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

Yinuo Zhao, Yubao Wang, Haifeng Wang, Yueming Wu, Harinder P. Makkar, Jianxin Liu

College of Animal Sciences, Zhejiang University, Hangzhou 310058, China
Institute for Animal Production in the Tropics and Subtropics (480b), University of Hohenheim, D-70593 Stuttgart, Germany

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 (JPI20, JPI40 and JPI60; 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 JPI20, JPI40 and CAS diets (P > 0.05). However, these parameters were lower in the rats fed JPI60 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 JPI40 and JPI60 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.

© 2018, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 has been optimized (Makkar et al., 2008; Wang et al., 2011). 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
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 divided into 5 groups, each group with 6 replications. Each group was supplemented with graded levels of 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.

2.2. 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).

2.3. Sample and analyses

### 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 the 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 performic acid before the acid hydrolysis.

### 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):

\[
\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein consumed (g)}}
\]

\[
\text{NPR} = \frac{(\text{Weight gain of test group} - \text{Weight loss of non-protein diet})}{\text{Protein consumed (g)}}
\]

### 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 determined.

---

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

| Item                   | Protein-free | CAS | JPI20 | JPI40 | JPI60 |
|------------------------|--------------|-----|-------|-------|-------|
| Protein-free CAS JPI   |              |     |       |       |       |
| Casein                 | 0.0          | 13.6| 10.8  | 8.1   | 5.4   |
| JPI                    | 0.0          | 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           | 4.0          | 4.0 | 4.0   | 4.0   | 4.0   |
| Vitamin mixture        | 1.0          | 1.0 | 1.0   | 1.0   | 1.0   |

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

| Item                        | JPI100 | JPI20 | JPI40 | JPI60 |
|-----------------------------|--------|-------|-------|-------|
| 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); JPI20, JPI40 and JPI60, diets containing Jatropha seed cake protein isolates to replace the 20%, 40% and 60% protein of casein.
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 the 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 JPI20 and JPI40 diets were not significantly different from those fed CAS \( (P > 0.05, \text{Table 3}) \), whereas these parameters were lower in rats fed the JPI60 diet than in those fed the CAS diet \( (P < 0.05, \text{Table 3}) \). 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.

| Item                        | Dietary treatments \(^1\) | SEM  | \( P \)-value |
|-----------------------------|--------------------------|------|---------------|
| Diet                          | CAS | JPI20 | JPI40 | JPI60 |
| Feed intake, g                | 355.3\(^a\) | 347.7\(^b\) | 319.4\(^c\) | 241.6\(^d\) | 14.29 | <0.01 |
| Protein intake, g             | 44.2\(^a\) | 42.6\(^b\) | 43.0\(^c\) | 29.2\(^d\) | 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\(^b\) | 11.4\(^c\) | 8.6\(^d\) | 0.51 | <0.01 |
| DM digestibility, %           | 90.7\(^a\) | 90.5\(^b\) | 87.5\(^c\) | 86.3\(^d\) | 1.25 | 0.07 |
| PER                          | 0.35\(^a\) | 0.30\(^b\) | 0.27\(^c\) | 0.21\(^d\) | 0.01 | <0.01 |
| NPR                          | 2.82\(^a\) | 2.43\(^b\) | 1.99\(^c\) | 1.76\(^d\) | 0.06 | <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 \( (P < 0.05) \).

3.3. Efficiency of nitrogen utilization

The rats fed diets containing JPI had significantly lower PER \( (P < 0.01) \), PER \( (P < 0.01) \) and NPR than those fed CAS \( (P < 0.01, \text{Table 3}) \). There was no significant difference \( (P > 0.05) \) in DM digestibility among CAS, JPI20 and JPI40 diet groups, but DM digestibility of JPI60 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 JPI40 and the lowest in those fed JPI60, with a significant difference between these 2 groups \( (P < 0.05, \text{Table 4}) \). 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 JPI20 and JPI40 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 JPI40 and JPI60 diets than that fed the CAS diet \( (P < 0.05, \text{Table 5}) \). 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% \( (\text{Wang et al., 2011b}) \), similar to the 89% reported by \text{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 \( (\text{Table 2}) \). 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) \( (\text{Makkar et al., 2008; Peralta-Flores et al., 2012}) \). 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 \( (\text{Peralta-Flores et al., 2012}) \). Protein solubility, water and oil binding capacities, foaming capacity and stability, and emulsion activity and stability of JPI have been reported to be good under neutral to basic pH condition \( (\text{Saetae and Suntornsuk, 2011}) \).

In the current study, there was no significant difference in feed intake among rats fed JPI20, JPI40 and CAS diets, but the weight gain rate was significantly lower in rats fed JPI20 and JPI40 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. \text{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 \text{Jatropha} meals, while feed intake of the diet containing heated \text{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 \( (\text{Angulo-Bejaranoa et al., 2008}) \) based on the EAA profile and protein digestibility analysis. The C-PER value for protein isolate from defatted JKLM \( (2.16) \) was comparable to, or higher than the values for regular animal feed ingredients, such as corn meal \( (1.1) \), wheat.
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 JPI40 and JPI60 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

Table 4
Serum characteristics of rats fed experimental diets.

| Item                   | CAS      | JPI20    | JPI40    | JPI60    | SEM  | P-value |
|------------------------|----------|----------|----------|----------|------|---------|
| Total protein, g/L     | 56.0b    | 59.0ab   | 61.2a    | 56.0b    | 1.52 | 0.07    |
| Albumin, g/L           | 30.4ab   | 31.6ab   | 32.0a    | 29.7b    | 0.72 | 0.11    |
| Alkaline phosphatase, IU/L | 345.4a  | 298.4ab  | 254.2b   | 241.2b   | 22.05| 0.02    |
| Blood urea nitrogen, mmol/L | 4.6   | 5.2      | 5.6      | 5.9      | 0.55 | 0.47    |

