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Original Research Article

Nutritional value of detoxified *Jatropha curcas* seed cake protein isolates using rats as an animal model

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ABSTRACT

A bioassay study was conducted to investigate the effects of substituting casein with graded levels of detoxified *Jatropha curcas* seed cake protein isolates (JPI) as a protein source on the growth performance, feed efficiency ratio (FER) and its protein values using rats as an animal model. Thirty 21-day-old male Sprague–Dawley weaned rats were randomly divided into 5 groups, each group with 6 replications ($n = 1$). Each group consumed one of the following diets: protein-free, casein (CAS) and JPI diets (JPI_{20}, JPI_{40} and JPI_{60}; different levels of JPI to replace the casein at concentrations of 20%, 40% and 60% on crude protein basis). Feed intake and protein intake showed no difference among the rats fed JPI_{20}, JPI_{40} and CAS diets ($P > 0.05$). However, these parameters were lower in the rats fed JPI_{60} than in rats fed CAS ($P < 0.05$). The rats fed diets containing JPI had lower body weight gain, protein efficiency ratio and net protein retention than those fed CAS diet ($P < 0.05$). When the level of JPI used to replace the casein was lower than 40%, protein efficiency ratio (PER) was close to or higher than 2.0, which suggests that JPI could be viewed as a high-quality protein. Inclusion of JPI in the diet decreased alkaline phosphatase activity. The values were significantly lower in rats fed JPI_{20} and JPI_{40} than in rats fed CAS ($P < 0.05$). No histopathological changes were observed in livers and kidneys in the rats fed JPI diets. The results demonstrate that JPI could be used as an efficient protein source at a level of no more than 40% of dietary protein source.

1. Introduction

Jatropha kernel meal (JKM) obtained as a by-product after oil extraction from *Jatropha* kernels has up to 60% crude protein (CP) content. It has been shown that the levels of essential amino acids (EAA) except lysine in JKM are higher than those of the FAO-WHO (1990) reference protein and the required EAA levels for chicks and young pigs (Makkar et al., 1997). The AA profile of JKM is comparable to that of soybean, except for lysine. The anti-nutritional factors and toxic factors in JKM can be removed using a procedure that uses chemical and heat treatments, and the detoxified JKM has been evaluated as a protein-rich feed source in a number of animal species (Makkar et al., 2012; Abd El-Hack et al., 2017).

In addition to JKM, *Jatropha* seed cake is generated when whole *Jatropha* seeds are passed through a mechanical press. This seed cake has a high amount of shells (up to 45%), hence it is not a good feed for animals. A process of protein isolate preparation from *Jatropha* seed cake has been optimized (Makkar et al., 2008; Wang et al., 2011b). *Jatropha curcas* seed cake protein isolates (JPI) has a high protein content (around 89%), and could be a good protein source for animals. The presence of anti-nutrients and toxins like phorbol esters in *J. curcas* seed cake limits its application in feeds (Sharath et al., 2016). Many work has been conducted to detoxify the JPI (Sharath et al., 2014; Abd El-Hack et al., 2017), but the effects

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are variable. The same approach as the detoxification of JKM has been developed by Makkar et al. (2012). Before the application of this protein in foods, animal studies should be conducted to confirm its safety. Our previous study confirmed that the detoxified JKM supplemented with lysine could replace 50% of soybean meal protein in the diets of growing pigs without any negative effects on health or production (Wang et al., 2011a).

The rat has been widely used as an animal model to evaluate protein efficiency. We hypothesized that JPI could substitute a part of casein in rat diets with no negative effect on performance. To evaluate detoxified JPI as a protein source in animal diets, this bioassay study was conducted to investigate the effects of substituting casein with graded levels of detoxified JPI on the growth performance, feed efficiency ratio (FER) and its protein values using rats as an animal model.

2. Material and methods

2.1. Feeds and experimental design

The JPI used in this study was prepared according to Makkar et al. (2008) and Wang et al. (2011b). The JPI preparation was free of phorbol esters and anti-nutritional factors such as trypsin inhibitor and lectins (Makkar et al., 2012). Thirty 21-day-old male Sprague–Dawley weaned rats, weighing 70 ± 4.39 g, were randomly divided into 5 groups, each group with 6 replications. Each group consumed one of the following diets: protein-free basal diet, casein (CAS) and JPI diets (JPI_{20}, JPI_{40} and JPI_{60}; different levels of JPI to replace the casein at concentrations of 20%, 40% and 60% on CP basis) (Table 1). The level of dietary protein of the CAS and JPI diets was 10%. The contents of the experimental diets are shown in Table 2.

