Evaluation of Spirulina on the performance and pigmentation of Rainbow Trout

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Declaration:

I, the undersigned, hereby declare that the work contained in this research assignment is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: ______________________________

Date: ______________________________

Summary

An experiment to determine the effect of *Spirulina* on flesh pigmentation and growth of rainbow trout was conducted at the Jonkershoek trout research station of the Division of Aquaculture, University of Stellenbosch. Treatments consisted of ten experimental diets containing increasing levels of *Spirulina* (0%, 0.05%, 0.1%, 0.15%, 0.3%, 0.6%, 1.25%, 2.5%, 5% and 10%). The data was analyzed through means of a one-way ANOVA and Tukey's pair wise comparison test for significant differences between treatments for the various parameters of flesh pigmentation and growth.

The results indicates that the inclusion of *Spirulina* in the diets of rainbow trout will cause a undesirable yellowing of fillets, particularly at levels of inclusion at 5 percent and above, which may limit its use in diets for rainbow trout. There was an inconsistent increase in b* (yellow-blue) and Chroma values with increasing levels of *Spirulina* inclusion, which may indicate the deposition of carotenoids (yellow-orange colour range) instead of the desired deposition of xanthophylls (pink-red colour range) in the flesh of the rainbow trout. This increase in b* (yellow-blue) values was significantly higher ($P<0.05$) at inclusion levels above 5 percent.

Spirulina had no significant effect on weight gain of rainbow trout at any level of inclusion. No significant differences ($P>0.05$) were observed in any of the other production performance parameters (growth rate, feed intake, feed conversion ratio, viscerosomatic index, hepatosomatic index, and liver lipid content) that were evaluated. Low mortality rates were observed with no significant differences between treatments. A trend of decreased feed intake with increased levels of *Spirulina* inclusion became significant at the 2.5 percent level of inclusion, caused by deterioration in the palatability of the feed. A significantly higher dress-out percentage was observed at levels of *Spirulina* inclusion above 5 percent. A trend of decreased liver lipid content with increased *Spirulina* inclusion was observed, though not significant at any level of *Spirulina* inclusion. This observation justifies further investigation because of its potential to improved carbohydrate metabolism in carnivorous fish

Results confirm that up to 10% *Spirulina* can effectively replaced soybean meal in the control diets for rainbow trout while simultaneously improving carcass dress-out at high inclusion levels.

Keywords: *Spirulina platensis,* rainbow trout (*Oncorhynchus mykiss)*, diets, flesh pigmentation, growth performance

Opsomming

‘n Studie is onderneem om die invloed van *Spirulina platensis* teen verskeie konsentrasies in die rantsoen van reënboog forel te evalueer. Die eksperiment is opgestel by die Akwakultuur Divisie, van die Universiteit van Stellenbosch, se navorsingstelsel in Jonkershoek, buite Stellenbosch. Reënboog forel (*Oncorhynchus mykiss)* vingerlinge van gemiddeld 20 gram is gebruik en ingedeel in 20 ronde tenks volgens kommersieële digthede (250 per tenk).
Die rantsoene is gemeng met verskeie vlakke van *Spirulina* insluiting (0%, 0.05%, 0.1%, 0.15%, 0.3%, 0.6%, 1.25%, 2.5%, 5% en 10%). Twee herhalings van elke rantsoen is gevolg vir ‘n periode van 3 maande. Die vis is *ad lib* (volgens behoefte) gevoer, drie maal per dag.

Daar was ‘n neiging tot ‘n verhoging in b* (geel-blou) en Chroma waardes met verhoogde insluitingsvlakke van *Spirulina.* Hierdie is wel ‘n aanduiding van die deponeering van karoteen, maar val in die geel-oranje kleur reeks en is nie die verlangde pienk-rooi (xantofil) kleur in die spier weefsel van die vis nie. Die verhoging in b* (geel-blou) waardes was statisties betekenisvol ($P<0.05$) bo die 5% insluitings vlak.

In vergelyking met die kontrole (0%) rantsoen, was daar geen betekenisvolle massa toename na afloop van die proef tussen die verskeie behandelings nie. Behalwe vir ‘n verskil in voer inname, was daar geen statisties betekenisvolle verskil ($P>0.05$) waarneembaar tussen die verskillende behandelings ten opsigte van die verskeie produksie prestasie parameters nie. Daar was wel ‘n afnemende neiging in voer inname met verhoogde vlakke van *Spirulina* wat betekenisvol geraak het bo 2.5% insluitingsvlak. Smaaklikheid kan die moontlike rede hiervoor wees. Baie lae mortaliteite is ervaar by al die insluitings vlakke. Daar was ook ‘n neiging in hoër uitslag persentasie by verhoogde insluitingsvlakke van *Spirulina* wat betekenisvol geraak het bo 5 % insluiting. ‘n Afnemende vlak van lewer lipied inhoud is waargeneem maar was nie betekenisvol by enige van die insluitingsvlakke nie.

Resultate van die proef dui daarop dat 10% *Spirulina* insluiting effektief kan dien as ‘n gedeeltelike plaasvervanger vir soyaboon meel in forel rantsoene. Die hoër uitslag persentasies hou belofte in. Die onverlangde vergeeling van die filette mag die gebruik van *Spirulina* beperk by reënboog forel rantsoene veral bo 5 % insluitings vlakke. Die verlaging in lewer lipied vlakke by ‘n verhoging van *Spirulina* insluiting regverdig vêrdere navorsing oor die potensiaal van *Spirulina* om die metabolisme van koolhidrate te verbeter by karnivoor visspesies.

Sleutelterme: *Spirulina platensis*, Reënboog forel (*Oncorhynchus mykiss*), Pigmentasie.

Dedicated to Charmaine

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List of Acronyms and Abbreviations

a*	Red-green chromaticity
ad lib	*ad libitum* = hand-fed to satiation
b*	Yellow-blue chromaticity
et al.	*et alii* (and others)
FCR	Feed Conversion Ratio
g	grams
kg	kilograms
ppm	parts per million
ppt	parts per thousand
L*	Lightness
mg	milligram
pp.	Pages

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*** Chapter 1 and 2 are presented in the format of scientific papers.**

INTRODUCTION TO SPIRULINA AND ITS USE AS A DIETRY COMPONENT IN FISH FEEDS

Introduction

The growth and intensification of aquaculture production over the past two decades has led to an increased importans of aqua feeds in terms of production performance and costs. The intensive culture of farmed species requires the use of complete balanced diets that includes all the macro and micro-ingredients essential to growth, health, quality and appearance. The limited supply and escalating cost of fish-meal in particular has initiated research on alternative protein sources (Shetty & Nandeesha, 1988). Spirulina contains a high percentage of protein of between 60 to 70% and can be used as a substitute for protein sources like fish-meal and soybean meal in fish feeds (Grinstead *et al*., 1999). Reports by Nandeesha *et al*. (1998), Mu *et al*. (2000) and Nandeesha *et al*. (2001) indicated that spirulina could be used as an effective partially or completely replacement for fishmeal in formulated aqua feeds.

Spirulina

Spirulina platensis (spirulina) is a blue green macro algae, rich in proteins, vitamins, essential amino acids, minerals and essential fatty acids such as GAMA Linolenicacid. Table 1 provides a summary of the composition of *Spirulina platensis*. Chemical analysis has shown that spirulina contains 60 to 70 % protein, higher than for any other natural food. Spirulina does not have a thick cell wall, can be digested easily and its nutrient rich contents can be readily absorbed (Clement *et al*., 1967; Bourges *et al.,* 1971; Anusuya Devi *et al.,* 1981). Spirulina has however not been widely evaluated in animal feeding studies. *Spirulina platensis* are produced commercially and grown organically in specialized ponds to provide a product that is free of contaminants (Henrikson, 1997).

Table 1: Typical laboratory analysis of ***Spirulina platensis* -** BioBiotic (Dekker, 2002).

