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Full Paper

Effects of organic and conventional rice on protein efficiency ratio and pesticide residue in rats

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Abstract: The comparative effects of organic rice and conventional rice on the protein efficiency ratio (PER) in rats were investigated by feeding 40 male Sprague-Dawley rats for four weeks with three experimental diets containing polished conventional rice (PCR), unpolished conventional rice (UCR), unpolished organic rice (UOR) and a control protein diet (casein) under standardised conditions. All diets were prepared according to AOAC guidelines. The results showed no statistically significant difference ($P > 0.05$) among the values of PER (2.75 ± 0.14 - 2.80 ± 0.09) in rats fed with diets containing PCR, UCR or UOR. Similar growth was also observed among the three groups fed with different experimental diets. Additionally, residues of pesticides, viz. carbofuran, methyl parathion, p-nitrophenol and β-cyfluthrin, in rat blood and rice samples were determined using liquid chromatography–electrospray ionisation tandem mass spectrometry. Pesticide residues were not detected in all serum samples of experimental rats and only p-nitrophenol was found (8.23 ± 0.65 - 12.84 ± 2.58 mg/kg) in all samples of the cooked rice diets, indicating that organic rice produced similar effect as conventional rice on PER and growth in rats.

Keywords: organic rice, conventional rice, protein efficiency ratio, pesticide residue

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INTRODUCTION

Rice (*Oryza sativa*) is the main food used by half of the world's population [1]. It is one of the most important cereal crops widely used in human nutrition as a source of energy due to its high starch content (approximately 90% in polished white grains) [2]. Total starch between 72-82% has been observed in brown rice grains of six cultivars grown in Philippines [3]. However, the level of this constituent can vary among grains of different varieties due to genetic and environmental factors. Furthermore, the rate and extent of starch digestion can be influenced by various factors including amylose/amylopectin ratio, grain processing, physicochemical properties (particularly gelatinisation characteristics), particle size and presence of lipid-amylose complexes [4].

Organic farming is a form of agriculture that relies on crop rotation, green manure, compost, biological pest control, and mechanical cultivation to maintain soil productivity and to control pests. The method excludes or strictly limits the use of synthetic fertilisers, synthetic pesticides, plant growth regulators, livestock feed additives and genetically modified organisms. Since 1990, the market for organic products has grown at a rapid pace, averaging 20-25 % annually to reach US$ 33 billion in 2005. This demand has driven a similar increase in organically managed farmland. Approximately 306,000 square kilometers (30.6 million hectares) worldwide are now farmed organically, representing approximately 2% of the world's total farmland. In addition, as of 2005, organic wild products were farmed on approximately 62 million hectares [5]. As for rice, only specially selected high-quality Jasmine rice is planted organically on a very limited area, although this type of agricultural practice is becoming more widespread as the number of health-conscious consumers is growing rapidly [6].

The protein quality of roasted wheat, maize and rice has been studied in feeding trials with rats. The protein efficiency ratio (PER), true digestibility, biological value and net protein utilisation decrease significantly with roasting. Relative nitrogen utilisation also decreases on roasting but the effect is greater with rice and maize than with wheat [7]. Kaur and Sekhon [8] studied in albino rats for 28 days using a reference casein diet, a raw rice diet and diet based on pressure parboiled rice as well as traditionally parboiled rice. The PER did not vary significantly among different groups but was highest for rats fed on the casein diet, followed by raw rice, pressure parboiled rice and traditionally parboiled rice diets respectively. Growth study and nutritional evaluation of 'Basmati-370', 'Pusa Basmati-1' and 'Haryana Basmati-1' have also been carried out using rats. The feed efficiency ratio and PER values did not differ among the rice diets. 'Pusa Basmati-1' had better apparent protein digestibility, true protein digestibility, biological value, net protein utilisation, utilisable protein and liver enzymes level than the other varieties [9].

Lam-Sonhez et al. [10] measured the protein content of the rice grain and found that it decreases from the outer to the inner layers of the grain. When weaning male Wistar rats were fed on diets with 7.9% protein supplied by different parts of the grain, the residue, which was mainly the central part of the endosperm (protein content 8.54 g/100 g), had a PER of 1.68, compared with 1.57 and 1.04 for the outer layers. This may be due to the presence of grain embryo in the grain residue. When the embryo was detached from the grain at the beginning of processing, there was a decrease in the nutritional quality of the grain. However, there has been no previous report on the

PER of organic rice compared with conventional rice, so one of the objectives of the present study was to compare the PER of organic rice with that of conventional rice.

