.* 1988). Several bacteria capable to digest fibre components have been isolated from other crop residues. They might also upgrade other agro-industrial wastes (Amato 1999).

The use of CoP in animal feeding is mainly restricted to fresh and/or dry untreated CoP. The use of biologically treated CoP in animal rations usually has been limited to ensilaged-CoP (Murillo 1979) and solid fermented-CoP with fungi (López & Pabón 1986). Calves and rats fed ensilaged-CoP showed a better performance (growth, feed efficiency, and survival) than those fed non-treated CoP, however with pigs no differences were detected (Murillo 1979). The use of fungi treated-CoP in rat's diets gave better performance than untreated CoP; however, with chickens both types of CoP gave similar results (López & Pabón 1986). To improve the nutritive value of CoP for monogastric animals, the biological treatments applied to CoP in this research were selected based on results of Rolz *et al.* (1982), Tauk (1986) and Amato (1999).

The aim of this research was to evaluate the effect of some biological treatments on the chemical composition and content of ANF's (total phenols or polyphenols, tannins and caffeine) of CoP in relation to the potential of CoP for monogastric animals feeding.

aerobic decomposition of CoP and bacteria inoculation of CoP were analyzed by one way ANOVA. Differences between treatment means were tested by the LSD Test at 95% confidence interval (STATGRAPHIC PLUS 7.1 software).

Results

1) Ensilage

The dry matter of ensilaged-CoP (E-CoP) was lower than in oven-dried-CoP (OD-CoP) ($P<0.05$). The increase in ensilage time significantly decreased the CoP dry matter content whereas the addition of molasses did not affect the CoP dry matter content.

True protein, fat and ash contents in E-CoP were higher than in OD-CoP, especially when CoP was ensilaged for two months or with 50 g kg^{-1} molasses (wet basis) ($P<0.05$). Cellulose contents were lower in ensilaged CoP than in OD-CoP ($P<0.05$). The molasses concentration of 50 g kg^{-1} (wet basis) had a significant effect on the protein and ash contents (increased both components), however, a further increase in molasses hardly affect the chemical composition of CoP. The storage time only affected the fat content (higher values) ($P<0.05$). The time and molasses concentration showed an interaction only for the ash content. Total phenols and tannin contents were lowered also by ensilage time and molasses addition when compared to OD-CoP ($P<0.05$), whereas caffeine values were not affected (Table 1).

Table 1. Chemical composition of CoP samples after ensilage treatments (expressed as g kg^{-1}, DM). Composition of ensilaged CoP is also compared to oven-dried CoP. (Means ± standard error).

Treatment / Variable	Oven-dried CoP	2 months			3 months			Significant effect [1]
		Molasses (0 g kg^{-1})	Molasses (50)	Molasses (100)	Molasses (0 g kg^{-1})	Molasses (50)	Molasses (100)	
Dry matter	259± 1.1	237± 0.9	227± 0.5	222± 0.9	225± 0.8	210± 1.9	212± 1.2	*T
True protein	80± 0.1	96 ±0.6	100±0.2	84±0.3	88±0.0	90±0.2	83±0.2	* C
Crude fat	29±0.2	56±0.2	63±0.1	56±0.3	87±0.3	78±0.3	81±0.2	* T
Crude ash	89± 0.2	101±0.1	118±0.06	125±0.2	100±0.05	114±0.5	139±0.3	* C, T x C
Cellulose	286± 1.0	224±0.1	213±1.0	216±0.9	237±0.8	208±1.6	222±0.9	NS
Total phenols	20± 0.0	8±0.05	12±0.2	13±0.2	10±0.05	10±0.1	14±0.0	NS
Tannins	7.4± 0.08	3±0.02	6±0.03	4±0.05	3±0.03	4±0.03	5±0.0	NS
Caffeine	18±0.06	13±0.02	22±0.05	11±0.05	19±0.05	20 ±0.04	18±0.05	NS
pH	4.5±0.01	4.2±0.02	4.2±0.0	4.2±0.02	3.9±0.0	4.0±0.02	4.0±0.02	* T
Drainage liquid (l)	--	0.53±0.03	0.68±0.07	0.93±0.01	0.46±0.06	0.58±0.04	0.98±0.01	* C

[1] * Significance according to factorial ANOVA ($P<0.05$), C: molasses concentration, T: months, T x C: interaction and NS: not significant.

The pH was similar for all molasses concentrations and varied from 3.9 to 4.2, but pH decreased with increase in ensilage time ($P<0.05$). The pH of treated samples was also lower than the pH of fresh CoP. The production of drainage liquid increased with addition of molasses from 0.5 l to 0.96 l ($P<0.05$). The production of drainage liquid was unaffected by the ensilage time (2 months: 0.7 l, 3 months: 0.67 l).

2) Aerobic decomposition

The room temperature and moisture varied from 17.2 °C (night time) to 29.4 °C (day time) and from 56 % (day time) to 98 % (night time), respectively, during the duration of the experiment. The temperature in the CoP increased up to 35 °C until 5th day and afterward it decreased to values between 22- 25 °C until 10th day, remaining unchanged for the rest of the experimental time. The dry matter was progressively lost (from 242 to 175 g kg^{-1}) with the increase in days of treatment ($P<0.01$).

Aerobic decomposed CoP had higher protein (from 47 to 124 g kg^{-1} DM up to 28 days) and fat (from 70 to 126 g kg^{-1} DM up to 35 days) contents than untreated sample ($P<0.01$). Both the protein and fat contents decreased again at 35 and 42 days (118 and 102 g kg^{-1} DM), respectively. The ash content of treated CoP steadily increased with time of decomposition, from 91 g kg^{-1} DM at 0 days to 187 g kg^{-1} DM at 42 days. The carbohydrate content, however, decreased with time of treatment ($P<0.01$). The cellulose contents apparently was not affected by treatment, however it showed a slightly reduction after 7 days of treatment until day 28 ($P<0.05$). Total phenols, tannins and caffeine contents decreased after 7 days of treatment and afterward they remaining unchanged. Caffeine was not detected after 14 days of treatment ($P<0.01$) (Table 2).

Table 2. Changes in the chemical composition of CoP after the aerobic natural decomposition treatment (g kg^{-1}, DM). Natural decomposed CoP is compared to oven-dried CoP. (Means ± standard error).

Treatment / Variable	0 days (OD-CoP)	7 days	14 days	21 days	28 days	35 days	42 days	p[1]
Dry matter	242±0.9 ^{a}	209±1.0 ^{bc}	199±0.8 ^{c}	187±1.1 ^{d}	199±0.5 ^{c}	184±0.6 ^{d}	175±0.2 ^{e}	**
True protein	47±0.2 ^{a}	101 ±0.4 ^{b}	110±0.4 ^{c}	124±0.6 ^{c}	124±0.1 ^{c}	118±0.4 ^{c}	116±0.2 ^{c}	**
Crude fat	70±0.3 ^{a}	84±0.7 ^{a}	76±0.5 ^{a}	79±0.5 ^{a}	108±0.6 ^{b}	126±0.8 ^{c}	102±0.2 ^{b}	**
Crude ash	91±0.5 ^{a}	147±0.3 ^{b}	160±0.2 ^{c}	164±0.3 ^{c}	175±0.1 ^{d}	176±0.4 ^{d}	187±0.2 ^{e}	**
Carbohydrate [2]	316±1.2 ^{a}	191±0.6 ^{b}	167±0.6 ^{c}	114±0.4 ^{d}	100±0.4 ^{de}	99±0.2 ^{de}	89±0.8 ^{e}	**
Cellulose	126±0.2 ^{a}	114±0.4 ^{b}	118±0.3 ^{ab}	114±0.1 ^{b}	115±0.2 ^{b}	120±0.2 ^{ab}	120±0.1 ^{ab}	*
Total phenols	11±0.1 ^{a}	1±0.01 ^{b}	2±0.03 ^{b}	2±0.01 ^{b}	2±0.01 ^{b}	3±0.02 ^{b}	1±0.0 ^{b}	**
Tannins	7±0.09 ^{a}	1±0.01 ^{b}	1±0.03 ^{b}	1±0.01 ^{b}	1±0.0 ^{b}	2±0.03 ^{b}	1±0.0 ^{b}	**
Caffeine	8±0.05 ^{a}	4±0.07 ^{b}	0.0	0.0	0.0	0.0	0.0	**

(1) P: Means in the same line with different letters are significantly different (*: $P<0.05$, **: $P<0.01$)

(2) Carbohydrate refers to starch and sugar expressed as g kg^{-1} of glucose.

3) Aerobic bacteria inoculum

In the solid fraction, protein content of CoP increased with time of degradation up to 21 days (137 to 392 g kg^{-1} DM) but decreased afterwards (28 days: 267 g kg^{-1} DM) ($P<0.05$). The initial dry matter (362 to 184 g kg^{-1}), cellulose (168 to 41 g kg^{-1} DM), total phenols (8.3 to 3.2 g kg^{-1} DM) and tannins (7.9 to 2 g kg^{-1} DM) contents decreased with increase in bacteria degradation time ($P<0.01$). However, caffeine contents in treated samples increased after 7 days of bacteria degradation (from 2.9 to 4.4 g kg^{-1} DM, $P<0.05$) and afterwards remained unchanged (Table 3).

Table 3. Chemical composition of the solid (g kg^{-1}, DM) and liquid (mg/10 ml) fractions of CoP after the bacterial inoculation treatment. (Means ± standard error).

Sample fraction	Treatment / Variable	0 days	7 days	14 days	21 days	28 days	Effect (1)
Solid	Dry Matter	362±0.6 ^{a}	233±1.2 ^{b}	209±0.9 ^{bc}	196±0.4 ^{bc}	184±1.0 ^{c}	**
	True protein	137±2.0 ^{a}	346±1.0 ^{b}	261±1.5 ^{ab}	392±1.7 ^{c}	267±0.9 ^{b}	*
	Cellulose	268±1.1 ^{a}	263±0.9 ^{ab}	204±1.6 ^{bc}	175±1.2 ^{c}	141±0.4 ^{c}	**
	Total phenols	8.3±0.03 ^{a}	6.9±0.04 ^{b}	5.1±0.05 ^{c}	4.4±0.01 ^{c}	3.2±0.01 ^{d}	**
	Tannins	7.9±0.03 ^{a}	6.2±0.04 ^{b}	4.4±0.02 ^{c}	3.2±0.01 ^{d}	2.0±0.1 ^{e}	**
	Caffeine	2.9±0.2 ^{a}	4.4±0.1 ^{b}	4.0±0.0 ^{b}	4.6±0.4 ^{b}	4.4±0.01 ^{b}	*
Liquid	True protein	56.3±1.5 ^{a}	17.6±0.2 ^{b}	14.6±1.4 ^{b}	22.8±1.8 ^{b}	42.0±1.6 ^{c}	**
	Total phenols	2.1±0.1 ^{ab}	2.7±0.1 ^{c}	2.3±0.2 ^{abc}	2.5±0.3 ^{bc}	1.9±0.2 ^{a}	*
	Tannins	0.95±0.1 ^{ab}	1.2±0.1 ^{b}	1.0±0.1 ^{b}	1.1±0.1 ^{b}	0.5±0.05 ^{a}	*

(1) Means in the same line with different letters are significantly different (*: $P<0.05$, **: $P<0.01$), NS: not significant.