General Analysis		**Pigments & Vitamins**	
Protein	58%	β-carotene	1 255mg/kg
Energy	19.18 MJ/kg	β-cryptoxanthin72mg/kg	
Carbohydrates	15%	Zeaxanthin	523mg/kg
Lipids	0.5%	Xanthophylls	1 183mg/kg
Ash	12%	Chlorophyll–a	10 150mg/kg
Moisture	7%	Phycocyanin	128 200mg/kg
Bulk density	0.6 kg/L	Vitamin B_{12}	0.4mg/kg
Particle size	<200 micron	Vitamin E	109mg/k
Minerals (mg/kg)		**Amino acids (g/100g)**	
Magnesium	8 040	Arginine	3.78
Calcium	5 370	Serine	2.77
Phosphorus	10 100	Aspartic acid	5.13
Potassium	19 500	Glutamic acid	6.94
Sodium	12 600	Glycine	3.03
Chloride	1 080	Threonine	2.58
Iron	986	Alanine	3.63
Cobalt	15	Tyrosine	3.14
Chromium	5.1	Proline	2.47
Arsenic	1.7	Methionine	1.50
Lead	1.7	Valine	3.06
Mercury	<0.05	Phenylalanine	2.71
Cadmium	0.2	Isoleucine	2.90
Nickel	23	Leucine	4.52
Manganese	71	Histidine	1.52
Molybdenum	5.9	Lysine	2.83
Copper		Cysteine	1.74
Aluminium	19	Tryptophan	1.04
Zinc	59		
Selenium	1.4		
Microbial Analysis			
Entero bacteria	negative	Shigella	negative
Coliform	negative	Yeast & moulds	<10 CFU/g
E-coli	negative	Staphylococcus	negative
Salmonella	negative		

The effect of *Spirulina platensis* on flesh pigmentation

The characteristic pink-red colour of salmon and trout flesh, caused by the intracellular deposition of carotenoids in the muscle, is an important parameter of product quality and consumer preference. Consumer preference is based on visual appearance and the known benefits of carotenoids to human health (Schiedt, 1998). Diet composition is one of the most important factors that affect flesh pigmentation (Torrissen, 1985). Carotenoids are a group of naturally occurring organic pigments that are responsible for the red, orange and yellow colours in the skin, flesh, shell and exoskeleton of aquatic animals. Astaxanthin and canthaxanthin are the main caroteniods found in crustaceans, whilst astaxanthin is the predominant carotenoid in trout and salmon.

Aquaculture species including fish and shrimp are not able to produce the caroteniods *de novo*, though certain aquaculture species are able to metabolize and deposit caroteniods such as astaxanthin in their tissue. Astaxanthin is the main carotenoid that is used as a micro-ingredient in aquaculture feeds to ensure colour quality and marketability of end-product. The synthetic product CAROPHYLL® Pink supplied by ROCHE is the main industrial source of astaxanthin to feed manufacturers. Alternative sources of astaxanthin for inclution in fish feeds are krill, shrimp, crawfish, abasidiomycetes yeast (*Phaffia rhodozyma)* and algae (*Haematococcus pluvialis),* Nickell and Springate, 1999.

Atlantic salmon and rainbow trout utilize astaxanthin more efficiently than canthaxanthin in terms of absorption and deposition (Torrissen *et al.*, 1989; Storebakken and No, 1992) though no differentiation has been reported in Arctic charr (Shahidi *et al.*, 1993). The type of pigments and levels of inclusion in feeds should be carefully assessed to ensure optimal colouration in the end product. The efficiency of absorption and deposition of astaxanthin vary with concentration, duration, fish size, age, season and physiological condition e.g. sexual maturation (Torrissen *et al.*, 1989; Storebakken and No, 1992). Increased pigment deposition with age has also been reported in rainbow trout (Sivtseva Dubrovin, 1981) though the relative interaction between age and body size is not clearly determined as yet. In small Atlantic salmon, which lacks the ability to deposit carotenoids in the flesh, large amounts of carotenoids may yet be detected in the skin (Storebakken *et al.*, 1987). This indicates that factors other than absorption of carotenoids contribute to low levels of flesh pigmentation as observed in juvenile salmon. According to Hemni *et al.* (1990), astaxanthin binds non-specifically to hydrophobic binding sites in the white muscle of salmonids. However, the number and size of white muscle fibers varies in different parts of the epaxial muscle of rainbow trout (Kiesling *et al.*, 1991), which could lead to variability in the number of astaxanthin binding sites around the fillet. This could explain the observed differences in astaxanthin concentrations between different areas of the fillet, irrespective of dietary treatment. Astaxanthin seems to give better results in terms of flesh pigmentation when the feed is alternated with normal diet (Wathne, 1998).

Various alternative sources of astaxanthin such as krill, shrimp, crawfish, abasidiomycetes yeast (*Phaffia rhodozyma)* and algae (*Haematococcos pluvialis)*, Nickell and Springate, 1999, can be considered for inclusion in fish feeds. The aim of this investigation was to investigate the effect of different levels of inclusion of *Spirulina* in diet of rainbow trout on the flesh-pigmentation.

Effect of *Spirulina platensis* on production performance

Spirulina phytocomponents is known to have general health benefits, such as improved carbohydrate metabolism, as indicated by Torres-Duran *et al*. (1998) through studies on reduction of lipid content in livers of laboratory mice. Torres-Duran *et al*. (1998) investigated the preventative effect of an oil extract of *Spirulina maxima* and its defatted fraction on fatty liver development, induced in rats by a single intraperitoneal dose of carbon tetrachloride (CCl_4). The increase of the serum lipoprotein VLDL and the decrease of the serum lipoprotein LDL percentages induced by the carbon tetrachloride administration were prevented with the inclusion of whole *Spirulina maxima* in the purified diet. Since the hepatotoxic effect of carbon tetrachloride is related to free radical generation (Glende & Recknagel, 1991; Gonzalez Padron *et al*., 1993), it is reasonable to assume that the potential hepatoprotective role of *Spirulina maxima* may be associated with its antioxidant constituents, such as selenium, chlorophyll, carotene, γ-linolenic acid, and vitamins E and C (Kay, 1991). It has also been reported that *Spirulina* sp. reduced or prevents the development of fatty liver, induced by a fructose-rich purified diet (González de Rivera *et al*., 1993; Nassir *et al*., 1993; Rodriguez-Hernandez *et al*., 2001), or by carbon tetrachloride treatment (Torres-Duran *et al*., 1998; Torres-Duran *et al*., 1999).Such a reducing effect on liver lipid content may be of value for inclusion of higher levels of carbohydrates in diets for carnivorous fish. The occurrence of fatty liver disease in these fish is often attributed to carbohydrate intolerance.

Spirulina contains various biologically active agents that have been investigated in a variety of practical applications in biotechnology and medical sciences. Spirulina is a rich source of the pigment C-phycocyanin C-PC (Richmond, 1986) that has antioxidant (Romay *et al*., 1998; Bhat and Madyastha, 2000; Pinero Estrada *et al*., 2001) and cancer inhibiting properties (Dasgupta *et al*., 2001) with therapeutic use in oxidative stress-induced diseases (Romay *et al*., 1998; Torres-Duran *et al*., 1999; Rimbau *et al*., 2001; Bhat and Madyastha, 2001; Premkumar *et al*., 2001). It has been widely studied for its possible immune-stimulating effect (Pascaud, 1993; Al-Batshan *et al*., 2001; Hirahashi *et al*., 2002), antibacterial, antiparasitic and antiviral properties (Ayehunie *et al*., 1998; Hayashi *et al*., 1996ab; Hernández-Carona *et al*., 2002). Qureshi (1995) observed that young poultry fed 1000 to 10 000 ppm Spirulina platensis had heavier spleen and thymus weights than poultry fed a control diet. Chickens fed *Spirulina platensis* also exhibited a higher clearance rate of *Esherichia coli* (intravenous inoculation) from their circulation than did chicks fed a basal diet (Qureshi, 1995). Vadiraja *et al*. (1998) observed spirulina to provide protection to the liver enzymes during induced hepatotoxicity in rats. The supplementation of diet with Spirulina has also been investigated its effect on several medical conditions such as allergies, ulcers, anaemia, heavy-metal poisoning, radiation poisoning (Zhang *et al*., 2001).

The aim of the current study was also to investigate the effect of the inclusion of *Spirulina* in diets of rainbow trout on production performance parameters such as growth rate, feed intake, feed conversion efficiency, viscerosomatic index (VSI), hepatosomatic index (HSI), and liver lipid content. The physiological condition of the fish is among the key factors underlying the attainment of the required performance levels. Assessment of the physiological state of the fish has become an integral part of the routine examination of fish health and of specific importance to the interpretation of the results of feeding trials, such as the testing of the biological and production efficiency of feeds. Spirulina was shown to have a very good potential as nutrient source in diets for abalone *Haliotis midae* (Britz *et al*., 1994; Britz, 1996), *Haliotis asinina* (Bautista-teruel *et al*, 2003), catla *Catla catla* and rohu *Labeo rohita* (Nandeesha *et al*., 2001).