Since organic rice is grown and processed without the use of any synthetic chemicals whereas conventional rice farming involves the use of synthetic chemicals such as fertilisers, insecticides, pesticides and herbicides. Consequently rats fed with organic rice should have better growth and contain less toxic chemicals in their blood than those fed with conventional rice. The objectives of this study are thus extended to test the above hypotheses.

MATERIALS AND METHODS

Preparation of Powdered Steamed Rice and Experimental Diets

One type of unpolished organic rice supplied by Progressive Farmer Association, Ubol Ratchathani province and two types of polished and unpolished conventional rice supplied by Ubol Agri Coop Federation Ltd., Ubol Ratchathani province were weighed, washed, steamed for 30 min., dried in an oven at 70°C for 7 hours and then powdered using a pin mill.

The above three kinds of rice: unpolished organic rice, polished conventional rice and unpolished conventional rice, and casein were used for the preparation of experimental diets by AOAC methods [11]. The diets consisted of 10 ± 0.3% protein, 8% soy oil, 5% mineral mixture (Table 1), 1% vitamin mixture (Table 2), 1% cellulose, 5% moisture, 35% sucrose and 35% corn starch. The composition of the experimental diets is shown in Table 3. Proximate analysis of rice and experimental diets were determined by AOAC methods [12].

Animals

Three-week old weanling male Sprague-Dawley rats were obtained from the National Laboratory Animal Centre, Mahidol University, Thailand. The rats with a mean initial weight of 50 - 60 g were used. They were divided randomly into four groups of 10 rats (one control group and three test groups). All rats were housed in individual stainless steel metabolic cages in an experimental controlled environment at 20-22°C, 60% relative humidity and 12-hours light-dark cycle and were given free access to the diet and water for a 28-day feeding period. Daily food intake and weekly body weight were recorded. The experimental protocol was developed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Institute of Food Research and Product Development, Kasetsart University.

Table 1. Composition of mineral mixture [11]

Mineral	g/kg
NaCl	139.300
KI	0.790
KH_2PO_4	389.000
Mg SO_4 anhydrous	57.300
$CaCO_3$	381.400
$FeSO_4 .7H_2O$	27.000
$MnSO_4 .H_2O$	4.010
$ZnSO_4 .7H_2O$	0.548
$CuSO_4 .5H_2O$	0.477
$CoCl_2 .6H_2O$	0.023

Table 2. Composition of vitamin mixture [11]

Vitamin	mg / 100 g ration
Vitamin A	2000 (IU)
Vitamin D	200 (IU)
Vitamin E	10 (IU)
Menadione	0.500
Choline	200.000
p-Aminobenzoic acid	10.000
Inositol	10.000
Niacin	4.000
Ca D-pantothenate	4.000
Riboflavin	0.800
Thiamine. HCl	0.500
Pyridoxine. HCl	0.500
Folic acid	0.200
Biotin	0.040
Vitamin B_{12}	0.003
Glucose, to make	1000.000

Table 3. Composition (g) of four experimental diets of 10 kg [11]

	Cooked polished conventional rice	Cooked unpolished conventional rice	Cooked unpolished organic rice	Casein
Rice	7002.80	6337.14	6693.44	-
Casein	614.63	614.63	614.63	1229.20
Cellulose	95.08	88.48	91.44	97.98
Vitamin mixture	100.00	100.00	100.00	100.00
Mineral mixture	495.70	488.65	489.42	495.14
Soy oil	728.00	771.58	771.02	798.77
Water	443.08	460.03	459.68	487.14
Corn starch	260.36	569.75	390.19	3395.89
Sugar	260.35	569.74	390.18	3395.88

Determination of Pesticide Residues in Rice and Rat Serum

Reagents

Carbofuran (99.5%) and ß-cyfluthrin were obtained from Dr. Ehrenstorfer GmbH, Germany. Methyl parathion (99.6%), p-nitrophenol (> 99.5%) and triphenyl phosphate (98%) were obtained from Chemical Service, Fluka and APS Ajax Fine Chemicals respectively.

High purity water was obtained from a Maxima Water Purification System (USF-Elga, High Wycombe, Bucks, U.K.). Standard stock solutions (1000 µg/mL in methanol) of p-nitrophenol, carbofuran, methyl parathion and ß-cyfluthrin were prepared and kept in amber bottles at 4ºC. Working solutions were diluted appropriately with methanol as required.