In the liquid phase, total phenols and tannins contents remained practically unchanged up to 21 days of treatment, but they decreased afterwards to levels similar to the control ($P<0.05$). The true protein contents were reduced after 7 days of treatment, however they increased at 28 days of CoP bacterial degradation ($P<0.01$).

Discussion

1) Ensilage of CoP

The ensilage treatment slightly increased the protein content but doubled the fat content of CoP. This could result from microbial growth in ensilaged CoP (E-CoP), which eventually resulted in higher protein and fat contents of the E-CoP as reported by Ramírez *et al.* (1999). The higher ash contents found in E-CoP could be a direct effect of the added molasses, which are reported to have high levels of minerals (GEPLACEA 1989). Bressani &

Braham (1980) concluded that the proximal composition of E-CoP practically remained similarly as in oven-dried CoP (OD-CoP). A review of their data suggests, however, that E-CoP had a higher protein and cellulose levels but also a lower caffeine level than OD-CoP. Murillo (1979) and Gómez *et al.* (1985) reported also reduced levels of cellulose, caffeine, polyphenols and tannins in E-CoP (Table 5). According to our results microbial growth in E-CoP caused a partial degradation of cellulose (reduction from 286 to 208 g kg^{-1}). This anaerobic conversion of carbohydrate (e.g. cellulose) in ensiling has been also reported by Adams & Dougan (1981).

The reduction of total phenols (polyphenols) and tannin contents agrees with previous findings (Table 4). This reduction may be a result of anaerobic microbial degradation and of some leaching in the draining liquid. The water-soluble caffeine, however, remained similar to OD-CoP after the ensilage process, which is in agreement with findings of Ramírez *et al.* (1999). The pH values of E-CoP concurred with previous reports (Rolz *et al.* 1988, Ramírez *et al.* 1999).

The observed differences with published data may be caused by differences in the molasses concentration used, the ensilage time and by the analytical methodology used to determine especially total phenols or polyphenols and tannins (Murillo 1979, Ramírez *et al.* 1999). The amount of molasses added to the CoP could affect the final relative contents of the chemical components measured when they were compared to OD-CoP. Molasses addition, relatively could increased the ash content and, could decreased cellulose. The ensilage time could affect mainly the final levels of true protein, cellulose and fat. Our results indicate that the ensilage process modified the composition of the original CoP.

Table 4. Effect [1] of ensilage treatments on different CoP components, compared to oven-dried CoP.

Crude protein	Fat	Ash	Cellulose	Total phenols	Tannins	Caffeine	References
++ [2]	++	++	--	--	--	0	This study
++	0	++	++		--	0	Murillo *et al.* (1976)
0, --			--		0, --	--	Murillo (1979)
++		--	++		0	--	Bressani & Braham (1980)
--	++	--		-- [3]	--	--	Gómez *et al.* (1985)

(1) ++: Increase, --: Decrease and 0: Similar, as compared to oven-dried CoP. Empty spaces indicate that parameters were not measured.

(2) True protein.

(3) Chlorogenic acid.

In general, results show that the nutritional quality of E-CoP for monogastric animals is found superior to OD-CoP. Its improvement in quality may be associated with higher fat

and protein contents and, a reduction in total phenols and tannins. Negative effects of ensiling are related to increasing ash contents. Furthermore, the increase in molasses level from 50 to 100 g kg^{-1} and the treatment time from two to three months did not produce significant improvement in the nutritional quality of ensilaged CoP. In summary, the best results were found in CoP ensilaged with 50 g kg^{-1} and for two months.

2) Aerobic decomposition of CoP

The CoP contains variable amounts of microorganisms (fungi, yeast, bacteria, etc.), which together contribute to its degradation. These aerobic microorganisms mainly appear to increase true protein and fat contents in treated-CoP as a result of biomass growth. Similar results were found by Adams & Dougan (1981), by Peñazola *et al.* (1985) with CoP fermented by *Aspergillus niger* and by Tauk (1986) treating CoP samples with either microorganism mixtures or isolated organisms obtained from the same pulp. The relative content of carbohydrate in treated-CoP decreased as a consequence of its utilization by the aerobic microbes. The higher ash content found in the decomposed CoP can be an indirect effect of losses in organic matter through the process. This agrees with findings of Rolz *et al.* (1982) and Peñazola *et al.* (1985). The degradation of the cellulose was partial and only reached about 8% in the final CoP product. The *A. niger* used in solid-state fermented CoP also showed a partial degradation of cellulose (Peñazola *et al.* 1985). When the temperature rises during the aerobic CoP decomposition, the thermophilic aerobic microbes decompose carbohydrate polymers (cellulose and hemicellulose) at higher rates than mesophilic ones (Rolz *et al.* 1982).

Mixtures of microbes present in CoP are capable to degrade total phenols up to 90%, tannins up to 85% and all caffeine after 14 days treatment. The capability to degrade these compounds however strongly depends on the type of microbes used or found in CoP. For instance, *A. niger* was not capable to degrade these compounds in CoP (Peñazola *et al.* 1985) but *A. oryzae*, *Penicillium roquefortii* and *P. crustosum* were able to degrade completely caffeine and partially the phenols in CoP (Rolz *et al.* 1982, Marin & Raimbault 1992). Fungi had shown different ability to digest cellulolytic components, caffeine and phenols from CoP (Rolz *et al.* 1988). The progressive dry matter losses with an increase in treatment time could result from the microbial degradation of the substrate, which used organic carbon components of CoP and from some leaching of organic matter from CoP. Similar results were found by Peñazola *et al.* (1985) and Tauk (1986).

In summary, a period of CoP degradation of 21 to 28 days seems acceptable to improve the nutritive value of CoP for monogastric animals, especially because ANF's were reduced strongly at these periods. The potential use of natural decomposed-CoP in animal feeding seems favorable because of its increase in protein and fat contents and of its reduction in total phenols, tannins and caffeine levels. However, the potential benefit of this treatment

to animal feeding has to be proven because up to date there is no published data available on the use of aerobic natural decomposed CoP on animal feeding.

3) Bacteria inoculation in CoP

The *Bacillus ssp.* increased the protein content in CoP due to the bacterial growth and the reduction of the cellulose content. Similar results were reported by Tauk (1986) who found an increase in the protein content of CoP after 23 days of treatment with *Aspergillus ssp.* or a mixture of microorganisms from the same pulp. The reduction in the protein content after 21 days of treatment may be caused by the reduction on the nutrient availability and on the cellulose content that could not sustain the bacterial growth.

This study demonstrated that the *Bacillus ssp.* had the ability to utilize extensively the cellulose of the CoP as also was found for other fibrous materials (Amato 1999). Some fungi also were capable to utilize the fibre fraction of CoP (López & Pabón 1986). The sharp reduction in the total carbohydrate content of treated CoP samples could indicate that *Bacillus ssp.* utilize not only the cellulolytic fraction but also other organic carbon components. Other microorganisms such as the fungi *Aspergillus ssp.*, the yeast *Hansenula tropicalis* and mixtures of microorganisms obtained from the same pulp were also capable to reduce carbon organic components in CoP (Tauk 1986).

The *Bacillus ssp.* population that grew on the CoP also reduced the content of total phenols and tannins with increase in treatment time, which indicated that these bacteria were capable to utilize these components. The reduced amount of these ANF's determined at the end of the trial supports this statement. Similar results were found with fungi by López & Pabón (1986). The caffeine content, however, was not affected by the *Bacillus ssp.* and its increase could result from the relative reduction of the other components. Bacteria and fungi apparently could be very specific to utilize some of the ANF's found in CoP and results from this study indicated that bacteria that belong to the *Bacillus* group are not able to use caffeine.

In summary, a period of CoP bacterial degradation of 21 days seems acceptable to improve the nutritive value of CoP for monogastric animals, especially because the treated CoP had the highest protein content and, the cellulose, total phenols and tannins were reduced strongly at this period. This improvement in the chemical composition of bacterial treated CoP appears to be favorable for its use in animal feeding, especially for monogastric animals. However, bacteria treated CoP has to be tested in feeding trials to elucidate if the potential benefits of treated CoP will be translated to animals.

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Chapter 6

Growth, feed utilization and nutrient digestibility in tilapia fingerlings (*Oreochromis aureus* Steindachner) fed diets with bacteria-treated coffee pulp

Growth, feed utilization and nutrient digestibility in tilapia fingerlings (*Oreochromis aureus* Steindachner) fed diets with bacteria-treated coffee pulp

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Abstract

The effectiveness of bacteria treated-coffee pulp (BT-CoP) in fish diets was evaluated in a feeding trial with *Oreochromis aureus* fingerlings. Five diets were formulated to contain 0, 60, 120, 180 and 240 g kg^{-1} BT-CoP, replacing wheat meal. Fish were reared in a recirculating unit consisting of 16 aquaria. Each aquarium was stocked with 10 fish of 1.1-2.4 g. Fish were fed "to apparent satiation" twice daily (10 and 15 h) for four weeks. Fish fed diets without BT-CoP and with 60 g kg^{-1} BT-CoP showed similar growth (body weight, growth rate: RGR_m) and feed utilization (feed conversion ratio, protein efficiency ratio, apparent net protein utilization). Diets with 0 and 60 g kg^{-1} of BT-CoP gave similar dry matter and protein digestibility coefficients but higher dietary BT-CoP levels than 60 g kg^{-1} produced lower digestibility values, except for carbohydrate. It is concluded that *O. aureus* fingerlings may assimilate only small amounts (60 g kg^{-1}) of BT-CoP in the diets without adverse effects on growth and feed utilization parameters. The CoP containing diets did not affect fish survival (100%). The depression in tilapia performance may be associated mainly to the high level of fibre presents into the CoP diets.

Keywords: *Oreochromis*, coffee pulp, fish nutrition, ANF's.