Objectives

The purpose of the study was to investigate the effect of level of inclusion of spirulina in diets for rainbow trout on:

1. flesh pigmentation in terms of visual evaluation via use of the Roche SalmoFan ™ values and electronic detection of hue and chroma values.
2. production performance parameters (growth rate, feed intake, feed conversion efficiency, viscerosomatic index (VSI), hepatosomatic index (HSI), and liver lipid content.

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Chapter 1:

THE EFFECT OF DIETARY SPIRULINA (*Spirulina platensis*) ON FLESH PIGMENTATION OF RAINBOW TROUT (*Oncorhynchus mykiss)*

1.1 Abstract

An experiment to determine the effect of *Spirulina* on flesh pigmentation of rainbow trout was conducted at the Jonkershoek trout research station of the Division of Aquaculture, University of Stellenbosch. Rainbow trout of an average weight of 80g were stocked at commercial densities in 20 circular ponds over a 90-day treatment period. Treatments consisted of ten grower diets containing increasing levels of *Spirulina* (0%, 0.05%, 0.1%, 0.15%, 0.3%, 0.6%, 1.25%, 2.5%, 5% and 10%). Ten fish were taken at random from each replication at the end of the trial for colour determination. Each sample was cleaned from bones, skin and brown muscle and a Scottish quality cut was prepared for analysis. The coloration of the flesh was determined both visually (Roche SalmoFan™ scale) and instrumentally with a Colorard 2000/05 system. Colour variables calculated by the instrument are CIE L*, a* and b* values of which L* describes lightness, a* red-green chromaticity and b* yellow-blue chromaticity.

All colour measurements show an incremental increase in value with increasing levels of spirulina inclusion in the diets. The increase in b*- (yellow-blue), a*- (red-green) and Roche SalmoFan colour values became significant in diets containing ≥ 5% spirulina levels. The increase in b*- (yellow-blue) and Chroma values gave an indication of the deposition of carotenoids in the yellow-orange colour range instead of the desired deposition of xanthophylls in the pink-red colour range in the flesh of the rainbow trout. From these results it can be concluded that the undesirable yellowing of fillets may limit the use of *Spirulina* in diets for rainbow trout, especially at above 5% inclusions.

Keywords: *Spirulina platensis*, Rainbow trout (*Oncorhynchus mykiss)*, diets, flesh pigmentation

1.2 Introduction

The blue-green algae *Spirulina platensis* (spirulina) is often considered as ingredient for inclusion in specialised aquafeeds due to its associated medicinal properties and rich spectrum of mixed carotene and xanthophyll pigments that contribute to skin pigmentation, especially in diets for ornamental fish (Clement *et al.*, 1967; Bourges *et al.*, 1971; Anusuya Devi *et al.*, 1981; Nandeesha *et al.,* 1998; Olvera-Novoa *et al*., 1998; Mu *et al.,* 2000; Nandeesha *et al.,* 2001). The pigmentation of rainbow trout flesh is exclusively achieved by the muscle retention of the xhantophyll pigments astaxanthin and canthaxanthin. These xantophyll pigments are commercially available for inclusion in diets for rainbow trout to ensure the desired deep-red pigmentation that the consumer demands. Other dietary carotenoid pigments such as carotenes from spirulina, yellow maize, maize gluten and alfalfa may also contribute to flesh pigmentation, although often resulting in the undesirable “yellowing” or “browning” of the flesh. Since flesh appearance is a major criterion on which consumer choice is based (Schiedt, 1998), such undesirable pigmentation is unacceptable to the producer. The aim of the current experiment was to investigate to what extent inclusion of Spirulina in diets for rainbow trout may contribute to the pigmentation of trout flesh.

1.3 Materials and methods

The experiment was conducted at the Aquaculture research facility of the University of Stellenbosch, Jonkershoek near Stellenbosch, South Africa. The experimental facility consists out 40 circular PVC tanks, 3 meters in diameter and 8 m^3 in volume. It operates as a continuous flow-through system, with a water supply from the Eerste River at the rate of approximately 1liter per second per tank.

Rainbow trout fingerlings were obtained from the standing population at the Jonkershoek hatchery. A total of 250 fish with an average weight of ±80g were randomly allocated to each of 20 circular flow-through ponds. The fish were stocked at commercial stocking densities of 20kg/m^3. The fish has received only a standard commercial trout feed without any carotene pigments in the period leading up to the start of the experiment. Feeding was conducted by hand, three times per day to the point of saturation. Treatments consist of the feeding 10 experimental trout grower diets containing different levels of spirulina inclusion, to two replicates over a period of three months. Growth rate, feed consumption and feed conversion efficiency was monitored.

The standard commercial trout grower feed formulation of AquaNutro (Pty) Ltd., Malmesbury was used to prepare two experimental diets in the form of 4 mm extruded pellets, containing 0% spirulina and 10% spirulina respectively. These feeds were then blended to produce 10 experimental diets containing a range of spirulina concentrations (Table 1.1). The results of an analysis of the nutrient composition of the 10 experimental diets are presented in Table 1.2.

Sampling of the fish was conducted after a 3 month experimental period. The average weight of the fish at the beginning of the trial was ±80 grams. All replicates were sampled on the same day. Ten fish were taken at random from each replication, killed and bled through a slid of the gills and placed on ice and kept at ±0°C. Fish were then processed according to standard filleting procedures and a skinless flesh sample was collected within a period of 4 hours from sampling. The bones, skin and brown muscle tissue were removed from the samples and a Scottish quality cut (Figure 1.1) was prepared for analysis according to a procedure described by Robb (2001).

The coloration of the flesh was the determined by a visual method with the use of the calibrated Roche SalmoFan ™ scale (Figure 1.2a) and an instrumental method with the use of the Colorard 2000/05 system (Figure 1.2b).

1.4 Results and discussion

The results of the flesh pigmentation in rainbow trout due to treatment with diets containing different concentrations of spirulina (*Spirulina platensis*) over a period of 3 months (90 days) are presented in Table 1.4 and Figure 1.3 to 1.7. The results are presented in terms of L*-values (lightness), a*-value (red-green chromaticity) and b*-values (yellow-blue chromaticity).

Linear regressions over all treatments showed a poor fit for L*- (R^2 = 14.9%) a*- (R^2-value = 56.2%) and b*-values (R^2-value = 36.4%). There was an inconsistent increase in both a* (red-green) and b* (yellow-blue) values with increasing level of Spirulina inclusion as seen in (Figures 1.4 and 1.5). The differences in a*- and b*-values became statistically significant ($P<0.05$) respectively above 7% and 5% levels of inclusion of Spirulina. These increase in b*-values gave an indication of the deposition of carotenoids (yellow-orange colour range) instead of the desired deposition of xanthophylls (pink-red colour range) in the flesh of trout.

From these results it may be concluded that the application of Spirulina in diets for rainbow trout may be limited due to its undesirable yellowing of trout flesh, especially above 5% inclusion. Figure 1.6 presents and image of differences in level of flesh pigmentation achieved by the two extreme treatments of 0% and 10% spirulina inclusion, after a period of three months. None of the treatments has resulted in the required flesh pigmentation to the minimum detectable value of 20 on the Roche SalmoFan™. There was a decreasing trend in L* (lightness) values with increasing level of Spirulina inclusion (Figure 1.3) that confirms the deposition of pigments. This decrease in L* (lightness) values was statistically significant ($P<0.05$) at 10% inclusion of Spirulina inclusion. Figure 1.7 shows a typical sample of each of the 10 different treatments comparing the difference in flesh colour.

A similar trend was also observed in egg yolks from White Leghorn hens (Saxena *et.al.,* 1982), where Spirulina diets gave the highest scores at all levels tested and produced a much deeper yolk colour than produced by even the highest level of the conventional carotenoid sources. The same trend was also observed in the boiled eggs where spirulina provided substantially higher levels of deposition and pigmentation. In a study conducted with Japanese quail in 1991, Anderson *et al.*, demonstrated that the optimal level of yolk colour (8-9 on the Roche egg yolk fan) was achieved with 1-1.5 % Spirulina diet and the colour levels of the egg yolks remained stable as long as the supplementation continued. The value of Spirulina does not only depend on the pigmentation effect of individual substances, but rather on the combined synergistic effect of all these substances together, many of which probably still needs to be identified.