Separation was performed by liquid chromatography (LC) using an Agilent 1100 LC binary pump (Agilent Technology, Waldbronn, Germany) equipped with a 1100 Agilent autosampler. Lichrosphere RP18 (4.0 x 125 mm, 5µm, Merck) was used as a column. The eluents A and B were methanol and 1 mM ammonium acetate respectively. The gradient program started with 40% methanol and increased linearly to 60% over 1 min., to 75% over 2 min., to 85% over 2 min. and to 100% over 5 min. The column was equilibrated for 10 min. prior to the next injection. The injection volume was 20 µL.

Mass spectrometric measurement was performed using an Applied Biosystem API 2000 triple-quadrupole instrument equipped with an electrospray ionisation interface (ESI). Analyst software (version 1.1) from Applied Biosystem was used for system control and data acquisition. The mass analyser, the first quadrupole (Q_1) and the last quadrupole (Q_3) were operated at unit resolution. The optimal parameters for MS/MS are summarised in Table 4.

Table 4. Optimised parameters and multi-reaction monitoring (MRM) transition for tested compounds

Period	Compound	Mode	MRM	Source paramerter						Compound parameter					
				CUR	CAD	IS	TEM	GS1	GS2	DP	FP	EP	CEP	CE	CXP
1	p-Nitrophenol	NI	137.9/92	13.8	2.8	-4200	350	41.4	27.6	-66	-130	7.5	-8	-23	-8
			137.9/107.9											-17	-5
2	Carbofuran	PI	222.2/165	17.2	2.8	5500	350	37.9	31.0	16	360	-11	16	29	12
			222.2/123.1											17	15
3	Methyl	NI	247.8/137.9	13.8	2.8	-4300	350	41.4	24.1	-21	-350	4.5	-14	-26	-14
	parathion		247.8/123.1											-26	-38
4	Triphenyl	PI	327.22/153	13.8	2.8	5000	350	24.1	31.0	56	320	-11.5	16	36	25
	phosphate		327.22/215											35	29
5	β-Cyfluthrin	NI	432.1/405	13.8	1.4	-4200	350	41.4	27.6	-6	-320	6.5	-24	-6.5	-25
			434.1/407											-7.5	-25

Mode : NI = negative ionisation mode, PI = positive ionisation mode

Source parameters : CUR (N/cm^2) = curtain gas; CAD (N/cm^2) = collision gas; IS(V) = ionspray voltage; TEM = temperature (°C); GS1 (N/cm^2) = source gas 1; GS2 (N/cm^2) = source gas 2

Compound parameters : DP(V) = declustering potential; FP(V) = focusing potential; EP(V) = entrance potential; CEP(V) = collision cell entrance potential; CE (V) = collision energy; CXP(V) = collision cell exit

Determination of p-Nitrophenol, Carbofuran, Methyl Parathion and ß-Cyfluthrin in Rice and Plasma

The extraction process for rice was a modification of that developed by Hirahara et al. [13]. An aliquot of steamed rice in powder form (2 ± 0.02 g) was placed in a conical flask and 0.5 mL of 20 mg/kg (0.0020%) triphenyl phosphate (internal standard) [14, 15] was spiked in the sample prior to the addition of 20 mL of methanol-acetone (1:1). The mixture was vortex-mixed for 1 min. and then ultrasonicated for 15 min. The process was repeated once. The combined extract was evaporated in a rotary evaporator and dried under a gentle stream of nitrogen. The residue was redissolved in 500 µL of methanol and filtered through a 0.2 µm nylon membrane prior to injection into LC-ESI-MS/MS.

The extraction process for plasma was a modification of that developed by Zhang et al. [16]. An aliquot of 50 µL of rat plasma sample was mixed with 220 µL of 45 ppb triphenyl phosphate (internal standard) in a 1.5 mL microcentrifuge tube. The solution was vortex-mixed for 2 min. and centrifuged at 13,400 rpm, 4°C for 10 min. The clear supernatant was removed and diluted to 500 µL with methanol and filtered through a 0.2 µm nylon membrane filter prior to injection into LC-ESI-MS/MS.

Determination of Percentage Recovery of Pesticides and Statistical Analysis

An experiment was performed to determine the percentage recovery of pesticides by the method employed. Serum sample from rats fed with unpolished conventional rice diet and another sample of polished conventional rice diet were taken as representatives for evaluation.