Introduction

A great variety and amount of agricultural residues are produced in the world and, because of their nutrient composition; many of them may have potential value as alternative ingredients for animal feeds. The main agricultural industries in the tropics generating large amount of wastes are coffee, bananas, sugarcane, cocoa and other fruit production. If their wastes could be incorporated into animal feeds, the environmental impact from their disposal may be reduced. To date, many agricultural wastes have been evaluated for their use in animal feeding (Fonseca 1976, Wee 1991, Tacon 1993). In many areas of Asia and Africa, wastes from agricultural origin are used in fish culture systems (Fagbenro & Arowosoge 1991, Tacon 1993, 1994).

Coffee pulp (CoP) has been used in animal feeds with variable results depending on the species being fed (Bressani & Braham 1980). For instance, CoP is recommended between 200-300 g kg^{-1} in beef rations (Cabezas *et al.* 1979), and a maximum of 150 g kg^{-1} in sheep rations (Vitto *et al.* 1999). For monogastric animals, the maximum recommended inclusion levels vary from 160 g kg^{-1} of the ration in swine (Jarquín 1979), and 100 g kg^{-1} in chicken diets (Romero *et al.* 1999) to 80 g kg^{-1} for growing/fattening diets for rabbits (Bautista *et al.* 1999a). For fish diets, however, the maximum inclusion levels are unclear. Tilapia (*Oreochromis aureus*) reared in earthen ponds and fed supplementary feeds containing 300 g kg^{-1} CoP showed similar growth to the control fish group (García & Bayne 1974, Bayne *et al.* 1976). Cachamay (*Colossoma* x *Piaractus*) reared in cages suspended in ponds and fed diets containing up to 200 g kg^{-1} CoP also showed similar growth to the control diet (Bautista *et al.* 1999b). Contrary to this, carp (*Cyprinus carpio* L.) and catfish (*Clarias isheriensis* Sydenham and *C. mossambicus* Peters) reared in aquaria and fed complete diets containing CoP (from 100 to 300 g kg^{-1}) showed a significant reduction in the growth and feed digestibility (Christensen 1981, Fagbenro & Arowosoge 1991).

Such negative effects of CoP may be caused by the presence of caffeine, polyphenols (total phenols), tannins (Bressani 1979, Vélez *et al.* 1985) and also by the high level of fibres found in CoP. Several treatments (chemical, physical and biological) have been applied to CoP to reduce the content of these components and to improve its nutritional value (Adams & Doughan 1981, Rolz *et al.* 1982, López & Pabón 1986, Rolz *et al.* 1988, Gómez *et al.* 1988).

Treating CoP with an inoculum of five bacteria species of the genus *Bacillus ssp.* improved its quality by increasing its protein content and reducing its antinutritional factors (total phenols and tannins) and cellulose contents after a treatment of 28 days (unpublished data). To test CoP's suitability for fish feeding, bacteria treated-CoP was evaluated in a feeding trial with *Oreochromis aureus* fingerlings.

Materials and Methods

Diet preparation and analysis

Samples of 148 g of fresh CoP were milled through a meat-grinder (1-mm die diameter) and mixed with 200 ml of buffer (0.1M $NaSO_4$ x $2H_2O$). To every 12 ml of ground CoP-buffer mixture (≈ 9.87 g CoP) was added 1 ml of yeast extract and 12 ml of bacteria mixture inoculum (156 x 10^8 bacteria/ml) and maintained in stagnant conditions to allow bacterial degradation. The inoculum included similar proportion of five bacteria strains named 201, 203, 206B, 207 and 208 (Amato, 1999) belonging to the genus *Bacillus ssp*. This procedure was upscaled several times to obtained the amount of CoP needed for the feeding trial. Treated CoP was filtered through three cotton towels and the solid fraction was immediately oven-dried at 60°C for 24 h and then milled to 1-mm diameter. Five diets containing 0, 60, 120, 180 and 240 g kg^{-1} BT-CoP were formulated to be approximately isoproteinous and isoenergetic (around 10.85 kJ g^{-1} DE, based on information for *Ictalurus punctatus*, NRC 1977). The BT-CoP replaced wheat meal in diet formulation. Dietary amino acid, minerals and essential fatty acid contents matched the requirements for tilapia (Tacon 1990, Ulloa 1995) (Table 1).

Table 1. Ingredients (g kg^{-1}, as feed basis) and chemical composition (g kg^{-1}, dry matter) of diets containing different bacteria treated-CoP levels used in the feeding trial with *O. aureus* fingerlings.

Diets (g kg^{-1}) / Ingredient [1]	D0 CoP	D60 CoP	D120 CoP	D180 CoP	D240 CoP
Fish Meal	400	400	400	400	400
Blood Meal	0	0	0	0	20
Wheat meal	360	295	230	165	105
Soybean meal	200	200	200	200	180
Soybeal oil	10	15	20	25	25
Coffee pulp	0	60	120	180	240
Vitamins [2]	20	20	20	20	20
Chromium oxide	10	10	10	10	10
			Nutrients		
Moisture (g kg^{-1})	80	78	62	51	64
NFE	382	353	324	296	264
Crude fat	63	72	80	89	97
Crude fibre	61	80	99	119	138
Crude ash	126	127	129	130	131
Crude protein	368	368	368	366	370
Carbohydrate [3]	318	291	263	235	202
Digestible energy (kJ g^{-1})	10.85	10.87	10.86	10.84	10.83

(1) Ingredients: fish meal (tuna meal mixed, crude protein (CP): 590 g kg^{-1}), blood meal (spray dried, CP: 815 g kg^{-1}), wheat meal (feed flour, CP: 115 g kg^{-1}), soybean meal (dehulled solvent extracted, CP: 460 g kg^{-1}).

(2) Composition as described by Ulloa & Verdegem (1994).

(3) Carbohydrate refers to starch and sugars expressed as glucose.

All dry ingredients were mixed during 15 minutes before adding the lipids. Subsequently, mixing continued for another 15 minutes. Next, water was added gradually until a desirable paste-like consistency was reached. This paste was forced through a 1-mm mesh screen using a meat grinder. Pellets were then dried during 16 hours at 50 °C. Finally, the feed could be easily manually crumbled to pellet size. The feeds were supplemented with 1 g kg^{-1} Cr_2O_3 for digestibility determinations.

Diet samples were analyzed for moisture, crude protein (N x 6.25), crude fat, crude ash and crude fibre by standard methods (AOAC 1990). The cellulose was measured gravimetrically according to Meites (1963). The carbohydrate content (sugar and starch) was determined according to Osborne and Voogt (1986). Polyphenols (total phenols) and tannins were measured according to Slinkard & Singleton (1977) and Field (1991). Caffeine was measured according to Morris (1973).

Experimental procedure

Fish were reared in a recirculating unit consisting of 16 aquaria (30 x 50 x 30 cm), a bio-filter and a sedimentation tank. Water quality was checked at the outflow of each system. Water temperature and dissolved oxygen were measured twice daily (8 and 17 h). The pH, nitrite and total ammonium levels were measured every week. By adjusting the water flow, nitrite and total ammonia levels were maintained below 0.15 and 2.5 ppm, respectively, and oxygen level above 3.5 ppm. The photoperiod was kept at 13 h day^{-1} (from 6 h to 19 h).

Oreochromis aureus fry were taken from an all male sex-reversed (17-α-methyltestosterone) population and acclimatised in the experimental units for one week. Fish were fed during this period with the control diet (0 g kg^{-1} CoP, Table 1) until they reached a weight between 1.4-1.9 g. At the beginning of the experiment each aquarium was stocked with 10 fish. The five diets were assigned randomly in three replicates in the system. Fish were fed "to apparent satiation" twice daily (10 and 15 h) for four weeks. When fish voided feces, these were collected from the aquarium bottom by using a pipette-like glass tube. Feces samples from every replicate were dried at 60 °C overnight and kept in a dessicator until further analysis.

Fish were taken from the initial population for body composition analysis. At the end of the feeding trial, all fish were weighed individually and, 4-6 fish per replicate were sampled and pooled for body composition analysis. All these analysis were done according to standard methods (AOAC 1990).

Data analysis

To assess growth and feed utilization, the following parameters were calculated: relative metabolic growth rate (RGR_m, g/$kg^{0.8}$/d), daily weight gain (DWG, g/fish/d), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (NPU_a, %) and survival rate (%).

Levels of chromic oxide were measured according to Mink *et al.* (1969) adjusted for small feces samples. Measurements were done in duplicate. Apparent digestibility coefficients (ADC) were calculated using formulas described by Mukhopadhyay & Ray (1997).

Statistical analysis of data was done by one-way ANOVA using the software STATGRAPHICS PLUS STATISTICAL GRAPHICS SYSTEM 7.0 (Manugistics, Inc. and Statistical Graphics Corporation, 1993). Treatment means were compared by the LSD test with 95% confidence intervals.

Results

The water quality parameters measured remained within common value ranges for tilapia culture (O_2 above 4.8 ppm, temperature: 27.3-30.3 °C, pH: 6.0-7.5, NO_2^-: 0.04-0.12 ppm, NH_4^+: 0.2-2.3 ppm).

The bacteria treated-CoP (BT-CoP) had significantly higher protein, fat and carbohydrate contents than untreated-CoP. The crude fibre was significantly reduced and the ash content remained similar after the bacteria treatment. The BT-CoP also showed lower total phenols and tannin contents than untreated-CoP ($P<0.05$) (Table 2)

Table 2. Chemical composition (g kg^{-1}, DM) of untreated-CoP and bacteria treated-CoP (with an inoculum of five species of *Bacillus ssp.*) as compared to wheat meal.

Foodstuff / Components	Untreated-CoP	Bacteria treated-CoP	Wheat meal
Crude protein (N x 6.25)	75	138	130
Crude fat	51	98	30
Crude ash	91	93	60
Crude fibre	548	372	50
NFE	235	299	730
Carbohydrate [1]	216	275	670
Cellulose	168	115	[2]
Caffeine	4.6	4.3	[2]
Total phenols	27	4.6	[2]
Tannins	12	1.8	[2]

[1] Carbohydrate refers to starch and sugars expressed as glucose.

[2] Not determined.

