Food and Chemical Toxicology 48 (2010) 2063–2072

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio* L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal

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ARTICLE INFO

Article history:
Received 19 December 2009
Accepted 3 May 2010

Keywords:
Common carp
Jatropha curcas
Metabolic enzymes
Haematology
Histology

ABSTRACT

Protein rich *Jatropha curcas* kernel meal is toxic. It was detoxified using heat treatment and solvent extraction. Two duration of detoxification process were investigated: shorter (30 min) and longer (60 min) and the detoxified meals so obtained were designated as J_a and J_b respectively. Common carp fingerlings (252 fish; 3.2 ± 0.07 g) were fed diets: Control containing fishmeal (FM); S_{50}, J_{a50} and J_{b50}: 50% of FM protein replaced by soybean-meal (SBM), detoxified Jatropha kernel meal (DJ_aKM and DJ_bKM); S_{75}, J_{a75} and J_{b75}: 75% of FM protein replaced by SBM, DJ_aKM and DJ_bKM. White blood cells count, mean cell volume and mean cell hemoglobin concentration, calcium and sodium ions and total bilirubin in blood did not differ significantly among the groups. Higher ($P > 0.05$) RBC count was observed in plant protein fed groups compared to control group. Highest alkaline phosphatase and alanine transaminase activities in blood were observed in J_{a75}, which were not different ($P > 0.05$) from those in J_{a50} group, but were higher than in other groups. No adverse histopathological changes in liver and muscle of any group were observed, but intestinal mucosa of J_{a75} groups showed severe pathological lesions. The results demonstrate that Jb was completely detoxified. Since the performance of J_{b50} group was similar to control group and better than other groups, optimum inclusion level of J_b is 50% replacement of FM protein.
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1. Introduction

Traditionally, fish meal (FM) has been the main source of dietary protein for fish. In recent years, its increasing cost, decreasing availability in the market and poor quality have stimulated several studies on its partial or complete substitution with alternative protein sources (Kaushik et al., 1995; Fournier et al., 2004). Soybean meal (SBM) is currently the most commonly used plant protein source in fish feeds (Yue and Zhou, 2009). The price of SBM, which is the main protein source for cultured animals, has increased sharply (Azaza et al., 2009). Nowadays, maize is used as an energy source in fish to reduce feed cost and also being used to satisfy the rising demand of the fast-growing bio-fuels industry (Azaza et al., 2009).

According to FAO (2008), the price of SBM increased to new record level, an increase of 60% from early May 2007. Based on the current supply and demand forecasts for the coming years, prices can be expected to remain high. This phenomenon has limited the expansion and profitability of aquaculture enterprises in most developing countries (Tacon, 2007). In many countries, SBM and soybean oil used in feed formulation are imported, which increase feed costs. This is the case in Europe and many tropical countries, especially in sub-Saharan Africa where soybean production is fairly limited due mainly to climatic and geographical constraints.

Most modern, nutrient-dense, aquaculture diets use some inclusion of plant protein ingredients. Many such ingredients have been assessed experimentally, but with the notable exception of SBM, few are used commercially (Carter and Hauler, 2000). The high cost of protein sources, their restricted availability and the unpredictability of their markets, increase the need for alternative sources of protein in fish feed.

Jatropha curcas (L.) (physic nut) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. It is a hardy plant, thrives on degraded land and requires limited amounts of nutrients and water. The International Jatropha Organization has projected that in 2017 there will be around 32.72 million hectares of land cultivated worldwide with *J. curcas,* producing 160 million tons of seeds and 95% of the total production will be in Asia (Siang, 2009). Jatropha seeds have been extensively investigated as a source of oil. The seed kernel contains about 60% oil that can be converted into biodiesel of high quality upon transesterification and used as a substitute for diesel fuel (Makkar et al., 2007). The kernel meal obtained after oil extraction is an excellent