2.2. Animal managements and performance measurement

All procedures used in the research were approved by the Animal Care and Use Committee at Zhejiang University, China. The experiments were conducted at the Laboratory Animal Research Center, Zhejiang Chinese Medical University, Hangzhou, China. All the conditions used for animal housing and handling followed the Regulations for the Administration of Affairs Concerning Experimental Animals (The State Food and Drug Administration of People's Republic of China, 2005).

Table 1
Ingredients of the experimental diets for rats, g/100 g.

Item	Dietary treatments[1]				
	Protein-free	CAS	JPI_{20}	JPI_{40}	JPI_{60}
Casein	0	13.6	10.8	8.1	5.4
JPI	0	0	2.4	4.7	7.1
Corn starch	80.0	66.4	66.8	67.1	67.5
Corn oil	10.0	10.0	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0	5.0	5.0
Salt mixture[2]	4.0	4.0	4.0	4.0	4.0
Vitamin mixture[3]	1.0	1.0	1.0	1.0	1.0

JPI = *Jatropha* seed cake protein isolate.
[1] CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet (dry matter basis); JPI_{20}, JPI_{40} and JPI_{60}, diets containing JPI to replace the 20%, 40% and 60% protein of casein.
[2] Salt mixture: $CaCO_3$ 78.6 g, $Ca_3C_{12}H_{10}O_{14}{\cdot}4H_2O$ 308.3 g, $CaHPO_4{\cdot}2H_2O$ 112.8 g, K_2HPO_4 218.8 g, KCl 124.7 g, NaCl 77.1 g, $MgSO_4$ 38.3 g, $MgCO_3$ 35.2 g, $Fe(C_6H_{17}N_3O_7)$ 15.3 g, $MnSO_4{\cdot}H_2O$ 0.201 g, $CuSO_4{\cdot}5H_2O$ 0.078 g, KI 0.041 g, $ALNH_4(SO_4)_2{\cdot}12H_2O$ 0.507 g.
[3] Vitamin mixture: vitamin A 1,000 IU, vitamin D 100 IU, vitamin E 10 IU, vitamin K 0.5 mg, riboflavin 1 mg, pyrodoxine 0.4 mg, pantothenic acid 4 mg, niacin 4 mg, choline 200 mg, inositol 25 mg, para-aminobenzoic acid 10 mg, vitamin B_{12} 2 μg, biotin 0.02 mg, folic acid 0.2 mg. Cellulose was added to bring it up to 1 g.

Table 2
Contents of amino acids in *Jatropha* seed cake protein isolate (JPI) and the experimental diets for rats, % of DM.

Item	JPI	Dietary treatments[1]			
		CAS	JPI_{20}	JPI_{40}	JPI_{60}
Essential amino acids					
Lysine	2.14	6.74	6.25	5.46	4.63
Methionine	1.52	2.06	2.04	1.79	1.77
Threonine	2.95	3.96	3.93	3.88	3.92
Valine	3.84	5.65	5.73	5.65	4.85
Isoleucine	3.47	4.35	4.27	4.34	4.45
Leucine	5.67	8.43	7.98	7.83	7.67
Phenylalanine	3.88	4.26	4.46	4.54	4.85
Histidine	2.01	2.28	2.38	2.42	2.57
Arginine	10.31	2.86	4.20	5.63	7.72
Non-essential amino acids					
Proline	3.27	8.90	7.79	7.02	6.15
Glycine	3.53	1.78	2.14	2.63	3.31
Cystine	1.22	2.54	3.09	3.14	1.45
Alanine	3.76	2.95	3.20	3.55	4.00
Asparagine	7.96	6.71	7.19	7.74	8.60
Serine	3.40	5.05	5.01	4.94	5.07
Glutamine	11.61	25.82	24.95	24.21	23.57
Tyrosine	2.98	3.80	3.52	3.35	3.32

[1] CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet (dry matter basis); JPI_{20}, JPI_{40} and JPI_{60}, diets containing *Jatropha* seed cake protein isolates to replace the 20%, 40% and 60% protein of casein.

The rats were singly housed in cages and kept in a well ventilated, thermostatically controlled room at 22 ± 1 °C under a 12-h light–dark cycle and had free access to drinking water. The experiment lasted for 28 d, including a 3-d adaptation period followed by an experimental period of 25 d. Feed intake was determined by weighing the amounts of diet given, refused and spilled. Feed intake per day was defined as feed intake rate. Live weight was recorded daily and the weight gain per day (daily gain) was defined as weight gain rate. The feces were collected on alternate days and stored at −20 °C until analysis.