Data were analysed statistically using analysis of variance (ANOVA) and Duncan's new multiple range test. A value of $P < 0.05$ was considered significant [17].

RESULTS AND DISCUSSION

Proximate Analysis of Conventional Rice, Organic Rice and Experimental Diets

The results of proximate analysis of conventional rice, organic rice and experimental diets is shown in Tables 5 and 6. The protein, fat, ash, crude fibre and carbohydrate contents of raw rice were lower than those of the cooked rice, except the fat content of raw polished conventional rice, which was higher than that of the cooked polished conventional rice. The moisture contents of all raw rice types were higher than those of the cooked rice samples because after being steamed, the cooked rice was heated to dry in an oven. The loss of water through evaporation also caused the protein, fat, ash, crude fibre and carbohydrate contents of the cooked rice to be higher than those of the raw rice. The fat content of the polished conventional rice was the lowest because it had been polished. Separate samples of the raw polished and unpolished conventional rice, unpolished organic rice were steamed, dried and powdered. Using the AOAC method [11], three experimental diets were prepared from the three types of cooked powdered rice, i.e. Diet 1- from cooked polished conventional rice, Diet 2- from cooked unpolished conventional rice, Diet 3- from cooked unpolished organic rice and Diet 4 (control)- casein diet containing 10 ± 0.3 % protein. Proximate analysis of Diets 1-4 is shown in Table 6. The protein contents of the experimental diets and control casein diet were 9.05 - 9.45% wet weight or 9.59 - 10.14% on a dry weight basis at 5% moisture. The level of total dietary fibre in Diet 4 was the lowest (1.89%) because this control diet was composed of casein that lacked fibre found in the rice.

Table 5. Proximate analysis of conventional and organic rice (g/100 g wet weight)

Rice	Protein	Moisture	Fat	Ash	Crude fibre	Carbohy-drate
Raw rice						
Polished conventional rice	6.33	11.54	1.16	0.23	0.31	80.43
Unpolished conventional rice	7.08	11.13	3.20	1.11	1.11	76.37
Unpolished organic rice	6.79	11.97	3.37	1.00	1.09	75.78
Cooked rice						
Polished conventional rice	7.14	7.22	1.02	0.27	0.56	83.79
Unpolished conventional rice	7.89	5.30	4.39	1.41	1.66	79.35
Unpolished organic rice	7.47	5.07	4.24	1.22	1.13	80.87

Table 6. Proximate analysis of experimental diets (g/100 g wet weight)

Experimental Diet	Protein	Moisture	Fat	Ash	Total dietary fibre
Diet 1	9.42	7.17	7.87	4.49	5.32
Diet 2	9.44	6.56	9.22	4.94	4.17
Diet 3	9.45	6.38	9.94	5.08	4.20
Diet 4	9.05	5.71	5.71	4.16	1.89

Growth of Rats Fed with and PER of Conventional and Organic Rice Diets

The final body weight of experimental rats fed with experimental diets composed of organic rice and the corrected PER were not significantly different from those fed with experimental diets from conventional rice (Table 7). PER of sample is calculated from weight gained of test animal (g) divided by protein consumed (g). Corrected PER is calculated from PER of test sample multiplied by 2.50 and divided by PER of casein. PER shown in the study was corrected PER, which were standardises to PER of 2.50 for casein (as standard) to eliminate laboratory variation [18]. Organic rice thus produced similar effect on PER and the growth rate of rats compared with conventional rice. Interestingly, the final body weight of and PER obtained from experimental rats fed with the diets composed of conventional and organic rice were higher than those fed with control casein diet, although the amount of casein in conventional and organic rice diets (614.63 g) were lower than that in the control casein diet (1229.20 g) (Table 3), indicating that the quality of protein in the three experimental rice diets was better than that of the casein diet. High quality protein in the rice diet thus gave rise to higher PER and consequent higher growth rate of the experimental rats.