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doi:10.1016/j.fct.2010.05.007

source of nutrients and contains 58–62% crude protein (Makkar et al., 2008). The levels of essential amino acids, except lysine in *J. curcas* kernel meal are higher than in SMB (except lysine). However, the presence of high levels of antinutrients such as trypsin inhibitor, lectin and phytate (Makkar et al., 2008) and the major toxic components phorbol esters (PE_S) (Makkar and Becker, 1997) restrict their use in fish feed. Heat labile antinutrients, protease inhibitors and lectins are easy to inactivate by moist heating (Makkar and Becker, 2009). A method for detoxification of Jatropha kernel meal has been developed in our laboratory. It is based on extraction of PEs using organic solvents and inactivation of trypsin inhibitors and lectin by heat treatment.

Jatropha plant can yield up to 4 tons seed per year from one hectare of plantation, which can produce approximately 1 ton of kernel meal rich in protein (Makkar and Becker, 1997). This means that there is a possibility of producing enough Jatropha kernel meal to meet growing aquaculture industry demand. Our previous studies have shown that detoxified Jatropha kernel meal (DJKM) is a good protein source for carp (*Cyprinus carpio*) (Kumar et al., 2008) and rainbow trout (*Oncorhynchus mykiss)* diets (Kumar et al., 2009). This study reports the physiological, haematological and histological responses of adding DJKM and SBM to common carp diets.

2. Material and methods

2.1. Preparation of the Jatropha meal

Jatropha seeds were obtained from India and deshelled manually to obtain kernels. Defatting of Jatropha kernels was done using petroleum benzene (b.p. 40–60 °C) in a Soxhlet apparatus. Organic solvents were used to detoxify defatted Jatropha kernel meal. Two durations of PE removal were investigated: shorter (30 min) and longer (60 min) and the detoxified meals so obtained were designated as J_a and J_b respectively (patent application has been filed for the detoxification process). After removal of PEs, the meal was autoclaved (121 °C) to remove heat labile antinutrients, trypsin inhibitor and lectin.

2.2. Diet formulation

Fish meal (Seelöwe fishmeal) was procured from Vereinigte Fischmehlwerke Cuxhaven GmbH & Co. KG, Cuxhaven, Germany; and wheat meal was purchased from a local market. Extracted soybean meal (dehulled, defatted and roasted) was obtained from the Institute of Animal Nutrition (450), University of Hohenheim, Stuttgart, Germany. Soya protein isolate (SUPRO® 500E IP) was purchased from Solae Europe S.A., 2, Chemin du Pavillon, CH-1218 Le Grand-Saconnex, Geneva, Switzerland. Prior to feed formulation, the proximate composition of defatted Jatropha kernel meal, wheat meal, SBM, soya proteins isolate and FM was determined. A total of seven isonitrogenous and isoenergetic diets were formulated. Experimental diets containing crude protein 38%, crude lipid 8%, vitamin premix 2%, mineral premix 2% and TiO_2 1% were prepared. Lysine monohydrochloride (lysine 80% in this salt) was supplemented at the rate of 1% of DJKM inclusion in the diet. Each experimental feed, except the control, contained 500 FTU phytase (NATUPHOS 5000G, BASF, Ludwigshafen) per kg. The inclusion levels of the DJKM and SBM were as follows: Control diet was prepared with FM and wheat meal, without any DJKM and SBM. S_{50}: 50% of FM protein replaced by SBM; S_{75}: 75% of FM protein replaced by SBM; J_{a50}: 50% of FM protein replaced by DJ_aKM; J_{a75}: 75% of FM protein replaced by DJ_aKM; J_{b50}: 50% of FM protein replaced by DJ_bKM; and J_{b75}: 75% of FM protein replaced by DJ_bKM. The final mixture of each diet was made to 2 mm diameter moist pellets and then freeze-dried (Table 1).