2.3. Sample and analyses

2.3.1. Chemical composition

The samples of diet ingredients and diets were analyzed for Kjeldahl nitrogen (N) using the AOAC method 954.01 (AOAC, 1995), and the CP was then calculated by multiplying N × 6.25. Concentration of N in acidified urine samples was determined using the micro-Kjeldahl analysis (AOAC, 1995). The AA levels were determined using an AA analyzer after hydrolyzing the samples with 6 mol/L HCl at 100 °C for 22 h. Sulfur-containing AA were oxidized using performic acid before the acid hydrolysis.

2.3.2. Protein biological indices

Dry matter digestibility values were obtained by subtracting the endogenous excretion corrected for the amount of diet consumed from the apparent fecal losses (Wolsac et al., 1981). Protein efficiency ratio (PER) and net protein retention (NPR) were calculated as follows (Friedman, 1996):

PER = Gain in body weight (g)/Protein consumed (g);
NPR = (Weight gain of test group + Weight loss of non-protein group) (g)/Protein consumed (g).

2.3.3. Blood biochemical parameters

Blood samples were collected from the eyeball of each rat and serum was collected by centrifugation at 1,200 × *g* for 15 min at 4 °C. Total protein, albumin, blood urea nitrogen and alkaline

phosphatase were analyzed by an automatic biochemistry analyzer (Hitachi 7020, Hitachi High Technologies, Inc., Ibaraki, Japan). Test kits were purchased from Diasys Diagnostic Systems (Shanghai Co. Ltd., Shanghai, China).

2.3.4. Histopathological studies

All 30 rats were slaughtered at the end of experiment. The liver, heart, lung, spleen and kidneys were weighed. The specimens of tissues from the liver and kidney were taken for histopathological examination. The tissues were immediately rinsed with physiological saline, fixed overnight in 4% paraformaldehyde and then dehydrated in a graded series of ethanol and embedded in paraffin for later slicing and hematoxylin and eosin staining.

2.4. Statistical analysis

For feed intake, average daily weight gain, feed conversion ratio, protein biological indices and serum parameters, each rat was considered as the experimental unit. The treatments were assigned as a completely randomized design using the general linear models (GLM) procedure of SAS Institute (1996). Differences among means of the 4 treatments were tested using Duncan's new multiple range test. Statistical significance was defined at $P \leq 0.05$, with highly significant values at $P \leq 0.01$.

3. Results

3.1. Amino acid composition of the experimental diets

Substitution of casein with JPI in the diet decreased the contents of EAA including methionine, lysine, and leucine, but an increasing trend was observed for arginine (Table 2). This was influenced by the high level of arginine (10.3%) in the *J. curcas* protein isolate. The contents of the non-EAA, proline and glutamine tended to decrease, whereas glycine, alanine, and asparagine contents tended to increase with the inclusion of JPI in diets.

3.2. Feed intake and growth performance of rats

Feed intake, protein intake and feed intake rate in rats fed JPI_{20} and JPI_{40} diets were not significantly different from those fed CAS ($P > 0.05$, Table 3), whereas these parameters were lower in rats fed the JPI_{60} diet than in those fed the CAS diet ($P < 0.05$, Table 3). Weight gain ($P < 0.01$) and weight gain ratio ($P < 0.01$) were lower in rats fed diets containing graded levels of JPI than in those fed the CAS diet.

Table 3
Feed intake, growth performance and digestive utilization of nitrogen in rats fed experimental diets.

Item	Dietary treatments[1]				SEM	*P*-value
	CAS	JPI_{20}	JPI_{40}	JPI_{60}		
Feed intake, g	355.3^{a}	347.7^{a}	319.1^{a}	241.6^{b}	14.29	<0.01
Protein intake, g	44.2^{a}	42.6^{a}	43.0^{a}	29.2^{b}	1.78	<0.01
Body weight gain, g	124.6^{a}	103.8^{b}	86.0^{c}	1.80^{d}	5.56	<0.01
Weight gain rate, g/day	4.5^{a}	3.7^{b}	3.1^{c}	1.9^{d}	0.20	<0.01
Feed intake rate, g/day	12.7^{a}	12.4^{a}	11.4^{a}	8.6^{b}	0.51	<0.01
DM digestibility, %	90.7^{a}	90.5^{a}	87.5^{ab}	86.3^{b}	1.25	0.07
FER	0.35^{a}	0.30^{b}	0.27^{c}	0.21^{d}	0.01	<0.01
PER	2.82^{a}	2.43^{b}	1.99^{c}	1.76^{d}	0.06	<0.01
NPR	2.27^{a}	1.86^{b}	1.44^{c}	0.96^{d}	0.09	<0.01