Table 7. Initial body weight (IBW), final body weight (FBW) and corrected PER of experimental rats fed with four experimental diets

Diets	IBW (g)	FBW (g)	Corrected PER
Diet 1	66.83 ± 1.57^{a}	196.07 ± 9.55^{a}	2.76 ± 0.10^{a}
Diet 2	66.57 ± 1.18^{a}	190.66 ± 12.89^{a}	2.75 ± 0.14^{a}
Diet 3	66.45 ± 2.85^{a}	189.43 ± 11.79^{a}	2.80 ± 0.09^{a}
Diet 4	66.62 ± 2.89^{a}	157.60 ± 9.19$^{b.}$	2.50 ± 0.12^{b}

Note: Values are means ± standard deviation, N = 10.
Values in the same column with different superscript letters are significantly different at $P < 0.05$.

This study illustrated that both organic and conventional rice diets could produce similar PER and growth rate in rats. The nutritional value of rice was not affected by the farming systems [19, 20] and organic farming can produce similar, if not better, rice quality as that obtained from conventional farming.

Pesticide Residues in Rice and Serum of Rats

In the determination of p-nitrophenol, carbofuran, methyl parathion and ß-cyfluthrin in rice and in serum samples, multireaction monitoring was used in the detection process, as this method is more selective compared with other techniques such as diode array detection, thereby simplifying the sample preparation [16]. A typical total ion chromatogram is shown in Figure 1. The detection of the compounds was divided into four periods to improve the sensitivity of the technique. The regression data of the compounds are presented in Table 8. Internal standard calibration was performed using triphenyl phosphate. The ratio of the peak area of analyte to that of triphenyl phosphate was plotted against the concentration of analyte. Good linear calibration curves were obtained for all compounds with correlation coefficients better than 0.99. The limit of detection (LOD) of each analyte was defined as the concentration giving a signal to noise ratio of 3.