Fish fed D0 g kg^{-1} BT-CoP diet and the D60 g kg^{-1} BT-CoP diet showed similar growth (body weight: 20.9 g vs. 18.9 g, RGR_m: 38.1 g/$kg^{0.8}$/d vs. 36.8 g/$kg^{0.8}$/d) and feed utilization (FCR: 1.1 vs. 1.0, PER: 2.8 vs. 3.4, NPU_a: 39.2% vs. 34.3%) ($P>0.05$) (Table 3). At BT-CoP inclusion levels higher than 60 g kg^{-1}, however, growth and feed utilization were depressed ($P<0.05$). Final weight decreased from 18.9 g at D60 g kg^{-1} BT-CoP to 6.7 g at D240 g kg^{-1} BT-CoP and RGR_m decreased from 36.8 g/$kg^{0.8}$/d to 16.8 g/$kg^{0.8}$/d. PER decreased from 3.2 to 1.8 and NPU_a from 34.3% to 15.6%. The FCR values increased from 1.0 at D60 g kg^{-1} BT-CoP to 1.8 at D240 g kg^{-1} BT-CoP ($P<0.05$). Survival was similar for all treatments (100%). Feed intake was also similar for fish fed D0 and D60 g kg^{-1} BT-CoP (0.7 g feed/fish/d and 0.6 g/fish/d). At higher dietary BT-CoP levels, feed intake decreased (at D120 g kg^{-1} BT-CoP: 0.5, at D180 g kg^{-1} BT-CoP: 0.4 and at D240 g kg^{-1} BT-CoP: 0.3 g/fish/d) (Table 3).

Nutrient digestibility decreased as dietary BT-CoP inclusion increased from levels above 60 g kg^{-1} BT-CoP ($P<0.05$), except for carbohydrate. Diets D0 and D60 g kg^{-1} BT-CoP gave similar dry matter and protein digestibility coefficients. Dry matter digestibility decreased from 757 at 0 g kg^{-1} BT-CoP to 582 g kg^{-1} at 240 g kg^{-1} BT-CoP. Protein digestibility decreased from 930 at 0 g kg^{-1} BT-CoP to 834 g kg^{-1} at 240 g kg^{-1} BT-CoP ($P<0.05$). Carbohydrate digestibility increased in fish fed diets containing 60 g kg^{-1} and 120 g kg^{-1} BT-CoP (857 and 837 g kg^{-1}, respectively), followed by fish fed diets containing 0 and 180 g kg^{-1} BT-CoP (775 and 771 g kg^{-1}) ($P<0.05$) (Table 3). Fish fed diet with 240 g kg^{-1} BT-CoP showed the lowest dry matter, protein and carbohydrate digestibility coefficients.

Table 3. Final growth, feed utilization parameters and nutrient digestibility values of tilapia (*O. aureus*) fed diets containing different levels of bacteria treated-coffee pulp during a four weeks feeding period. (Means ± standard error).

Variable [1] \ Diets (g kg^{-1})	D 0 CoP	D 60 CoP	D 120 CoP	D 180 CoP	D 240 CoP
Initial weight (g)	1.7 ±0.07 ^{a}	1.6 ±0.04 ^{a}	1.7 ±0.04 ^{a}	1.7 ±0.06 ^{a}	1.6 ±0.02 ^{a}
Final weight (g)	20.9 ±0.74 ^{a}	18.9 ±0.25 ^{a}	13.2 ±0.82 ^{b}	10.1 ±0.44 ^{c}	6.7 ±0.65 ^{d}
DWG (g/fish/d)	0.6 ±0.02 ^{a}	0.6 ±0.01 ^{a}	0.4 ±0.03 ^{b}	0.3 ±0.01 ^{c}	0.2 ±0.02 ^{d}
RGRm (g/$kg^{0.8}$/d)	38.1 ±0.75 ^{a}	36.8 ±0.13 ^{a}	28.0 ±1.46 ^{b}	22.3 ±0.74 ^{c}	16.8 ±1.46 ^{d}
Feed intake (g/fish/d)	0.7 ±0.02 ^{a}	0.6 ±0.01 ^{ab}	0.5 ±0.03 ^{bc}	0.4 ±0.01 ^{c}	0.3 ±0.03 ^{d}
FCR	1.1 ±0.05 ^{a}	1.0 ±0.02 ^{a}	1.3 ±0.07 ^{ab}	1.6 ±0.1 ^{bc}	1.8 ±0.1 ^{c}
PER	2.8 ±0.15 ^{ab}	3.2 ±0.08 ^{a}	2.5 ±0.14 ^{bc}	2.1 ±0.13 ^{cd}	1.8 ±0.11 ^{d}
NPUa (%)	39.2 ±2.04 ^{a}	34.3 ±0.8 ^{a}	25.0 ±1.44 ^{b}	23.3 ±1.5 ^{b}	15.6 ±1.06 ^{c}
Digestibility (g kg^{-1})					
Dry Matter	757 ±2.04 ^{a}	751 ±2.6 ^{a}	717 ±3.04 ^{b}	646 ±0.9 ^{c}	582 ±1.6 ^{d}
Protein	932 ±1.04 ^{a}	914 ±1.24 ^{a}	911 ±3.9 ^{a}	861 ±3.44 ^{b}	838 ±1.8 ^{b}
Carbohydrate [2]	775 ±3.3 ^{a}	857 ±1.11 ^{b}	837 ±0.9 ^{b}	771 ±1.2 ^{a}	741 ±2.51 ^{c}

(1) Means in the same line with different letters differ significantly ($P<0.05$).

(2) Carbohydrate refers to starch and sugars expressed glucose.

At increasing dietary BT-CoP levels, body protein, fat and ash contents tended to decrease, except for D180 g kg^{-1} BT-CoP (P=0.07) (Table 4). Body ash and fat contents at the start of the experiment were higher and lower, respectively, than at the end of the experiment. Regression analysis between dietary BT-CoP level and body components only showed a negative trend for body fat (r = -0.595, P=0.069). Allometric relation only showed a positive correlation between body fat vs. fish weight (R^2= 0.587, log fat = 0.53 + 0.252 x log fish weight) (P=0.009).

Table 4. Proximate body composition (g kg^{-1}, wet basis) of fingerlings *O. aureus* fed diets containing different bacteria treated-CoP levels during a four weeks feeding trial. (Means ± standard error).

Components [1] / Diets	Moisture	Crude fat	Crude protein	Crude ash
Initial fish	796 ± 8.3 [a]	39 ± 1.0 [a]	120 ± 6.3 [a]	32 ±1.5 [a]
D0 (0 g kg^{-1} CoP)	747 ±38.8 [a]	82 ±21.3 [a]	136 ±30.6 [a]	30 ±7.0 [a]
D1 (60 g kg^{-1} CoP)	786 ±37.3 [a]	71 ±17.1 [a]	107 ±18.8 [a]	29 ±9.2 [a]
D2 (120 g kg^{-1} CoP)	793 ±10.8 [a]	63 ± 4.8 [a]	103 ± 6.3 [a]	26 ±0.6 [a]
D3 (180 g kg^{-1} CoP)	778 ±33.6 [a]	63 ± 1.1 [a]	114 ±15.4 [a]	32 ±3.8 [a]
D4 (240 g kg^{-1} CoP)	822 ± 9.8 [a]	50 ± 2.1 [a]	95 ± 5.6 [a]	26 ±3.6 [a]

[1] Means in the same line with different letters differ significantly (P<0.05).

Discussion

The BT-CoP produced a negative effect on growth and feed utilization in fingerlings of *Oreochromis aureus* when fed at levels higher than 60 g kg^{-1} of the diet. Consequently, *O. aureus* fed BT-CoP containing diets exhibited a reduced feed intake. In addition, fish fed diets D180 g kg^{-1} and D240 g kg^{-1} BT-CoP initially refused to eat these feeds. The depressed feed intake agrees with results found in ruminants, swine, poultry and turkey (Bressani *et al.* 1975, Jarquín *et al.* 1977, Wiseman 1983). This must be related to the ANF's (polyphenols or total phenols, tannins and caffeine) and the high level of the fibre fraction present in CoP that may produce a low feed palatability (Bressani & Braham 1980).

Tilapia fed diets with 130 to 390 g kg^{-1} of untreated-CoP showed similar negative effects in growth and feed utilization (Ulloa & van Weerd 1997). Similar results were found also with common carp (*Cyprinus carpio*), catfish (*Clarias mossambicus*) and *Clarias isheriensis* (Christensen 1981, Fagbenro & Arowosoge 1991). Contrary to these data, Bayne *et al.* (1976) and Bautista *et al.* (1999b) found no differences on growth in tilapia (fed a diet with 300 g kg^{-1} CoP) and in Cachamay (*Colossoma* x *Piaractus*) fed a diet with 200 g kg^{-1} CoP and the control diets, respectively. In the latter two studies, fish were raised in earthen ponds. It is very well possible that the natural food present in these ponds might have supported tilapia and Cachamay growth and minimized the negative effect of CoP on the fish.

Unfortunately, these authors did not measure the contribution of natural food to fish growth.

Reduced protein utilization can be caused by the presence of tannins, total phenols and caffeine in the diets with high BT-CoP dietary levels. Total phenols (polyphenols) and tannins interfere with the utilization of proteins by forming indigestible complexes and making them less available for growth (Bressani & Braham 1980, Vélez *et al.* 1985). The low protein digestibility of diets with BT-CoP levels higher than 60 g kg^{-1} had a negative effect on the nitrogen retention and voluntary intake. This is confirmed by the lower protein and dry matter digestibility coefficients measured for *O. aureus* fingerlings fed diets with high BT-CoP levels. Polyphenols could interference also with feed intake, digestibility and absorption of nutrients such as iron and proteins (de Rozo *et al.* 1985, Vélez *et al.* 1985). Caffeine interferes with the protein utilization and also has a diuretic effect, which increases the urinary output.

Another factor that could have contributed also to the reduced growth, feed and nutrient utilization in *O. aureus* fingerlings is the high fibre content in the diets with higher BT-CoP levels (120 to 240 g kg^{-1}). These high fibre levels are above the recommended maximum value for other tilapia species, e.g., *O. niloticus* x *O. aureus*, *O. niloticus* (L.) and *O. mossambicus* (Peters) (Shiau *et al.* 1989, Dioundick & Stom 1990, De Silva & Anderson 1995, Al-Ogaily 1996). High dietary fibre levels may reduce feed efficiency and digestibility in fish (Anderson *et al.*1984, De Silva & Anderson 1995). This could result in dilution of nutrients, thereby evoking poor fish growth, especially in diets containing 180 and 240 g kg^{-1} BT-CoP.

In addition, both fibre and ANF's may interact and exert synergistic negative effects on the fish. Ruminants can handle higher levels of ANF's than many other animals do, e.g., caffeine levels of 12 g kg^{-1} did not reduce their growth but 24 g kg^{-1} did. However, when different ANF's are fed combined (12 g kg^{-1} caffeine plus 75 g kg^{-1} tannins) they exerted an additive negative effect (Cabezas *et al.* 1976, Bressani & Braham 1980).