FER = Feed efficiency ratio; PER = protein efficiency ratio; NPR = net protein ratio.
^{a,b,c,d} Within a row, means without a common superscript differ ($P < 0.05$).
[1] CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet; JPI_{20}, JPI_{40} and JPI_{60}, diets containing *Jatropha curcas* seed cake protein isolates to replace the 20%, 40% and 60% protein of casein.

3.3. Efficiency of nitrogen utilization

The rats fed diets containing JPI had significantly lower FER ($P < 0.01$), PER ($P < 0.01$) and NPR than those fed CAS ($P < 0.01$, Table 3). There was no significant difference ($P > 0.05$) in DM digestibility among CAS, JPI_{20} and JPI_{40} diet groups, but DM digestibility of JPI_{60} diet group was lower ($P < 0.05$) than that of the CAS diet group.

3.4. Serum characteristics, the ratio of internal organ to body weight and histopathology

Contents of total protein and albumin were the highest in rats fed JPI_{40} and the lowest in those fed JPI_{60}, with a significant difference between these 2 groups ($P < 0.05$; Table 4). No significant difference was found in blood urea nitrogen among different groups ($P = 0.47$). Inclusion of JPI in diets decreased the alkaline phosphatase content ($P = 0.02$) with significantly lower values in rats fed JPI_{20} and JPI_{40} than in rats fed CAS ($P < 0.05$).

The ratio of the liver or kidney to the body weight was significantly higher in the rats fed JPI_{40} and JPI_{60} diets than that fed the CAS diet ($P < 0.05$, Table 5). The rats fed the JPI diets had a significantly higher ratio of the spleen to body weight than those fed the CAS diet ($P < 0.05$). There was no significant difference in the lung to body weight ratio ($P > 0.05$). No histopathological changes were observed in the liver (Fig. 1) and kidney (Fig. 2) among rats in all dietary groups.

4. Discussion

The protein content of the JPI was 84.4% (Wang et al., 2011b), similar to the 89% reported by Saetae and Suntornsuk (2011). There was a decrease in methionine, lysine and leucine contents, but an increase in the arginine content was noted in the diets containing increasing amounts of JPI (Table 2). Previous reports also showed that the JPI had the highest arginine content, followed by aromatic (phenylalanine + tyrosine) and nonpolar AA (leucine, isoleucine, alanine, glycine, valine, and proline) (Makkar et al., 2008; Peralta-Flores et al., 2012). Compared with the FAO/WHO reference protein for infants (1990), the protein fractions of *J. curcas* provide all EAA in sufficient amounts with the exception of lysine and tryptophan (Peralta-Flores et al., 2012). Protein solubility, water and oil binding capacities, foam forming capacity and stability, and emulsion activity and stability of JPI have been reported to be good under neutral to basic pH condition (Saetae and Suntornsuk, 2011).

In the current study, there was no significant difference in feed intake among rats fed JPI_{20}, JPI_{40} and CAS diets, but the weight gain rate was significantly lower in rats fed JPI_{20} and JPI_{40} diets than in rats fed CAS. Based on these observations, it can be assumed that the significant decrease in weight gain rate with the inclusion of increased levels of JPI may not have resulted from a low feed intake, but perhaps from a low protein utilization efficiency of the diet. Makkar and Becker (1999) found similar results in the evaluation of the nutritional value of the *J. curcas* meal obtained from the non-toxic genotype. The growth rate was the highest with the casein diet, followed by diets containing heat-treated and unheated *Jatropha* meals, while feed intake of the diet containing heated *Jatropha* meal did not differ significantly from that of the casein diet.

The calculated nutritional indices, such as PER (C-PER), for *J. curcas* protein suggest excellent quality for animals (Angulo-Bejaranoa et al., 2008) based on the EAA profile and protein digestibility analysis. The C-PER value for protein isolate from defatted JKM (2.16) was comparable to, or higher than the values for regular animal feed ingredients, such as corn meal (1.1), wheat

Table 4
Serum characteristics of rats fed experimental diets.