The treatment of rainbow trout with incremental levels of spirulina inclusion in the diets over a 90 day period have caused a increase in overall flesh pigmentation as reflected by the reduction in L*- values and increase in a*- and b*- values. None of the treatments have however yielded a desirable level of flesh pigmentation in terms of commercial standards. The increase in b*- values may limit the use of spirulina in trout diets due to the undesirable yellowing of trout flesh, especially above 5 % inclusion.

1.5 Conclusion

From these results it may be concluded that the application of Spirulina in diets for rainbow trout may be limited due to its undesirable yellowing of trout flesh and low palatability, particularly at higher levels of inclusion.

1.6 Acknowledgements

The technical and financial support of AquaNutro Aquafeeds and BioDelta Organic Products is greatly acknowledged. Thanks are extended to Anvor Adams and Frans Mouton for their dedicated assistance during the trial.

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Tables

Table 1.1 Method of preparation of experimental diets for rainbow trout containing a range of 10 incremental concentrations of spirulina.

Treatment	Blending ratio		Effective Level of spirulina inclusion (%)
	Standard diet + 0% spirulina	Standard diet + 10% spirulina	
Treatment 1	100	0	0
Treatment 2	99.5	0.5	0.05
Treatment 3	99	1	0.10
Treatment 4	98.5	1.5	0.15
Treatment 5	97	3	0.30
Treatment 6	94	6	0.60
Treatment 7	87.5	12.5	1.25
Treatment 8	75	25	2.5
Treatment 9	50	50	5
Treatment 10	0	100	10

Table 1.2 The nutrient composition of the range of 10 experimental diets for rainbow trout. (All values expressed as a percentage)

Nutrient composition	Experimental diets									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Spirulina	0.0	0.05	0.10	0.15	0.30	0.60	1.25	2.5	5.0	10.0
Dry matter	91.36	91.36	91.36	91.36	91.36	91.36	91.36	91.36	91.36	91.36
Crude protein	40.64	40.64	40.65	40.65	40.66	40.66	40.72	40.80	40.96	41.27
Lysine	2.63	2.63	2.63	2.63	2.63	2.63	2.64	2.64	2.65	2.67
Methionine	1.40	1.40	1.40	1.40	1.40	1.40	1.39	1.38	1.37	1.34
Cystine	0.46	0.46	0.46	0.46	0.46	0.46	0.47	0.48	0.51	0.56
Threonine	1.61	1.61	1.61	1.61	1.61	1.61	1.62	1.63	1.64	1.67
Tryptophan	0.42	0.42	0.42	0.42	0.42	0.42	0.43	0.43	0.44	0.47
Arginine	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.37	2.38	2.39
Glycine	2.20	2.20	2.20	2.20	2.20	2.20	2.22	2.24	2.27	2.34
Serine	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.71
Histidine	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.04	1.04	1.06
Isoleucine	1.86	1.86	1.86	1.86	1.86	1.86	1.87	1.87	1.89	1.91
Leucine	3.49	3.49	3.49	3.49	3.49	3.49	3.48	3.47	3.46	3.43
Phenylalanine	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.83	1.82
Tyrosine	1.44	1.44	1.44	1.44	1.44	1.44	1.45	1.47	1.49	1.54
Valine	2.07	2.07	2.07	2.07	2.07	2.07	2.08	2.08	2.10	2.12
Crude fat	17.18	17.18	17.18	17.17	17.17	17.17	17.13	17.07	16.96	16.75
Crude fiber	2.02	2.02	2.02	2.02	2.02	2.02	2.01	2.00	1.97	1.92
Ash	7.66	7.66	7.67	7.67	7.68	7.68	7.75	7.85	8.04	8.42
Calcium	1.61	1.61	1.61	1.61	1.62	1.62	1.64	1.66	1.71	1.81
Phosphorous	1.19	1.19	1.19	1.19	1.20	1.20	1.22	1.24	1.29	1.39
AMEn (MJ/kg)	15.13	15.13	15.13	15.13	15.13	15.13	15.15	15.16	15.20	15.26
Lysine:AME	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.74	5.73	5.72

Table 1.3 Flesh pigmentation in rainbow trout as the result of treatment with diets containing different levels of inclusion of spirluina (*Spirulina platensis*) over a period of 3 months (90 days). Measurements presented as L*-values (lightness), a*-value (red-green chromaticity) and b*-values (yellow-blue chromaticity).

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Spirulina (%)	0.0	0.05	0.10	0.15	0.30	0.60	1.25	2.5	5.0	10.0
L*-value (lightness)	53.323 ± 3.865^{abc}	53.453 ± 2.187^{abc}	51.725 ± 1.905^{ab}	54.243 ± 2.278^{abc}	51.758 ± 2.636^{abc}	52.532 ± 3.108^{a}	53.308 ± 3.308^{abc}	52.616 ± 3.048^{a}	51.013 ± 2.987^{bc}	48.859 ± 3.575^{c}
a*-value (red-green)	-0.924 ± 1.121^{d}	-1.209 ± 0.973^{d}	-0.231 ± 1.115^{d}	-0.924 ± 1.116^{d}	-0.115 ± 1.105^{cd}	-0.653 ± 1.206^{d}	0.706 ± 1.531^{cd}	0.892 ± 1.357^{d}	2.406 ± 1.411^{ab}	4.029 ± 1.901^{a}
b*-value (yellow-blue)	10.399 ± 2.320^{c}	10.194 ± 2.759^{c}	10.572 ± 1.739^{c}	9.889 ± 1.932^{c}	12.062 ± 1.888^{c}	11.405 ± 2.141^{c}	10.262 ± 2.678^{c}	11.706 ± 2.422^{b}	14.807 ± 3.504^{ab}	16.471 ± 3.232^{ab}

Mean values with different superscripts in each row differs significantly ($P<0.05$)

Figures

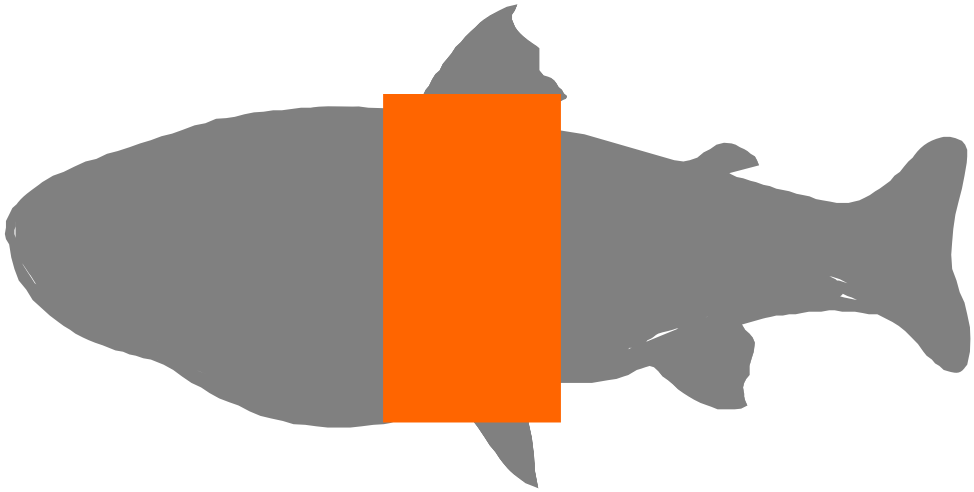

Figure 1.1: The area of the body from with the "Scottish Quality Cut" was prepared for colour analysis according to the procedure described by Robb (2001).

Figure 1.2a: SalmoFan ™ scale.

Figure 1.2b: Tristimuli as light reflection method to determine flesh pigmentation.