The high survival rate (100%) found in all CoP-treatments suggests that *Oreochromis aureus* fingerlings are relatively resistant to the CoP substances (caffeine, total phenols, tannins and high potassium levels), which are suspected to cause high mortality in other domestic animals (poultry, rats and turkeys) (Bressani *et al.* 1973, Gómez *et al.* 1985, Donkoh *et al.* 1988).

It is concluded that *O. aureus* fingerlings may assimilate small amounts of BT-CoP (60 g kg^{-1}) in the diets without adverse effects on growth and feed utilization parameters. However, BT-CoP levels higher than 120 g kg^{-1} produced a steady reduction of tilapia growth.

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Chapter 7

General discussion

Introduction

The ultimate objective of this thesis was to assess the feasibility of coffee pulp (CoP) as an ingredient in diets for tilapia (*Oreochromis aureus*). The CoP is the most relevant waste from the coffee processing industry and its disposal results in severe pollution problems. The Costarrican coffee industry is also concerned about these CoP disposal problems and the Costarrican Coffee Research Centre studies several techniques to reduce the impact of these wastes on the environment. Attempts to use CoP in animal feeds for ruminants and monogastrics (including fish) gave variable results depending on the species (Bayne *et al.* 1976, Cabezas *et al.* 1979, Bressani & Braham 1980, Christensen 1981, Fagbenro & Arowosoge 1991, Vitto *et al.* 1999, Romero *et al.* 1999, Bautista *et al.* 1999a,b). The content of antinutritional factors (ANF's, e.g., polyphenols or total phenols, tannins, caffeine) and the high level of the cellulolytic fraction are mentioned as main components constraining the use of CoP at high levels in animal feeds (Bressani 1979, Vélez *et al.* 1985, Gómez *et al.* 1985, de Rozo *et al.* 1985, López & Pabón 1986, Mehansho *et al.* 1987).

This thesis focused on different aspects of CoP related to fish feeding. As CoP contains different ANF's, their effect on the performance of *Oreochromis aureus* was studied first. Secondly, several techniques to upgrade the nutritional value of CoP were tested (chemical and biological treatments) and, finally treated CoP was used in diets for *O. aureus*. The selection of CoP as possible foodstuff ingredient was based on an inventory of the main agricultural wastes generated in Costa Rica during one production year (1993-1994). The selection of *O. aureus* was based on the relevance of this species to the aquaculture industry in Costa Rica (and beyond) and in view of its omnivorous feeding behaviour. The chemical and physical composition of CoP is influenced by coffee species and variety, location, management and processing machinery (Elías 1979, Abate 1988, Ramírez 1988, Ramírez & Clifford 1989, Clifford & Ramírez 1991a,b). Therefore, the variation in the chemical composition of CoP was analysed during the harvesting season (1999-2000) from samples taken from one specific processing plant.

Differences in the growth response of tilapia to CoP containing diets as found in the literature are discussed and a comparative study under laboratory and field culture conditions was done.

Since CoP contains ANF's, which may produce negative effects when CoP diets are fed to animals, in the present study the critical levels of some of these ANF's (fibre, tannins and caffeine) were determined for *O. aureus* reared under laboratory conditions. In view of the fact that ANF's from CoP affected also negatively the *O. aureus* performance, several chemical and biological treatments and their combinations were applied to upgrade the chemical composition of CoP. The chemical treatments used were the immersion in NaOH solutions, in NaOH-HCl mixtures and NaOH-ensilage, whereas, the biological treatments applied were ensilage, natural decomposition and bacterial inoculation. These treatments

were chosen in view of previous results with CoP and other fibrous residues (Klopfenstein 1978, Rolz *et al.* 1982, Owen *et al.* 1984, López & Pabón 1986, Rolz *et al.* 1988, Gómez *et al.* 1988, Klopfenstein 1994, Amato 1999).

Finally, the best upgrading treatment for CoP (bacterial inoculation) was tested in diets for *O. aureus* fingerlings and, the upscaling problems of this treatment were discussed.

Major residues in Costa Rica, coffee pulp selection and CoP composition variability

In Costa Rica, during the 1993-94 production season, the banana, coffee and sugarcane industries produced the major amounts of wastes with 388-575,000 MT, 436-452,000 MT and 298,000-313,000 MT, respectively. For the 1998-1999 production season, these crops still generated the major volumes of wastes (477,700-654,500 MT for banana, 406,000-412,800 MT for coffee and 383,100-388,200 MT for sugarcane). According to their chemical composition, CoP and rejected green banana (RGB) may have potential as ingredients in animal feeds. Their large volume available and the potential to reduce their pollution risk are also reasons to support their re-utilisation. However, as a result of the processing, the CoP physical structure is more accessible to degradation than RGB and, therefore it has a higher pollution risk (Vázquez 1997). For this reason we preferred to work with CoP.

The use of CoP in animal feeding, however, may be constrained by its variability in chemical composition and the drying method used. The analysis of the chemical composition of CoP during part of the harvesting season in Costa Rica showed that the CoP composition is highly variable, especially for moisture, fibre, carbohydrate, total phenols and tannins. Changes introduced in the depulping process, (e.g., improvement of devices; use or no use of water) and the start of the dry season could affect the chemical composition of CoP during the coffee-harvesting season. These changes not only could reduce the water content of the CoP but also the amounts of water-soluble components (sugars, some tannins and total phenols), of mucilage and of loose particles that are not washed out completely from the CoP. As a result of this all, the effluent water of the processing plants contain less amounts of dissolved and suspended solids thereby reducing environmental pollution (Vázquez 1997).

Other factors affecting the chemical composition of CoP may be differences in location (soil type, altitude), in coffee species (*Coffea arabica* or *C. robusta*) and strains, in type of depulping machinery and process, in culture practices and in extraction and analytical methods (Elías 1979, Abate 1988, Ramírez 1988, Ramírez & Clifford 1989, Clifford & Ramírez 1991a,b). The relative amount of impurities contained in the CoP (e.g., grass, leaves pieces, grain pieces, damaged grains) may also influence its chemical composition.

The sun-dried CoP showed higher crude and true protein, ash, neutral detergent fibre and acid detergent fibre contents but lower cellulose, total phenols, tannins and caffeine contents than oven-dried CoP. These differences between both drying methods appear to be

related to the duration of drying (6 days, during the rainy season). During the period that CoP is dried in the sun, some microbial growth could happen and aerobic biodegradation of the CoP could take place. This could increase the protein content and reduce the cellulose and ANF's contents, e.g., total phenols or polyphenols, tannins and caffeine as found by Rolz *et al.* (1982) and Tauk (1986). Further, the method to dehydrate CoP differs from place to place (Bressani *et al.* 1975) and this could also induce differences in the chemical composition of CoP. The selection of the drying method will depend on the availability of space (land) and economic reasons (power costs). Depending upon the season of the year, it will affect strongly the composition of the CoP.

For these reasons, in studies involving CoP in animal feeding, these factors should be standardised or should be taken into account when chemical composition data are taken from literature. Therefore, the chemical composition of CoP must be analysed and digestibility studies must be done before animal feeds containing CoP are formulated.

Coffee pulp in fish diets

In Costa Rica as well as in other coffee producing countries attempts have been made to utilise CoP as feed for cattle, swine, poultry and fish. The use of CoP in fish feeding has given controversial results, which appeared to be influenced by the fish species and the culture systems used. When CoP diets are used in recirculation and tank systems, the fish species studied so far (catfish and carp) showed reduced growth and feed utilisation (Christensen 1981, Fagbenro & Arowosoge 1991), but when CoP diets were applied in pond cultures, higher levels of CoP (up to 200 g kg^{-1}, depending of species) may be used in diets without negative effects to the fish (in the reported cases: tilapia and *Colossoma* x *Piaractus*) (Bayne *et al.* 1976, Bautista *et al.* 1999a). It may be possible that the natural food present in the ponds might have minimised the negative effect of CoP on the fish and consequently supported adequate growth. Unfortunately, these authors did not measure the contribution of natural food to fish growth.

The results obtained in this thesis indicated that *O. aureus* reared in aquaria and fed diets containing 0, 130, 260 and 390 g kg^{-1} of oven-dried CoP showed a progressive reduction in growth and feed utilisation (PER and nutrient digestibility). The high dietary fibre level together with the presence of ANF's may explain these results. However, when the same diets were used in pens located in an earthen pond, the effects of ANF's from CoP on *O. aureus* were much smaller and dietary CoP up to 130 g kg^{-1} supported similar growth and feed utilisation as the control diet. The natural productivity of the pond could explain these differences found between aquaria and pens. Anyway, the results suggest that the inclusion of CoP in tilapia diets might be limited up to 130 g kg^{-1} when fish are raised in earthen ponds at a stocking density of 1.5 fish/m^2 and when natural food is available.

Using a diet containing 130 g kg^{-1} CoP, the effect of a higher stocking density was tested and compared to the conditions of the previous trial. *O. aureus* reared at 3 fish/m^2 showed similar growth and feed utilisation as those kept at 1.5 fish/m^2 (unpublished data, Table 1). In this case, obviously the natural productivity of the pond was still enough to maintain the growth of tilapia. This has a great implication for small tilapia farmers because fish density can be increased without adverse effects to fish and, additionally, it may allow the farmers to use their resources in a more profitable way.

Table 1. Growth and feed utilisation of *O. aureus* fed diets containing 130 g kg^{-1} CoP and reared in pens at two densities (fish/ m^2) in an earthen pond with low water exchange. (Means ± standard error).

Density / Parameter [1]	1.5 fish/ m^2	3.0 fish/ m^2	1.5 fish/ m^2 (no feed)
Final body weight (g)	159.6 ± 1.9 [a]	163.6 ± 2.0 [a]	54.9 ± 2.9 [b]
Daily weight gain (g/d)	1.24 ± 0.07 [a]	1.29 ± 0.08 [a]	0.36 ± 0.07 [b]
RGRm ($g/kg^{0.8}/d$)	10.4 ± 0.70 [a]	10.7 ± 0.8 [a]	5.7 ± 0.70 [b]
Feed intake (g/fish/d)	2.2 ± 0.10 [a]	2.0 ± 0.11 [a]	--
FCR	1.8 ± 0.08 [a]	1.5 ± 0.09 [a]	--
PER	1.4 ± 0.06 [a]	1.6 ± 0.07 [a]	--

[1] Means in the same line with different letters differ significantly ($P<0.05$).

How some ANF's affects the growth and feed utilisation of *O. aureus* fingerlings?