2.3. Experimental system and animals

Common carp (*C. carpio* L.) fingerlings (about 2.0–3.0 g) from the Institute of Fisheries Ecology and Aquaculture of the Federal Research Center for Fisheries at Ahrensburg, Germany, were transferred to the University of Hohenheim, Stuttgart, Germany, and kept in two 500 l capacity tanks for acclimatisation. They were fed a standard fish diet containing approximately 38% protein, 8% lipid, 10% ash and with a gross energy content of 18.5 kJ g^{-1} dry matter. After an acclimatisation period of 20 days, 252 fish were randomly distributed into seven groups with four replicates; each replicate contained nine fish in an aquarium (45 l capacity). All the aquaria were supplied with water from a recirculatory system. The system was subjected to a photoperiod of 12 h light:12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 26.2–27.1 °C, pH 7.0–7.5, dissolved oxygen 6.9–7.4 mg l^{-1}, total NH_3 0.1–0.2 mg l^{-1}, nitrite 0.07–0.1 mg l^{-1} and nitrate 1–3 mg l^{-1}). Water flow was adjusted to keep the oxygen saturation above 80%. One day before start of the experiment, the fish were starved and during the experimental period fish were fed at 16 g feed per kg metabolic body mass ($kg^{0.8}$) per day (equal to five times their maintenance energy requirement). No feed was left in the aquaria when the feeds at five times maintenance energy requirement were offered. Since the aim of the study was to evaluate the performance of fish fed diets containing DJKM, high level of feed consumption was preferred, in order to elicit adverse effects if any due to the presence of the detoxified kernel meal.

Total feed per day was split into five equal portions and each portion was given at 8:00, 10:30, 13:00, 15:30 and 18:00 h. The feeds were dispensed using an automatic feeder. Fish were weighed individually at the beginning of the experiment (av. wt. 3.2 ± 0.07 g) and at weekly intervals during the experimental period to ad-

Table 1
Composition of the experimental diets (g kg^{-1} feed).

Ingredients	Experimental diets						
	Control	J_{a50}	J_{a75}	J_{b50}	J_{b75}	S_{50}	S_{75}
Fish meal	507.5	253.7	126.3	253.7	126.3	253.7	126.3
Soyabean meal	–	–	–	–	–	342.1	513
Wheat meal[a]	402.5	381.5	372	390	384.1	271	206
Jatropha meal	–	249.5	372	242.5	361.9	–	–
Soya concentrate	–	3.5	7	2	5	22	32
Sunflower oil	40	61.8	72.7	61.8	72.7	61.2	72.7
Vitamin premix[b]	20	20	20	20	20	20	20
Mineral premix[c]	20	20	20	20	20	20	20
TiO_2	10	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000	1000
Phytase (FTU/kg)	–	500	500	500	500	500	500
Lysine monohydrochloride (g)	–	2.5	3.7	2.4	3.6	–	–

Control: FM and wheat meal, without any DJKM and SBM.
J_{a50}: 50% of fish meal protein replaced by detoxified Jatropha kernel meal (30 min).
J_{a75}: 75% of fish meal protein replaced by detoxified Jatropha kernel meal (30 min).
J_{b50}: 50% of fish meal protein replaced by detoxified Jatropha kernel meal (60 min).
J_{b75}: 75% of fish meal protein replaced by detoxified Jatropha kernel meal (60 min).
S_{50}: 50% of fish meal protein replaced by soybean meal.
S_{75}: 75% of fish meal protein replaced by soybean meal.

[a] Whole wheat meal.

[b] Vitamin premix (g or IU kg^{-1} premix): retinol palmitate, 500,000 IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50,000 IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25.

[c] Mineral premix (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4{\cdot}7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4{\cdot}H_2O$, 3.12; $ZnSO_4{\cdot}7H_2O$, 4.67; $CuSO_4{\cdot}5H_2O$, 0.62; KI, 0.16; $CoCl_2{\cdot}6H_2O$, 0.08; ammonium molybdate, 0.06; 3 $NaSeO_3$, 0.02.

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