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

| Item       | Dietary treatments 1 | SEM  | P-value |
|------------|----------------------|------|---------|
| CAS        | JPI20                | JPI40| JPI60   |
| Liver      | 3.10d                | 3.33ab| 3.46a   | 3.53a   | 0.11 | 0.08    |
| Kidney     | 0.80     | 0.88ab  | 0.95b  | 1.04a   | 0.03 | <0.01   |
| Spleen     | 0.53a    | 0.63a   | 0.65a  | 0.71a   | 0.03 | 0.01    |
| Lung       | 0.23    | 0.24    | 0.24   | 0.24    | 0.02 | 0.92    |

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; JPI20, JPI40 and JPI60 diets containing Jatropha curcas seed cake protein isolates replacing 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.

Acknowledgements

This work was supported by the grants from Ministry of Science & Technology of China (No. 2009DFA32260), and the Bundesministe-

References

Abd El-Hack ME, Alagawany M, El-Sayed SAA, Fowler J. Influence of dietary inclusion of untreated or heat-treated *Jatropha curcas* seed cake on productive and reproductive performances and biochemical blood parameters of laying Japanese quail. Poult Sci 2017. https://doi.org/10.3382/ps/pex089 (in press).

Angulo-Bejarana PI, Verdugo-Montoya NM, Cuevas-Rodrigueza EO, Milan-Carrillo J, Mora-Escobedor R, Lopez-Valenzuelab JA, Garzon-Tiznado JA, Reyes-Moreno C. Tempelh flour from chickpea (*Cicer arietinum L.*) nutritional and physicochemical properties. Food Chem 2008;106:106–12.

AOAC. Official methods of analysis. 16th ed. Arlington, VA: Association of Official Analytical Chemists; 1995.

Devappa BK, Swamylingappa B. Biochemical and nutritional evaluation of *Jatropha curcas* protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. J Sci Food Agric 2008;88:911–9.

FAO/WHO. Protein quality evaluation. Report of the joint FAO/WHO expert consultation. FAO Food and Nutrition (Paper 52). Rome, Italy: Food and Agri-culture Organization of the United Nations (FAO); 1990.

Friedman M. Nutritional value of proteins from different food sources. J Agric Food Chem 1996;44:11–6–29.

Kumar V, Makkar HPS, Anselmgrabuer W, Becker K. Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio L.*) fingerlings fed differently detoxified *Jatropha curcas* kernel meal. Food Chem Toxicol 2010;48: 2063–72.

Makkar HPS, Becker K. Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. Plant Foods Hum Nutr 1999;53:183–92.

Makkar HPS, Becker K, Sporer F, Wink M. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J Agric Food Chem 1997;45:3152–7.

Makkar HPS, Francis G, Becker K. Protein concentrate from *Jatropha curcas* scree-pressed seed cake and toxic and antinutritional factors in protein concentra-

J. Sci Food Agr 2008;88:1542–8.

Makkar HPS, Kumar V, Becker K. Use of detoxified *jatropha* kernel meal and protein isolate in diets of farm animals. In: Makkar HPS, editor. Biofuel co-products as livestock feed: opportunities and challenges. Rome, Italy: FAO; 2012.

Peralta-Flores L, Gallegos-Tintorea S, Solorza-Feria J, Davila-Ortiz G, Chel-Guerrero L, Martinez-Ayala A. Biochemical evaluation of protein fractions from physic nut (*Jatropha curcas L.*). Grasas Aceites 2012;63(3):253–9.

Sart he D, Sundornisk W. Toxic compound, anti-nutritional factors and functional properties of protein isolated from detoxified *Jatropha curcas* seed cake. Int J Mol Sci 2011;12:66–77.

SAS Inc. SAS User’s guide: statistics. Version 6. 12th ed. Cary, NC, USA: SAS Inc.; 1996.

Sharath BS, Mohankumar BV, Somase khar D. Bio-detoxification of phorbol esters and other anti-nutrients of *Jatropha curcas* seed cake by fungal cultures using solid-state fermentation. Appl Biochem Biotechnol 2014;172(5): 2747–57.

Sharath BS, Muthukumar SP, Somase khar D. Study on utilization of detoxified *Jatropha curcas* seed cake subjected to solid state fermentation as a dietary supplement in wistar rats. Recent Pat Food Nutr Agric 2016;8(3):190–8. 
The State Food and Drug Administration of People’s Republic of China. Regulatory
guide on the techniques for drug researches. Beijing: Chinese Medical Science
and Technology Press; 2005. p. 83–93.
Wang HF, Chen Y, Zhao YN, Liu HY, Liu JX, Makkar HPS, Becker K. Effects of replacing
soybean meal by detoxified *Jatropha curcas* kernel meal in the diet of growing
pigs on their growth, serum biochemical parameters and visceral organs. Anim
Feed Sci Technol 2011a;170(1):141–6.

Wang YB, Zhao YN, Wang HF, Liu JX. Determination of conditions for extraction of
protein isolate from *Jatropha curcas* seed cake. Chin J Anim Sci 2011b;47(21):
53–5 (in Chinese with English abstract).
Wolsac A, Bressan R, Brenes R. A comparison of in vivo and in vitro estimates of
protein digestibility of native and thermally processed vegetable protein. Plant
Foods Hum Nutr 1981;31:31–43.