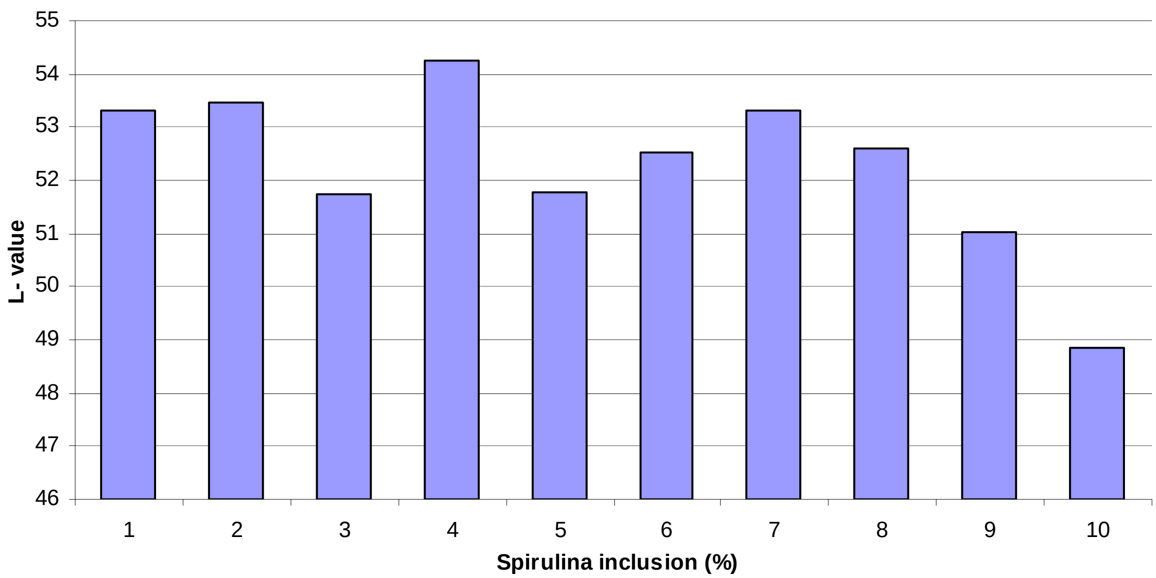

Figure 1.3 Flesh pigmentation in rainbow trout expressed as L*-(lightness) values against dietary treatments containing different levels of inclusion of spirulina (*Spirulina platensis*) over a period of 3 months (90 days).

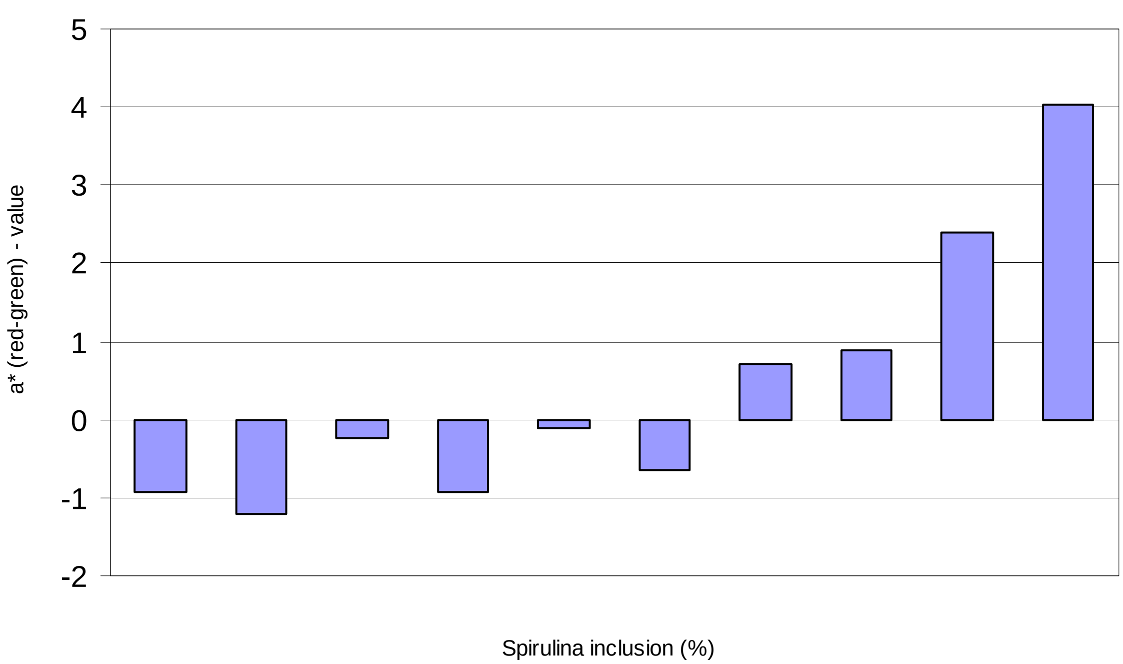

Figure 1.4 Flesh pigmentation in rainbow trout expressed as a*- (red-green chromaticity) values against dietary treatments containing different levels of inclusion of spirulina (*Spirulina platensis*) over a period of 3 months (90 days).

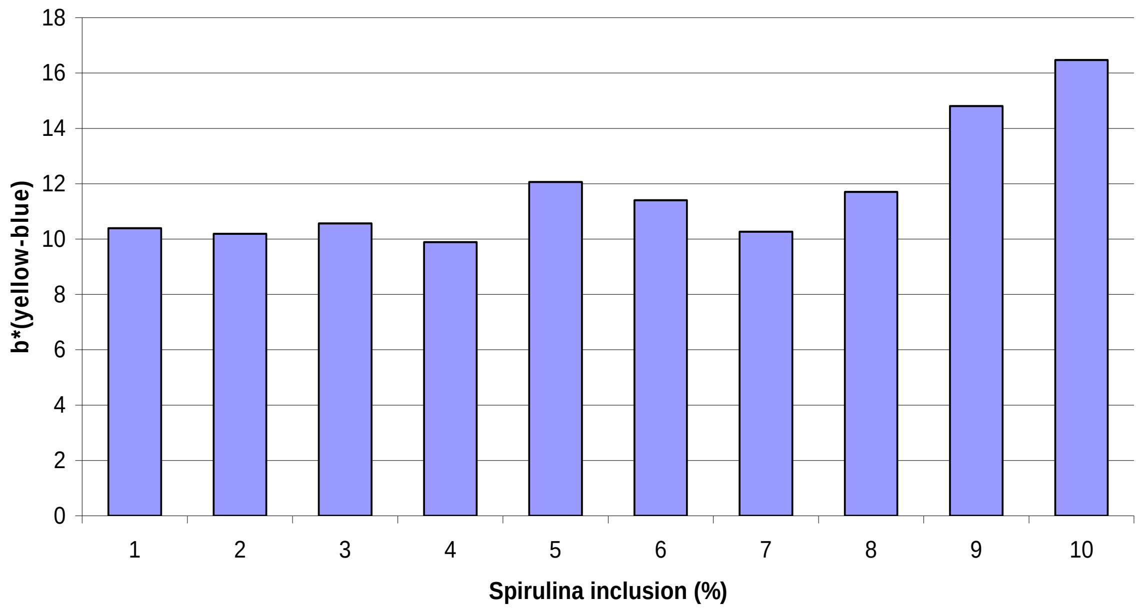

Figure 1.5 Flesh pigmentation in rainbow trout expressed as b*- (yellow-blue chromaticity) values against dietary treatments containing different levels of inclusion of spirulina (*Spirulina platensis*) over a period of 3 months (90 days).

Figure 1.6 Example of differences in the flesh pigmentation of rainbow trout on a diet with 0% (left) and 10% (right) spirulina inclusion.

Figure 1.7 Pigmentation of rainbow trout against a series of treatments on incremental dietary spirulina inclusion.