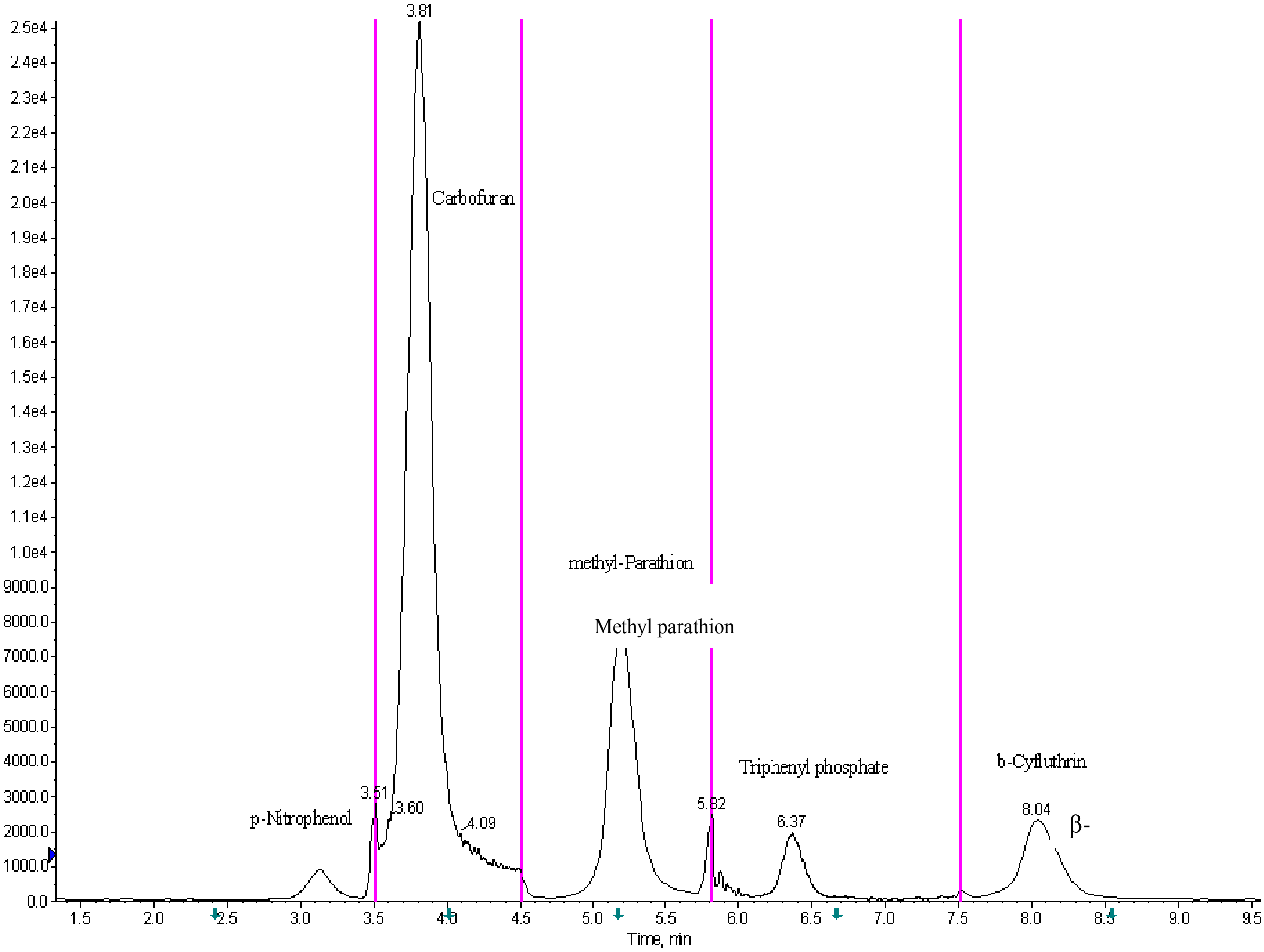

Figure 1. Typical total ion chromatogram of pesticide residues

Table 8. Regression data and detection limit of the tested compounds

Pesticide	Period	t_r *(min)	Linear range (µg/L)	R^2**	LOD*** (µg/L)
p-Nitrophenol	1	3.10	0.80 - 8.00	0.9905	1.162
Carbofuran	2	3.85	200 - 10000	0.9894	2.824
Methyl parathion	3	5.21	490 - 4920	0.9999	443.5
β-Cyfluthrin	4	8.19	1 - 20	0.9973	561.9

*Retention time, **Correlation-coefficients, ***Limit of detection

Results from LC-ESI-MS/MS indicated that, p-nitrophenol, carbofuran, methyl parathion and β-cyfluthrin were not present in any serum sample of the experimental rats. In the rice samples, only p-nitrophenol (a metabolite of methyl parathion) was detected in all samples of the cooked rice diets (Table 9). The presence of p-nitrophenol in organic rice sample might be due to contamination of nitroaromatic compounds and pesticides in the drinking water source used [21, 22]. Another interesting finding from the present study was that although p-nitrophenol was found in all samples of the cooked rice diets (Table 9), it was not detected in any serum sample of the rats. This might reflect the ability of the animal to metabolise or excrete the residue of p-nitrophenol contaminated in the cooked rice diets. Future study should focus on the effect of organic rice on the health aspects of humans.

Table 9. Concentration (mg/kg) of p-nitrophenol, carbofuran, methyl parathion and β-cyfluthrin in cooked conventional and organic rice diets

Cooked rice diet	p-Nitrophenol	Carbofuran	Methyl parathion	β-Cyfluthrin
Polished conventional rice	12.84 ± 2.58	nd*	nd	nd
Unpolished conventional rice	8.23 ± 0.65	nd	nd	nd
Unpolished organic rice	10.13 ± 0.26	nd	nd	nd
Casein diet	4.28 ± 0.66	nd	nd	nd

* not detectable

Percentage Recovery of Pesticides in Serum and Rice Diets

Percentage recoveries of carbofuran and methyl parathion were found to be high in both samples. However, variations between samples were rather high for p-nitrophenol and β-cyfluthrin (Table 10). The recovery of each analyte was calculated from the peak area of analyte in spiked rice and serum sample, compared to expected concentration based on the combination of spiked and original amounts present in the sample.

Table 10. Percentage recoveries of pesticides in serum samples and polished conventional rice diet

Pesticide	Serum sample*	Polished conventional rice diet
p-Nitrophenol	68.82 ± 2.14	119.46 ± 0.81
Carbofuran	94.62 ± 5.69	99.09 ± 7.75
Methyl parathion	86.92 ± 0.03	82.48 ± 4.45
β-Cyfluthrin	101.03 ± 4.25	43.21 ± 12.18

* obtained from rats fed with the unpolished conventional rice diet

CONCLUSIONS

Apart from providing information that may be used in the production of low-cost novel food products with sufficiently high PER values from mixtures of animal and rice proteins, this study also shows that the effect of organic rice on the growth of rat is not significantly different from that of conventional rice and that pesticide residues found in the rice samples are not detected in the serum of rats fed with either conventional or organic rice diets. Another practical benefit is that it may be used as one of the factors in the compilation of supporting data in planning for a national or international organic rice policy.

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