The presence of ANF's and high levels of fibre in CoP impeded a successful inclusion of CoP in animal diets. These ANF's (caffeine, total phenols and tannins) and high levels of fibre reduce animal growth and feed utilisation (Cabezas *et al.* 1979, Vitto *et al.* 1999, Romero *et al.* 1999, Bautista *et al.* 1999a,b). However, often ANF's and fibre can be reduced through special treatments. When such techniques are applied to CoP, higher inclusion levels might be possible in animal diets. For this reason, we tried to establish the critical levels of fibre, tannins and caffeine for *O. aureus* fingerlings. When dietary fibre levels of 106.5 g kg^{-1} were fed to tilapia, feed utilisation and growth decreased. However, at the same time our results indicated that the applied dietary fibre levels did hardly affect the digestibility neither of dry matter (522-604 g kg^{-1}) nor of carbohydrate (627 to 696 g kg^{-1}). Therefore, this growth reduction does not seem to be caused by a lower nutrient digestibility. High fibre levels reduce the gut passage time in tilapia, possibly leading to a lower nutrient uptake by the gut absorptive epithelium (Shiau *et al.* 1988). Moreover, the larger amount of indigestible components (e.g., cellulose) in diets with high fibre content may also cause a lower availability of dietary energy (Buddington 1979, Shiau *et al.* 1988). It is believed that these two reasons together with the lower feed intake found were inducing the lower growth at high fibre levels.

Dietary tannin levels of 4.4 g kg^{-1} and above depressed growth, feed intake and feed utilisation of *O. aureus* fingerlings. This agrees with the reduction in digestibility of the dry matter and protein as found in diets with tannic acid. Similar results were found in domestic animals (De Rozo *et al.* 1985, Vélez *et al.* 1985, Gómez *et al.* 1985). Dietary tannins bind proteins and iron forming indigestible complexes, and, consequently, growth decreases. In addition, tannins are responsible for a bitter taste of the feed and this may explain the reduction in feed intake found at higher dietary tannin levels (Krogdahl 1987, Mehansho *et al.* 1987).

Oreochromis aureus fingerlings receiving dietary caffeine levels of 2.4 g kg^{-1} or higher showed a trend for lower nutrient digestibility and growth. In terrestrial animals, caffeine was reported to give also a bitter taste to the diets, to produce a diuretic effect and to interfere with proper utilisation of nutrients (Bressani 1979). However, in fish or other aquatic animals, diuretic and stimulatory effects of caffeine have not been proven yet.

Since the ANF's tested exert a negative effect on growth and feed utilisation of *O. aureus*, CoP has to be treated chemically or biologically to reduce or destroy its ANF's. If this is achieved, the inclusion of CoP in diets for tilapia cultured in earthen ponds systems may increase to levels higher than 130 g kg^{-1}.

The upgrading of coffee pulp

Chemical treatments

CoP treated with alkali and acid-alkali solutions contained higher ash, fat and fibre levels but lower contents of total phenols, tannins and caffeine than oven-dried (ODT)-CoP did. The true protein, fat and cellulose contents of NaOH treated CoP were similar to ODT-CoP. The HCl-NaOH treatment induced an increased leaching of soluble organic matter compared to NaOH treatment. This could explain the relative higher levels of the non-soluble fractions (ash and fat) and the reduction of true protein. In the mixed chemical treated CoP, however, also the washing/draining process applied to the CoP after the HCl treatment could have caused these reactions.

The combined alkali-ensilaged CoP contained higher true protein, fat and ash contents than ODT-CoP but had a similar content of cellulose. Total phenols, tannins and caffeine contents were lower in treated CoP than in ODT-CoP. The higher ash content found could result from the additive effect of the alkali and of the added molasses due to its high level of minerals (GEPLACEA 1989). Similarly, the small increase in true protein and fat levels (due to bacterial growth during anaerobic fermentation) could result from the alkali inhibition of the bacterial growth. The reduction of total phenols, tannins and caffeine in alkali-ensilaged CoP can be a result of their utilisation by micro-organisms and/or due to their leaching in the draining liquid (Murillo 1979). Micro-organisms from CoP have been found to be able to use caffeine and tannins (Rolz *et al.* 1982, Marin & Raimbault 1992).

The reduction of ANF's was much bigger in the chemically treated CoP than in the alkali-ensilaged CoP. The alkali treatment yielded the best overall results in upgrading the nutritive value of CoP.

Biological treatments

True protein and fat levels in CoP increased after ensilage. This may have been caused by the microbial growth in CoP. In earlier reports, bacterial growth has been detected in CoP ensilaged with molasses (Ramírez *et al.* 1999). This eventually resulted in higher protein contents of the ensilaged CoP (ECoP). The higher ash content found in ECoP could be a direct effect of the added molasses, which are reported to have high levels of minerals, especially potassium (GEPLACEA 1989). According to our results it seems that microbial growth in ECoP caused a partial degradation of cellulose.

In ECoP, total phenols levels decreased and statistically a trend for lower tannin levels was found. Also this effect could be the result of anaerobic microbial degradation. Also here, leaching in the draining liquid (Murillo 1979) could have contributed to this reduction of the ANF's. In addition, our data showed that the relative composition of nutrients in CoP changed upon treatment with molasses (ensilage). More into particular, the percentage of NFE and the measured carbohydrate (sugars and starch) increased relatively to the concentration of the other macronutrients. We believe that this effect might be the result of the added molasses. Further, duration of ensilage could also affect the degradation activity of micro-organisms on the CoP components. All these factors could explain the observed differences between our results and data from literature. Another factor that could have contributed to these differences was the different methods used to extract and determine polyphenols (total phenols) and tannins (Murillo 1979, Ramírez *et al.* 1999, Mueller-Harvey 2001). Anyway, our results indicated that the ensilage process certainly modified the composition of the original CoP.

The microbial treatment increased true protein and fat contents in the CoP. It is well known that mixtures of micro-organisms or organisms isolated from the pulp itself, increase amino acid and nitrogen contents of CoP (Tauk 1986). In the same way, it was also shown that fermentation by *Aspergillus niger*, *Penicillium roquefortii*, *P. crustosum* and *A. oryzae* increased the nitrogen content of CoP (Rolz *et al.* 1982, Peñazola *et al* 1985, Marin & Raimbault 1992). Similarly, the same aerobic microbes also utilise carbohydrates, and as a result, the relative content of the latter decreased in treated CoP. The relative higher ash content found in the decomposed CoP can be attributed to losses in organic matter caused by the process. Similar results were found by Rolz *et al.* (1982) and Peñazola *et al.* (1985).

Mixtures of microbes present in CoP are capable to degrade the total phenols in the CoP up to 90%, the tannins up to 85% and all caffeine (100%) after 14 days of treatment. This capability apparently depends on the type of microbes used because *A. niger* was unable to degrade these compounds in CoP whereas *P. roquefortii*, *P. crustosum* and *A. oryzae* were

able to degrade caffeine completely and polyphenols partially in CoP (Rolz *et al.* 1982, Marin & Raimbault 1992). In addition, Murillo (1979) found that during the ensilage process a significant part of the ANF's in CoP leach into the drainage liquid. Also in our case, this factor may have partly contributed to the reduction in ANF's in the final product. Further, our results showed that a period of CoP degradation ranging between 21 to 28 days of decomposition of CoP seems to be sufficient to improve its nutritive value, especially because ANF's were reduced strongly over these periods.

A mixture of cellulolytic bacteria (*Bacillus ssp.*) may be used to treat CoP. This process will automatically lead to bacterial growth, thereby increasing the protein content and decreasing the cellulose content in CoP. Similar results are reported by Tauk (1986). The reduction in the protein content after 21 days of treatment of CoP may be caused by a lower availability of nutrients and cellulose to bacteria that could not maintain their growth. The present study demonstrated that *Bacillus ssp.* was able to utilise the cellulose of the CoP. Amato (1999) found a similar results for other fibrous materials. Some fungi also are capable to utilise the fibre fraction of CoP (López & Pabón 1986). The sharp reduction in the total carbohydrate content (sugar, starch and non-starch polysaccharides) of treated CoP could indicate that *Bacillus spp.* utilise not only the cellulolytic fraction but also other organic carbon components. Similar results were found in CoP treated with *Aspergillus spp.*, with *Hansenula tropicalis* and/or with mixtures of micro-organisms obtained from the same pulp (Tauk 1986).

The *Bacillus spp.* also reduced the content of total phenols and tannins in the CoP, which indicates that these bacteria are capable to digest these components. Fungi grown on CoP were able to metabolise polyphenols and tannic acid (López & Pabón 1986). Bacteria and fungi apparently are very specific and utilise only some of the ANF's found in CoP. In this regard, we may conclude from our results that bacteria belonging to the *Bacillus* group are not able to use caffeine.

Use of treated CoP in diets for *O. aureus* fingerlings

Those treatments applied to CoP, which resulted in the highest reduction of ANF's, were tested subsequently in aquaria studies with *O. aureus* fingerlings (CoP treated with NaOH and CoP inoculated with *Bacillus spp.*).

NaOH treated CoP was included at levels of 0 g kg^{-1}, 130 g kg^{-1}, 260 g kg^{-1} and 390 g kg^{-1} in diets fed to *O. aureus* fingerlings (Table 2). Despite the great reduction of ANF's (total phenols from 20 to 1.4 g kg^{-1}, tannins from 7 to 0.5 g kg^{-1} and caffeine from 18 to 2.2 g kg^{-1}), the growth and feed utilisation of the fish was reduced at increasing CoP dietary levels. It was concluded that the high fibre content constrained the *O. aureus* fingerlings' performance. This was confirmed by feeding the same levels of untreated CoP to *O. aureus*

reared under the same conditions, resulting in a similar reduction of growth and feed utilisation as when fish were fed NaOH treated CoP.

CoP treated with *Bacillus spp.* was included at levels of 0, 60, 120, 180 and 240 g kg^{-1} in the diet. From the results, it was concluded that *O. aureus* fingerlings may accept only small amounts (60 g kg^{-1}) of bacterially treated CoP in the diets without adverse effects on growth and feed utilisation. Again, it appeared that the reduction in performance might be mainly associated with the high level of fibre present in CoP. These levels are above the recommended maximum for some tilapia species (Shiau *et al.* 1989, Dioundick & Stom 1990, De Silva & Anderson 1995, Al-Ogaily 1996). Also remaining ANF's (total phenols, tannins and caffeine) in the CoP after treatment may have contributed to this poor performance by causing a low feed palatability (Bressani & Braham 1980). *O. aureus* showed a reduced feed intake, especially when receiving diets containing 180 and 240 g kg^{-1} CoP. This agrees with findings in ruminants, swine, poultry and turkey (Bressani *et al.* 1975, Jarquín *et al.* 1977, Wiseman 1983).