Chapter 2:

Effect of dietary inclusion of spirulina (*Spirulina platensis)* on the production performance of rainbow trout (*Oncorhynchus mykiss)*

2.1 Abstract

An experiment to determine the effect of *Spirulina* on the production of rainbow trout was conducted at the Jonkershoek trout research station of the Division of Aquaculture, University of Stellenbosch. Rainbow trout of an average weight of 80g were stocked at commercial densities in 20 circular ponds over a 90-day treatment period. Treatments consisted of ten grower diets containing increasing levels of *Spirulina* (0%, 0.05%, 0.1%, 0.15%, 0.3%, 0.6%, 1.25%, 2.5%, 5% and 10%). Various production traits was recorded including, weight gain, feed intake,

Spirulina had no significant effect (P>0.05) on weight gain of rainbow trout at any level of inclusion. No significant differences were observed in any of the other production parameters, i.e. growth rate, feed intake, feed conversion ratio, viscerosomatic index, hepatosomatic index, and liver lipid content. Low mortalities rates were observed with no significant differences between treatments. A trend of decreased feed intake with increased levels of *Spirulina* inclusion became significant at the 2.5 percent level of inclusion, caused by deterioration in the palatability of the feed. A significantly higher dress-out percentage was observed at levels of *Spirulina* inclusion above 5 percent. A trend of decreased liver lipid content with increased *Spirulina* inclusion was observed, though not significant at any level of treatment. This observation justifies further investigation because of its potential to improved carbohydrate metabolism in carnivorous fish

Results confirm that up to 10% *Spirulina* can effectively replaced soybean meal in the diets for rainbow trout whilst maintaining production performance in terms of weight gain, survival, liver fat %, Viscerosomatic Index, Hepatosomatic Index and improving carcass dress-out at high inclusion levels.

Keywords: *Spirulina platensis*, rainbow trout (*Oncorhynchus mykiss)*, diets, production performance.

2.2 Introduction

Spirulina holds a great variety of biologically active agents that has been investigated in a variety of practical applications in biotechnology and medical sciences. *Spirulina* is a rich source of the pigment C-phycocyanin C-PC (Richmond, 1986). C-PC has antioxidant properties (Romay *et al*., 1998; Bhat and Madyastha, 2000; Pinero Estrada *et al*., 2001) and anticancer properties (Dasgupta *et al*., 2001), and is a potential therapeutic agent in oxidative stress-induced diseases (Romay *et al*., 1998; Torres-Duran *et al*., 1999; Rimbau *et al*., 2001; Bhat and Madyastha, 2001; Premkumar *et al*., 2001). It was widely studied for its possible immune-stimulating effect (Pascaud, 1993; Al-Batshan *et al*., 2001; Hirahashi *et al*., 2002), antibacterial, antiparasitic and antiviral properties (Ayehunie *et al*., 1998; Hayashi *et al*., 1996ab; Hernández-Carona *et al*., 2002). Qureshi (1995) observed that young poultry fed 1000 to 10 000 ppm *Spirulina* platensis had heavier spleen and thymus weights than poultry fed a ordinary diet. Chickens fed Spirulina platensis also exhibited a higher clearance rate of Esherichia coli (intravenous inoculation) from their circulation than did chicks fed a basal diet (Qureshi, 1995). Vadiraja *et al*. (1998) observed spirulina to provide protection to the liver enzymes during induced hepatotoxicity in rats. *Spirulina* phytocomponents is also known to have general health benefits, such as improved carbohydrate metabolism (Torres-Duran *et al*., 1998) as well as the suppression of allergies, ulcers, anaemia, heavy-metal poisoning, radiation poisoning (Zhang *et al*., 2001).

Studies have shown (Nandeesha *et al.,* 1998; Olvera-Novoa *et al*., 1998; Mu *et al*., 2000; Nandeesha *et al.,* 2001) that the blue - green algae *Spirulina platensis* holds potential for inclusion in diets of various fish species due to its attractive nutrient profile and digestibility. In addition, the nutraceutical value of *Spirulina* phytocomponents has led to various health claims, such as improved carbohydrate metabolism from studies that observed reduction in lipid content in livers of laboratory mice (Rodrigues-Hernandez *et al*., 2001). Such a reducing effect on liver lipid content may be of value in inclusion of higher levels of carbohydrates in diets for carnivorous fish. The occurrence of fatty liver disease in these fish is often attributed to carbohydrate intolerance. Kay (1991) suggested that the hepatoprotective properties of *Spirulina* sp. may be associated with its antioxidant constituents, such as selenium, chlorophyll, carotene, γ-linolenic acid, and vitamins E and C. Some strains of *Spirulina* may produce bioactive substances that may inhibit or promote intestinal microbial growth, with consequent potential for proliferation of beneficial intestinal bacteria (Belay *et al*., 1993; Parada, 1998).

Various authors have reported on the potential of Spirulina as nutrient source in diets for abalone such as for *Haliotis midae* (Britz *et al*., 1994; Britz, 1996), *Haliotis asinine* (Bautista-teruel *et al*., 2003), catla *Catla catla* and rohu *Labeo rohita* (Nandeesha *et al.,* 2001). According to Bautista-teruel *et al*, the carcass composition of abalone showed an inverse relationship between protein and fat deposition. They noted that though the percentage of fat was lower in diets containing *Spirulina*, it gave rise to higher fat in the carcass. Atack *et al*. (1979) reported an increase in fat deposition in mirror carp (*Cyprinus carpio)* on diets containing *Spirulina maxima* while the opposite effect has been reported Mustafa *et al.* (1994) in red sea bream (*Pagrus major)* and Nandeesha *et al*. (1998) in common carp (*Cyprinus carpio)* for *Spirulina platensis*.

The aim of the current study was to investigate the effect of dietary *Spirulina platensis* in diets for rainbow trout on production traits such as growth rate, feed intake, feed conversion efficiency, viscerosomatic index (VSI), hepatosomatic index (HSI), and liver lipid content.

2.3 Materials and methods

The experiment was conducted at the Aquaculture research facility of the University of Stellenbosch, Jonkershoek near Stellenbosch, South Africa. The experimental facility consists out 40 circular PVC tanks, 3 meters in diameter and 8 m^3 in volume. It operates as a continuous flow-through system, with a water supply from the Eerste River at the rate of approximately 1liter per second per tank.

Rainbow trout fingerlings were obtained from the standing population at the Jonkershoek hatchery. A total of 250 fish with an average weight of ±80g were randomly allocated to each of 20 circular through-flow ponds. The fish were stocked at commercial stocking densities of 20kg/m^3. The fish has received only a standard commercial trout feed without any carotene pigments in the period leading up to the start of the experiment. During the trials feeding was conducted by hand, three times per day to the point of saturation. Treatments consist of the feeding 10 experimental trout grower diets containing different levels of spirulina inclusion, to two replicates over a period of three months. Growth rate, feed consumption and feed conversion efficiency was monitored.

The standard commercial trout grower feed formulation of AquaNutro (Pty) Ltd., Malmesbury was used to prepare two experimental diets in the form of 4 mm extruded pellets, containing 0% spirulina and 10% spirulina respectively. These feeds were then blended to produce 10 experimental diets containing a range of spirulina concentrations (Table 1.1). The results of an analysis of the nutrient composition of the 10 experimental diets are presented in Table 1.2.

Sampling of the fish was conducted after a 3 month growth period. The average weight of the fish at the beginning of the trial was ±80 grams. All replicates were sampled on the same day. Ten fish were taken at random from each replication. From these fish individual data was recorded for round, gutted and headed weight as well as the weight of the livers. Feed conversion ratio (FCR) was calculated on the basis of overall feed consumption and weight gain per tank.

2.4 Results and discussion

A summary of the various production parameters versus treatments are presented in Table 2.1 and Figure 2.1 to 2.7.

With regard to the initial and final weights of treatment groups no indication of any trend or significant differences were detected between treatments. It can therefore be concluded that the inclusion of dietary spirulina had no significant effect on weight gain of rainbow trout. A negative trend in feed intake with increasing levels of spirulina inclusion became statistically significant ($P<0.05$) above 5% spirulina inclusion (Table 2.1 and Figure 2.1). No significant differences ($P<0.05$) in the FCR were observed, over all the treatments (Figure 2.2). Nandeesha *et al* also found no significant difference in the final weight attained by catla at all levels of Spirulina incorporation as compared to the fish-meal-based control diet.