Item	Dietary treatments[1]				SEM	*P*-value
	CAS	JPI_{20}	JPI_{40}	JPI_{60}		
Total protein, g/L	56.0[b]	59.0[ab]	61.2[a]	56.0[b]	1.52	0.07
Albumin, g/L	30.4[ab]	31.6[ab]	32.0[a]	29.7[b]	0.72	0.11
Alkaline phosphatase, IU/L	345.4[a]	298.4[ab]	254.2[b]	243.2[b]	22.05	0.02
Blood urea nitrogen, mmol/L	4.6	5.2	5.6	5.9	0.55	0.47

[a,b] Within a row, means without a common superscript differ ($P < 0.05$).
[1] CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet; JPI_{20}, JPI_{40} and JPI_{60}, diets containing *Jatropha curcas* seed cake protein isolates to replace the 20%, 40% and 60% protein of casein.

Table 5
Ratios of internal organs to body weight (%) of rats fed experimental diets.

Item	Dietary treatments[1]				SEM	*P*-value
	CAS	JPI_{20}	JPI_{40}	JPI_{60}		
Liver	3.10[b]	3.33[ab]	3.46[a]	3.53[a]	0.11	0.08
Kidney	0.80[c]	0.88[bc]	0.95[b]	1.04[a]	0.03	<0.01
Spleen	0.53[b]	0.63[a]	0.65[a]	0.71[a]	0.03	0.01
Lung	0.23	0.24	0.24	0.24	0.02	0.92

[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$).
[1] CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet; JPI_{20}, JPI_{40} and JPI_{60}, diets containing *Jatropha curcas* seed cake protein isolates replacing the 20%, 40% and 60% protein of casein.

flour (0.8), soy flour (1.3) and quality protein maize (1.43) (Angulo-Bejaranoa et al., 2008; Devappa and Swamylingappa, 2008). Although the rats fed diets containing JPI had lower FER and PER value than those fed the CAS diet, the PER value was 1.76 even when JPI was included in the diet at a level of 60% to substitute casein protein. According to Friedman (1996), a protein source with PER < 1.5 is considered to be of low quality, whereas a protein source with a PER > 2.0 is considered as good quality. In the present study, the PER was higher than or close to 2.0 when JPI replaced less than 40% of the casein protein, reflective of the high quality of the JPI as a protein source.

In terms of the limiting AA, the lysine contents of the JPI_{40} and JPI_{60} groups were lower than the FAO/WHO recommended value of 5.80% of CP (FAO/WHO, 1990). A low content of lysine and a high content of arginine in JPI-fed rats may cause an imbalance in the proportion of these AA. Moreover, lysine and arginine exert antagonistic function in digestion, absorption and renal reabsorption; therefore, the AA composition may lead to a decline in the utilization of this protein. Furthermore, the decrease in protein utilization may account for a decrease in digestibility, FER, PER and NPR when casein was replaced with JPI in the diet of rats.

A significantly higher alkaline phosphatase activity was observed in blood samples collected from common carp (*Cyprinus carpio* L.) fingerlings fed incompletely detoxified JKM compared with the fish on fishmeal diet, but no significant change was found in fish fed diets containing completely detoxified JKM (Kumar et al., 2010). In this study, phorbol esters were not identified in JPI-fed groups (data not shown). Decreased alkaline phosphatase levels found in groups fed JPI diets suggested that the JPI was not detoxified completely. However, the histopathological results showed no adverse effects and blood parameters were in the normal ranges, suggesting that JPI used in this study