Table 2. Growth and feed utilisation of *O. aureus* fingerlings fed diets containing graded levels of NaOH-treated CoP [1]. (Means ± standard error).

	Diets (g kg^{-1} CoP)			
Parameters [2]	D1 (0)	D2 (130)	D3 (260)	D4 (390)
Initial mean body weight (g)	7.2 ±0.11 ^{a}	7.0 ±0.09 ^{a}	7.0 ±0.02 ^{a}	7.2 ±0.13 ^{a}
Final mean body weight (g)	45.0 ±0.44 ^{a}	31.3 ±0.32 ^{b}	15.6 ±0.21 ^{c}	7.4 ±0.18 ^{d}
WG (g/fish/d)	0.7 ±0.01 ^{a}	0.4 ±0.06 ^{b}	0.2 ±0.02 ^{c}	0.01 ±0.002 ^{c}
RGRm (g/$kg^{0.8}$/day)	17.2±0.51 ^{a}	12.5 ±0.27 ^{b}	5.6 ±0.18 ^{c}	0.9 ±0.10 ^{d}
FCR	1.2 ±0.03 ^{a}	1.5 ±0.04 ^{a}	2.2 ±0.12 ^{b}	7.7 ±0.14 ^{c}
PER	2.1 ±0.21 ^{a}	1.9 ±0.14 ^{a}	1.3 ±0.19 ^{b}	0.4 ±0.09 ^{c}
NPU_a (%)	33.4 ±0.52 ^{a}	30.6 ±0.17 ^{a}	21.1 ±0.27 ^{b}	6.1 ±0.19 ^{c}
Feed intake (g/fish/period)	46.3 ±0.09 ^{a}	33.0 ±0.38 ^{b}	16.6 ±0.41 ^{c}	6.4 ±0.16 ^{d}
Digestibility (g kg^{-1})				
Dry matter	740 ±0.85 ^{a}	689 ±0.65 ^{ab}	645 ±0.15 ^{bc}	594 ±0.19 ^{c}
Protein	827 ±0.54 ^{ab}	834 ±0.21 ^{ab}	854 ±0.18 ^{b}	785 ±0.27 ^{c}
Carbohydrate [3]	874 ±0.32 ^{a}	817 ±0.73 ^{ab}	733 ±1.19 ^{bc}	550 ±0.10 ^{c}

[1] Adapted from Ulloa & van Weerd (1997).

[2] Means in the same line with different letters differ significantly ($P<0.05$).

[3] Carbohydrate refers to starch and sugars expressed as glucose.

Obviously, the reduced growth and protein, carbohydrate or dry matter utilisation as we found at higher CoP inclusion levels in the diets of *O. aureus*, may also be caused by a synergistic negative effect of the different ANF's and fibre, rather than by each of these

compounds alone (Bressani & Braham 1980, de Rozo *et al.* 1985, Vélez *et al.* 1985). Our data do not allow a clear distinction between the different factors.

The high survival rate (above 92%) found with untreated CoP diets suggests that *O. aureus* fingerlings can tolerate higher levels of CoP substances (caffeine, total phenols, tannins and high potassium levels) than other domestic animals (poultry, rats and turkeys) (Bressani *et al.* 1973, Gómez *et al.* 1985, Donkoh *et al.* 1988).

The upscaling of the bacteria inoculation treatment

In the present study CoP was treated with bacteria both at laboratory and at pilot scale. The scale of treatment seemed to affect the chemical composition of the treated CoP (Table 3). The pilot-scale treated CoP had a lower protein but a higher cellulose content than laboratory-scale treated CoP. Compared to untreated CoP, both pilot and laboratory scale contained less tannins and total phenols.

Table 3. Chemical composition (g kg^{-1}, DM) of CoP samples treated with an inoculum of five species of *Bacillus* bacteria at laboratory and at pilot-scale.

CoP type / Components	Untreated CoP	CoP at laboratory scale	CoP at pilot scale
Crude protein	75	392	138
Crude fibre	567	(1)	396
Cellulose	168	75	115
Caffeine	4.6	4.6	4.3
Total phenols	27	4.4	4.6
Tannins	12	3.2	1.8

(1) Not determined.

Some practical problems during the upscaling of the treatment could have contributed to these differences:

1) In the pilot scale process, the aerobic condition was not maintained in all the CoP samples. Due to the bigger amount of CoP to be treated, CoP was equally subdivided in several containers. However, the thick CoP layer impaired an aerobic condition in the entire mass of CoP. This may have disturbed the degrading action of the aerobic bacteria.
2) In the laboratory scale process, the CoP was liquefied to small particle size but in the pilot scale process this was not possible because there was not a similar equipment available. Therefore, in the pilot process CoP was forced through a 5-mm mesh die using a meat grinder. For this reason, the CoP used in the pilot-scale trial had a bigger particle size and this could have interfered the action of the bacteria (Ghose & Ghosh 1978, Boopathy 1988).

3) In the laboratory-scale process, all samples were centrifuged at the end of the treatment while this didn't happen in the pilot-scale process because there was no suitable equipment available. Instead, the treated CoP was filtered through cotton towels (≈ 0.5-mm mesh). This could have caused losses of suspended organic matter and bacteria biomass, thereby affecting the protein content of the final product.
4) In the pilot-scale process contamination with fungi in the liquid portion of the treated samples could have resulted in competition for nutrients between the fungi and the bacteria.

If these problems of upscaling can be solved, the composition of the final product (treated CoP) may be similar to that obtained in the laboratory-scale process. As a result, a product with a lower fibre and ANF's and higher protein content can be obtained and, higher CoP inclusion levels may be used in diets for fish and other animals.

Further research on the technology of the bacterial treatment may focus on the use of different rates of aeration to increase bacterial growth and to reduce the treatment time. Other interesting aspects for future research may be to design and develop containers for CoP treatment that only allow the diffusion of air through the cover. This may avoid contamination of the CoP-treated samples by fungi and consequent competition for nutrients. Another aspect of the bacterial treatment that may also merit further research is the standardisation of CoP particle size that may optimise the substrate surface for bacterial action.

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Summary

This research focused on the feasibility of using coffee pulp (CoP) in diets for tilapia (*Oreochromis aureus*). First, a literature survey analized the limitations of CoP as an animal foodstuff (several ANF's and high fibre contents), different ways to upgrade the CoP nutritional value and the maximal inclusion level of CoP in diets for ruminants, monogastric animals and fish (**Chapter 1**).

Next, an inventory of the agricultural activities in the country was realized for one production year (1993-94). The most important agricultural activities were identified and classified in two categories: crop and animal husbandry sectors. The residues (wastes and by-products) of both sectors were characterized by their seasonality, their amounts produced, the methods of treating wastes, their use (if any) and their potential pollution risk. In addition, the most relevant wastes were more precisely studied and, their chemical composition and the presence of potential ANF's indicated. The CoP was selected for further study because of its annual production and its potential pollution risk. Changes of the chemical composition of CoP during the harvesting season and using different drying methods were examined in more detail (**Chapter 2**). The limitations to use CoP as a foodstuff for fish were studied and the possible causes of the differences on growth responses to CoP diets found in tilapia reared either in extensive and intensive systems (pens in ponds and recirculation-aquaria system) are mentioned (**Chapter 3**).

As several ANF's were identified in CoP, the critical value of some of them was determined for *Oreochromis aureus* fingerlings. Based on the growth and feed efficiency (digestibility), the following dietary critical levels of fibre and tannins were determined: 4.4 g kg^{-1} of tannins and 106.5 g kg^{-1} of fibre. Dietary caffeine levels increasing from 2.4 to 4.6 g kg^{-1} tended to reduce fish growth, feed intake and also nutrient digestibility of *O. aureus* (**Chapter 4**).

Chemical treatments have been applied to straws and coffee hulls with positive results. The uses of some of these treatments were tested in fresh CoP. The NaOH, the combined HCl-NaOH and NaOH-ensilage treatments were applied to CoP to reduce the content of caffeine, total phenols (polyphenols), tannins and cellulose. Biological treatments were also used to diminish the ANF's in CoP but they also may increase the CoP protein and fat contents. The ensilage of CoP with molasses was also tested at higher concentration (100 g kg^{-1}) than normally done; however, it did not result in any additive effect. Microbial decomposition was done in a closed lab controlling parameters such as environmental moisture and temperature, photoperiod and temperature of CoP samples. Specific cellulolytic bacteria were used to degrade the fibrous components and the ANF's of CoP. The inoculation of CoP was done with a coktail of five species of *Bacillus* for 28 days under aerobic conditions. The best result in relation with the upgrading of the nutritional quality of CoP was found with the bacterial treatment (**Chapter 5**).

Subsequently, CoP treated with *Bacillus ssp.* was included in diets for tilapia. The final product was included at different concentrations (0, 60, 120, 180 and 240 g kg^{-1}) in the fish diets and fed to *O. aureus* fingerlings for 4 weeks. At the end of the experimental period, the fish response was evaluated in terms of growth, feed intake, protein utilization and nutrient digestibility (**Chapter 6**). The best results were found with diets containing 0 and 60 g kg^{-1} CoP. Problems related to the upscaling of the bacterial treatment are mentioned and discussed.

The overall results are integrated in one general discussion and several conclusions and recommendations are drawn to upgrade coffee residues for animal feeding purposes (**Chapter** 7). The potential of using CoP in diets for tilapia cultured in extensive or semi-intensive systems is discussed. Technological and engineering aspects impaired the upscaling of the bacterial treatment for CoP and produced a final product with a lower quality than the one obtained at laboratory scale.

Resumen

El objetivo de este trabajo fue analizar la utilización del desecho agrícola pulpa de café (CoP) en dietas para tilapia (*Oreochromis aureus*). En una revisión de literatura inicial se establecieron las limitaciones de la CoP para ser usada como ingrediente en alimentos para animales (factores antinutricionales: ANF's y un alto contenido de fibra), las diferentes maneras de mejorar su calidad nutricional y los niveles máximos de inclusión conocidos para rumiantes, animales monogástricos y peces (**Capítulo 1**).

Durante el período 1993-1994 se realizó un inventario de las actividades agrícolas más importantes en Costa Rica, tanto en producción animal como en producción vegetal. Los residuos (desechos y subproductos) de ambos sectores se caracterizaron de acuerdo a su estacionalidad, volumen producido, tratamientos aplicados, cantidad de residuos utilizados en otras actividades y por último, su riesgo potencial como contaminantes del ambiente. Los desechos producidos en mayor cantidad se analizaron con más detalle determinando su composición química y la presencia de potenciales ANF's. La selección de la CoP para realizar esta tesis se basó en su producción anual y en su más alto riesgo como contaminante del ambiente. Se examinó en detalle la variación en la composición química de la CoP durante la época de cosecha y en dependencia del método de secado (al sol y en un horno con aire forzado) **(Capítulo 2)**. Se analizarón las limitaciones del uso de la CoP como ingrediente en alimentos para peces, así como, las causas de las diferencias en crecimiento encontradas en tilapias alimentadas con dietas conteniendo CoP y cultivadas en sistemas de cultivo extensivos e intensivos experimentales, tales como sistemas recirculados y encierros en estanques de tierra (**Capítulo 3**).