However, the replacement of fish meal by more than 25% Spirulina resulted in significantly superior growth of rohu (*Labeo rohita).*

Except differences in feed intake (Figure 2.1), no statistically significant differences ($P<0.05$) between treatments were observed for any of the other production performance parameters that were evaluated, although negative trends were also observed (Figure 2.5). This may probably be attributed to the decreasing trend in feed intake (Figure 2.1) with increasing level of spirulina inclusion. Very low mortalitys were observed. The lack of improvement in production performance parameters is supported by results found by Grinstead *et al*. (2000) from pig trials. However, they suggested that the health status of the animals, the inclusion of medication and growth promotants and feed processing methods, might have been factors affecting the effectiveness of *Spirulina platensis* in their pig trials. These results supports the findings of Nandeesha *et al.,* 2001, who tested the influence of *Spirulina platensis* meal on the growth and carcass composition of two Indian major carps, *Catla catla* and *Labeo rohita.* They found that all their diets were nearly isonitrogenous and isocaloric. However, with the increasing level of Spirulina in the diet, fat percentage declined owing to the low fat content of Spirulina. On this topic, Torres-Duran, 1999, concluded that serum lipoprotein changes induced by carbon tetrachloride, were prevented by the inclusion of whole *Spirulina maxima* in the diet, which suggests that either their hepatic synthesis is not affected or that its peripheral metabolism is preserved. This hypothesis of them would explain the lower accumulation of fatty acids in rat livers, in rats receiving Spirulina in their diet. Spirulina also produced high growth and conversion efficiencies (FCR 0,8) when fed to abalone, *Haliotis midae* (Britz, 1996). The results of Torres-Duran *et al.,* 1999, also support the potential hepatoprotective role of Spirulina.

2.5 Conclusion

Results confirm that up to 10% *Spirulina* effectively replaced soybean meal in the control diets for rainbow trout while simultaneously improving carcass dress-out at high inclusion levels.

The health status of the fish and the feed processing methods (e.g. high temperature at pelleting) may be factors affecting the effectiveness of *Spirulina platensis.* It produced high growth and conversion efficiencies, making it a suitable protein source in a practical diet. However, its use depends on it being commercially available and sold for approximately the same price as fishmeal. Due to the large price difference between spirulina and fish-meal, these research results have not been practically implemented in nowadays- commercial aqua feeds for food-fish production. These results indicate that spirulina can be an effective partial replacement (10%) for the soybean meal used in the control diet. The high cost of spirulina compared to that of soybean meal may however restrict it's inclusion on diets for Rainbow trout from at least-cost formulation point of view.

2.6 Future Research

Research in this area in aquaculture is lacking and needs to be further investigated. It may be of interest to investigate the effect of level of *Spirulina* inclusion on the support of immunity and disease resistance parameters such as white blood cell count and differentiation. Assessing the extent and specificity of potential modification of gut microbes, especially towards the prolifiration of gut-beneficial bacteria, is also suggested. The main limitation of the present study was the relatively short experimental period, which should be expanded in future experiments. The influence of spirulina on lipid metabolism regulation for the reduction of body lipid and hence of improving dress-out of slaughtered fish should however be further investigated before proper conclusion can be drawn.

2.7 Acknowledgements

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Table 2.1: The effect of dietary of inclusion of *Spirulina platensis* on production performance of rainbow trout (*Onchorhynchus mykiss*) over an over a 90-day growth period.

	Diet									
Spirulina inclusion level	**1** (0.0%)[1]	**2** (0.05%)	**3** (0.1%)	**4** (0.15%)	**5** (0.3%)	**6** (0.6%)	**7** (1.25%)	**8** (2.5%)	**9** (5.0%)	**10** (10.0%)
Fish per pond (*n*)	250	250	250	250	250	250	250	250	250	250
Weight-initial (kg/pond)	19.82 ± 0.530	20.07 ± 0.672	18.98 ± 0.544	19.75 ± 0.707	20.075 ± 0.318	20.75 ± 0.071	19.35 ± 0.283	19.41 ± 0.643	20.12 ± 0.318	20.35 ± 0.636
Weight-final (kg/pond)	68.07 ± 4.957	68.44 ± 0.290	66.73 ± 6.668	69.53 ± 1.952	69.20 ± 0.721	71.85 ± 0.544	72.50 ± 5.013	62.19 ± 0.396	65.63 ± 4.179	64.03 ± 3.458
Feed intake (kg/pond)	50.90 ± 1.273	50.18 ± 0.255	47.56 ± 4.412	50.89 ± 1.400	50.89 ± 1.485	50.37 ± 0.693	49.86 ± 1.640	42.21 ± 0.693	39.6 ± 0.325	42.26 ± 2.659
Feed intake (% BW/day)*	1.97 ± 0.014[ab]	1.90 ± 0.000[b]	1.93 ± 0.091[b]	1.98 ± 0.014[ab]	1.95 ± 0.042[b]	1.87 ± 0.014[bc]	1.93 ± 0.049[b]	1.73 ± 0.049[c]	1.59 ± 0.000[d]	1.67 ± 0.028[cd]
FCR[2]	1.06 ± 0.070[a]	1.04 ± 0.014[a]	0.9 ± 0.035[a]	1.02 ± 0.000[a]	1.03 ± 0.007[a]	0.98 ± 0.021[a]	0.94 ± 0.063[a]	0.98 ± 0.021[a]	0.87 ± 0.077[ab]	0.97 ± 0.000[a]
AGR[3] (kg/pond/day)	0.62 ± 0.056	0.62 ± 0.014	0.61 ± 0.077	0.64 ± 0.014	0.63 ± 0.014	0.65 ± 0.007	0.68 ± 0.070	0.55 ± 0.000	0.58 ± 0.056	0.56 ± 0.042
SGR[4] (%/day)	3.1 ± 0.205	3.1 ± 0.162	3.2± 0.325	3.2 ± 0.035	3.1 ± 0.113	3.2 ± 0.049	3.5 ± 0.403	2.8 ± 0.113	2.9 ± 0.332	2.8 ± 0.091
Survival (%)	100 ± 0.000	100 ± 0.000	100± 0.000	100.± 0.000	99.7 ± 0.361	99.7 ± 0.269	99.8 ± 0.311	99.6 ± 0.488	100. ± 0.000	99.5 ± 0.460
HIS (%)	1.7 ± 0.248	1.7 ± 0.337	1.6 ± 0.225	1.8 ± 0.379	1.9 ± 0.283	1.8 ± 0.269	1.7 ± 0.255	1.7 ± 0.206	1.8 ± 0.258	1.8 ± 0.258
VSI (%)	14.2 ± 1.399	14.2 ± 2.201	13.9 ± 1.180	15.2 ± 1.774	14.7 ± 2.226	14.4 ± 1.471	13.9 ± 1.287	14.6 ± 1.467	13.5 ± 0.973	13.2 ± 1.205
Dress-out (%)	85.8 ± 1.399	85.8 ± 2.201	86.1 ± 1.180	84.8 ± 1.774	85.2 ± 2.226	85.5 ± 1.471	86.1 ± 1.287	85.3 ± 1.467	86.4 ± 0.973	86.8 ± 1.205
Liver Fat %	3.4 ± 0.233	3.3 ± 0.353	3.3 ± 0.735	3.2 ± 0.162	3.3 ± 0.424	2.99 ± 0.509	3.3 ± 0.572	3.0 ± 0.091	3.0 ± 0.254	2.8 ± 0.077

[1] Level of spirulina inclusion in parenthesis; [2] Feed conversion ratio; [3] Absolute growth rate; [4] Specific growth rate, *mean values with different superscripts in each row differs significantly ($P<0.05$)

Figures

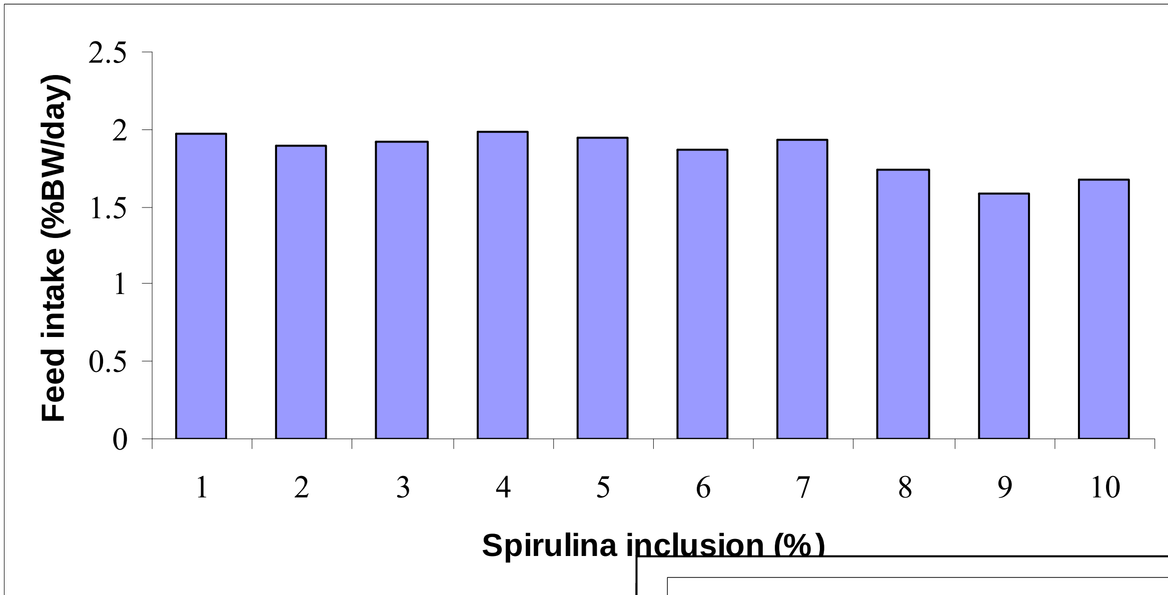

Figure 2.1 The effect of dietary inclusion of spiruli
mykiss).