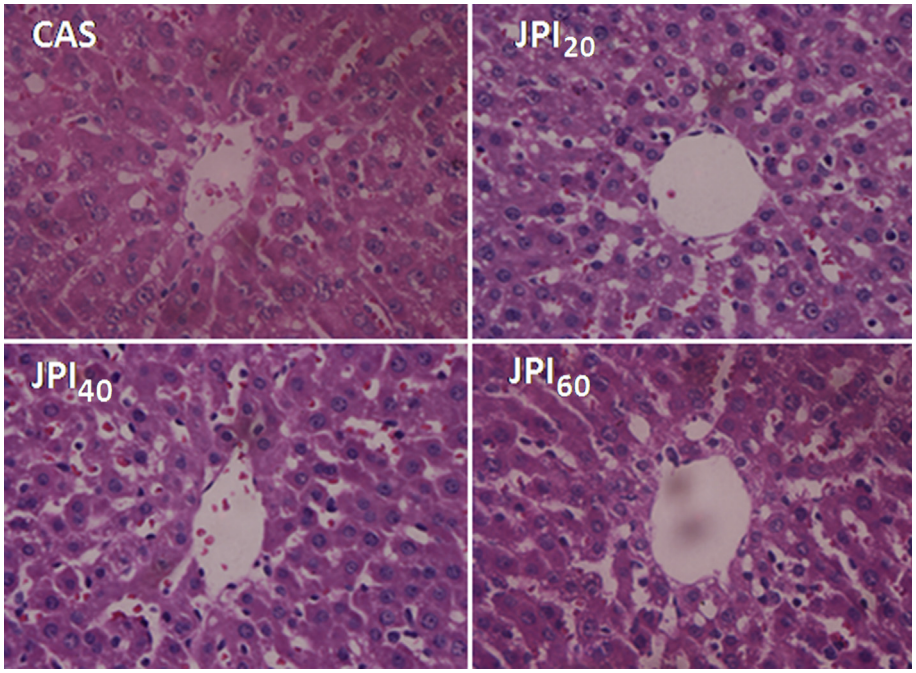

Fig. 1. Histopathological changes in the livers of rats fed diets containing casein or *Jatropha curcas* seed cake protein isolates (JPI) (magnification, 400×). CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet; JPI_{20}, JPI_{40} and JPI_{60}, diets containing JPI to replace the 20%, 40% and 60% protein of casein.

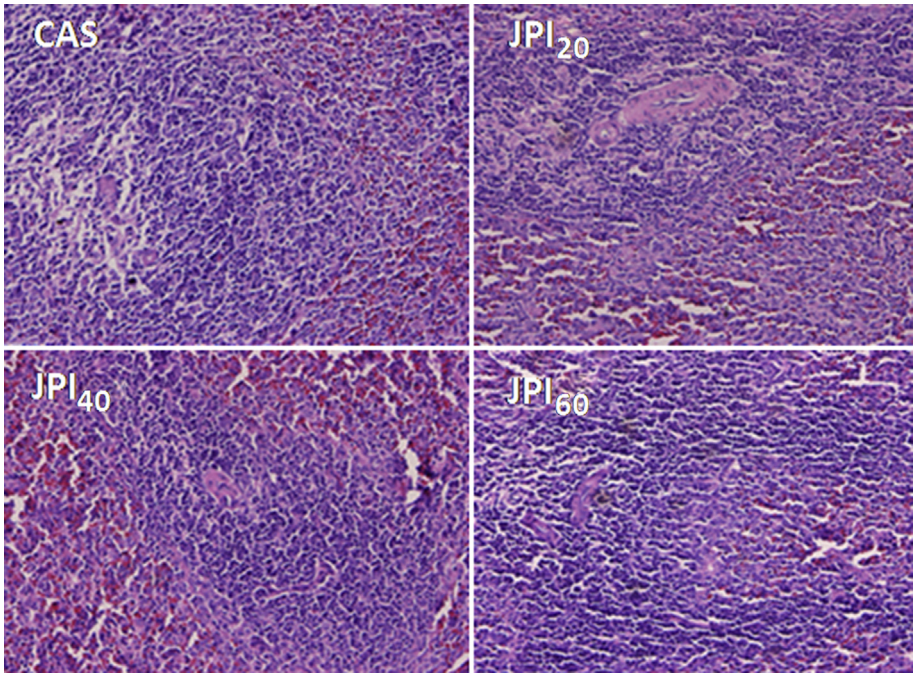

Fig. 2. Histopathological changes in the kidneys of rats fed diets containing casein or *Jatropha curcas* seed cake protein isolates (JPI) (magnification, 200×). CAS, casein diet containing casein at the level of 10% protein, substituting equivalent amount of starch in basal diet; JPI_{20}, JPI_{40} and JPI_{60}, diets containing JPI to replace the 20%, 40% and 60% protein of casein.

was deemed to have non-toxic compounds. Further work is warranted to include the detoxified JPI in animal experiments for its practice.

5. Conclusion

J. curcas seed cake protein isolates had slightly lower quality than casein. However, when less than 40% of casein protein was replaced with JPI, PER was still close to or higher than 2.0, reflecting its high quality as a feed protein source. No histopathological changes were observed in the liver and kidney by substitution of casein with JKM, suggesting innocuous nature of this protein isolate. These results indicated that JPI could be used as a protein feed source for animals at a level no more than 40% of dietary protein source.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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