Se determinaron posteriormente los niveles máximos de fibra, taninos y cafeína que se pueden permitir en alimentos para alevines de *Oreochromis aureus*. Con base en el crecimiento y utilización del alimento (digestibilidad) se determinaron como niveles críticos: 4.4 g kg^{-1} de taninos y de 106.5 g kg^{-1} de fibra. Aunque no se pudo establecer con claridad un nivel máximo de inclusión de cafeína, se encontró que niveles de cafeína similares o mayores a 2.4 g kg^{-1} en el alimento produjeron una reducción en la digestibilidad de los nutrientes, así como una tendencia a un menor crecimiento e ingesta de alimento en *O. aureus* (**Capítulo 4**).

Con el fin de mejorar su calidad nutricional, se han aplicado algunos tratamientos químicos a forrajes, al pergamino y pulpa de café con resultados positivos. En este estudio, se aplicaron a la pulpa de café fresca tratamientos con NaOH, mezclas de NaOH y HCl y la combinación de NaOH y ensilaje para reducir su contenido de cafeína, taninos, fenoles totales o polifenoles y de celulosa. Tratamientos biológicos como descomposición natural, ensilaje e inoculación con bacterias también se aplicaron a la CoP con el fin de reducir su contenido de ANF's y de celulosa, con la ventaja de que estos tratamientos por lo general incrementan el contenido de proteína y grasa en el producto final. El ensilaje de la CoP con melaza se realizó a una concentración de melaza más alta que la habitual, pero este cambio no

resultó en algún mejoramiento adicional de la CoP. El tratamiento de descomposición microbiana de la CoP se realizó en un laboratorio cerrado controlando el fotoperíodo, la humedad relativa y temperatura ambiental y la temperatura de la CoP. Para el tratamiento con bacterias, se usó una mezcla de cinco bacterias celulolíticas (*Bacillus ssp.*) por un período de 28 días, manteniendo las condiciones aeróbicas para descomponer la fibra y los ANF's de la CoP. La CoP tratada con éste cóctel de bacterias presento los mejores resultados en relación al mejoramiento nutricional de la CoP **(Capítulo 5)**.

La CoP tratada con la mezcla de bacterias se incluyó en alimento para alevines de *O. aureus* a niveles de 0, 60, 180 y 240 g kg^{-1} y alimentando los peces por cuatro semanas. El rendimiento de las dietas se evaluó con base en el crecimiento, la ingesta de alimento, la utilización de la proteína y la digestibilidad de los nutrientes (**Capítulo 6**). Con niveles de 60 g kg^{-1} de CoP en la dieta los peces mostraron un rendimiento similar a los de la dieta control (0 g kg^{-1} CoP), pero por encima de este nivel el rendimiento de las tilapias fue menor. Se mencionan y discuten además los problemas relacionados con la aplicación del tratamiento bacterial a mayor escala.

Los resultados se integran en una discusión general, en la que se presentan varias conclusiones y recomendaciones para mejorar la calidad de los residuos de café con el fin de utilizarlos en la alimentación animal. Se analiza el potencial de la CoP como un posible ingrediente en alimentos para tilapia cultivada en sistemas extensivos e intensivos. Finalmente, se identificaron varios problemas prácticos (técnicos y de diseño) que dificultaron la aplicación del tratamiento bacterial a mayor escala, con la consecuencia de que la calidad final de la CoP tratada fuera peor a la calidad obtenida con el tratamiento bacterial a escala de laboratorio (**Capítulo 7**).

Samenvatting

De doelstellingen van dit onderzoek waren (a) inzicht en begrip te ontwikkelen m.b.t. de afvalsituatie in de Costa Ricaanse landbouw en (b) de haalbaarheid van het gebruik van koffiepulp (CoP) als ingredient in tilapiavoeders te onderzoeken. De beperkingen van CoP als ingredient in diervoeders worden benoemt (verschillende anti-nutritionele factoren (ANF's) en hoog vezelgehalte) en methoden om de voedingswaarde van CoP te verhogen worden besproken in de inleiding (**Hoofstuk 1**). Het maximale gehalte waarop CoP kan wordt opgenomen in diëten van herkauwers, mono-gastrische dieren of vissen wordt besproken.

Om een breed inzicht te krijgen in de algemene situatie m.b.t. landbouw-afvalproducten in Costa Rica werd een overzicht gemaakt van alle landbouwactiviteiten in één productiejaar ('93-'94). De belangrijkste landbouwactiviteiten werden geïdentificeerd en onderverdeeld in 2 categorieën: plantaardige en dierlijke activiteiten. De afval- en bijproducten van beide categorieën werden geclassificeerd volgens seizoensgebondenheid, de hoeveelheid geproduceerde afval- en bijproducten, de toegepaste methode van afvalbehandeling, hun eventuele toepassing en hun potentiële bijdrage aan vervuiling. Daarnaast werden de belangrijkste afvalproducten (op basis van hoeveelheid en vervuilingsrisico) in detail bestudeerd. Hun chemische samenstelling en de aanwezigheid van ANF's werden ook bepaald (**Hoofstuk 2**). In dit hoofstuk wordt CoP geïdentificeerd als een potentiëel belangrijk afvalproduct. Daarnaast geeft het hoofstuk een overzicht van toepassingen van CoP binnen de visteelt. De chemische samenstelling van CoP en de variatie daarvan in de loop van het oogstseizoen werden in detail bestudeerd, en de beperkingen van CoP als voedingsingrediënt voor vissen werden aangetoond (**Hoofstuk 3**). De verschillen tussen extensieve en intensieve systemen worden ook diepgaand besproken in dit hoofstuk.

Voor enkele van de geïdentificeerde ANF's in CoP werd de kritische grenswaarde bepaald bij *Oreochromis aureus* pootvis. De maximale kritische waarden van vezels, tanines en cafeïne voor jonge *O. aureus* werden bepaald in verschillende aquariumproeven (**Hoofstuk 4**). Hoge gehaltes van tanines (4.4 g kg^{-1}) en vezels (106.5 g kg^{-1}) veroorzaakten verminderde groei en verteerbaarheid. Een verhoging van het cafeïnegehalte van 2.4 g kg^{-1} tot 4.6 g kg^{-1} verminderde de groei, voedselopname, en verteerbaarheid bij *O. aureus*.

Chemische behandelingen van stro en koffiepeulen hadden positieve resultaten. Sommige van deze behandelingen werden uitgetest op verse CoP, echter gebruik makend van hogere concentraties en langere blootstellingstijd dan normaal. Om de hoeveelheid caffeine, tanines en polyphenolen in CoP te verlagen werd CoP behandeld met NaOH, HCL-NaOH of NaOH gevolgd door inkuiling. Biologische behandelingen werden ook gebruikt om de ANF's in CoP te verlagen en eventueel het eiwit- en vetgehalte te verhogen. Het inkuilen van CoP met melasse werd getest bij een hogere (100 g kg^{-1}) dan normale concentratie maar zonder resultaat. De microbiële afbraak gebeurde met controle van o.a. de vochtigheidsgraad van de omgeving, de fotoperiode en het vochtgehalte in de monsters. Specifieke cellulolytische

bacteriën werden gebruikt om de vezels en ANF's in CoP af te breken. CoP werd geïnoculeerd met een coctail van 5 soorten *Bacillus* en daarna 28 dagen onder anaerobe omstandigheden gehouden. Van alle behandelingsmethoden leverde de bacterie-behandeling het beste resultaat op.

Koffiepulp behandelt met de *Bacillus* coctail werd bij verschillende concentraties opgenomen in tilapiavoeder (0, 60, 120, 180 en 240 g kg^{-1}) en gedurende 4 weken gevoerd aan *O. aureus* pootvissen. Na het experiment werden groei, voedselopnamen, eiwitbenutting en verteerbaarheden bepaald (**Hoofstuk 6**). De beste resultaten werden gevonden bij diëten met 0 en 60 g kg^{-1} CoP. Problemen ten gevolge van het opschalen van de bacterie-behandeling worden besproken.

In de algemene discussie worden conclusies en aanbevelingen gegeven m.b.t. het opwaarderen van plantaardige afval via diervoeding (**Hoofstuk 7**). De mogelijkheid CoP te gebruiken in tilapiavoeder in extensieve en semi-intensieve teeltsystemen wordt bediscussieerd. De algemene discussie wordt uitgebreid met extra resultaten uit eigen onderzoek betreffende het gebruik van chemisch behandeld CoP in tilapiavoeders. Een vroeger experiment waarbij een vijver werd opgedeeld met in kwadranten met behulp van netten wordt ook besproken. Deze kwadranten werden bezet met tilapias bij verschillende dichtheden. De waterdoorstroming in de vijver was laag. Tot slot worden de problemen bij opschaling van de bacteriële CoP behandeling besproken. Hierbij gaat het vooral om technologische en mechanische problemen. Deze problemen verhinderden het opwaarderen van de bacteriële CoP behandeling en leidden tot een minder resultaat dan wat te verwachten was op basis van de voorafgaande laboratoriumproeven.

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Curriculum vitae

Juan B. Ulloa Rojas was born on the 13th of July 1962, in San Ramon, Alajuela, Costa Rica, as son of Carlos Ulloa Cordero and Alice Rojas González. He entered the School of Biological Sciences of the National University (UNA) in Heredia, in which he obtained the degree of B.Sc. Marine Biologist in 1984. Almost two years later, he obtained the degree of Licenciate in Marine Biology with emphasis in Aquaculture, at the same university. He started working at School of Biological Sciences of the UNA, first as research assistant, and then also as a teacher until 1990; when he enrolled the Wageningen Agricultural University, The Netherlands, where in 1992 he was granted the diploma of Master of Science in Aquaculture. After two years of work as researcher and teacher at the School of Biological Sciences of the UNA, he started his Ph.D. study in September 1994 at the Wageningen Agricultural University, The Netherlands. During the duration of his doctoral study, he also was teaching partly at the School of Biological Sciences of the UNA, place where he realized all the doctoral research. After finishing his Ph.D. study, he will stay in Costa Rica and will continue teaching and researching at his former place of work.

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