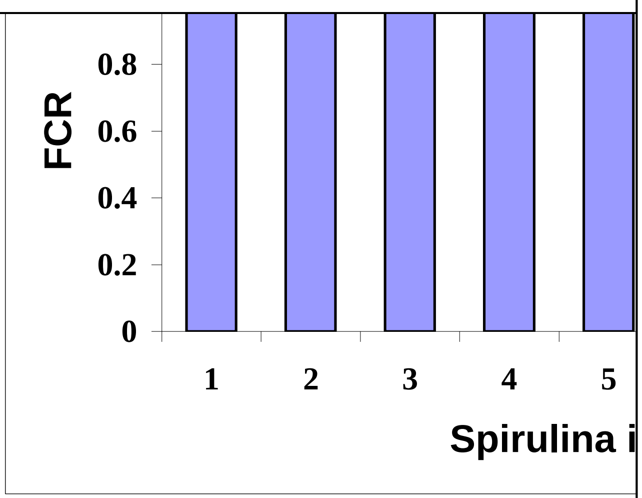

Figure 2.2 The effect of dietary inclusion of spiruli

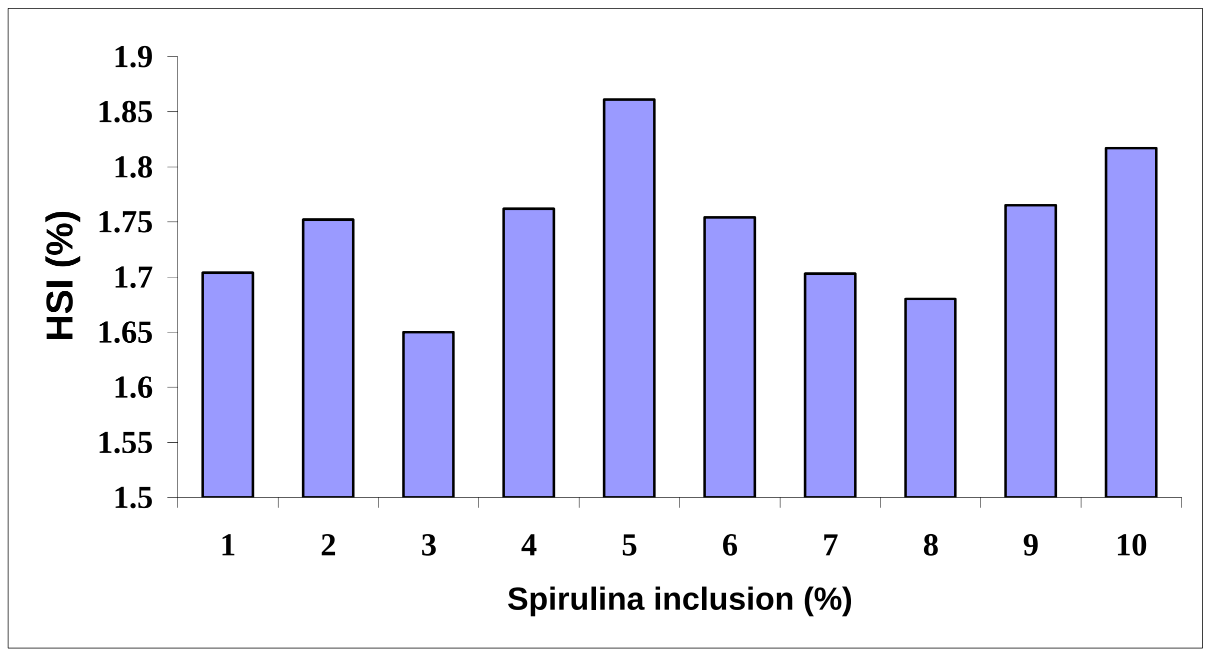

Figure 2.4 The effect of dietary inclusion of spirulina on the Hepatosomatic Index (HSI) of rainbow trout (Onchorhynchus mykiss).

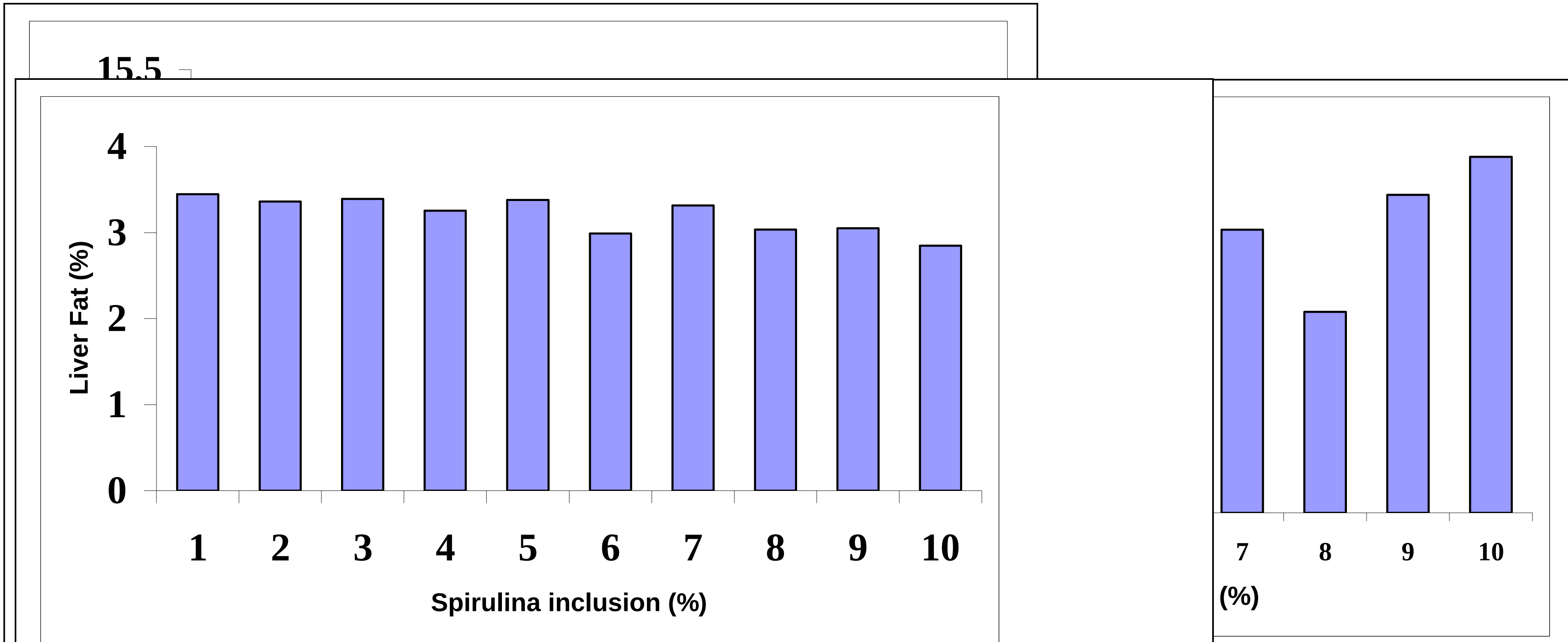

Figure 2.7 The effect of dietary inclusion of spirulina on the Liver Fat (%) of rainbow trout (Onchorhynchus mykiss).

ss-out (%) of rainbow trout