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Quality Assessment of Rice Industry By-Products as Ingredients of Animal Diets Based on Nutrient Content, Undesirable Substances and Hygienic Parameters

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Abstract: Various parameters were assessed in samples of 2 rice mill by-products, namely broken rice and rice bran, taken from 4 rice mill establishments in Western Greece, for their characterization as feed ingredients. The analyses (152 in total), based on European Union (EU) methods, included determination of major nutrients, ash insoluble in HCl, trace elements, heavy metals, aflatoxin B_1 and microbial load. Mean values of crude protein (152), crude fibre (70.3), ash (82.7) and especially fat (155 g kg^{-1} on dry matter basis), revealed that the assumed rice bran was not actually a brown or white bran, but rice pollards, i.e. a mixture of bran and polishing. Presence of Pb, Cd and aflatoxin B_1 was not detected in any of the broken rice or bran samples. Average values for enumeration of total bacterial counts were 4.65 and 5.67: whereas that of yeasts/moulds were 2.73 and 4.13 log cfu $g^{-1}$for broken rice and bran, respectively, being below permitted levels for broken rice, but raising concern about storage of rice bran. Information from this study aims at contributing to measures taken by the State to cope with EU legislation on food and feed safety, developing traceability procedures and establishing HACCP system as well as GMP programme for Greece and elsewhere.

Key words: Rice mill by-products, nutrients, trace elements, heavy metals, aflatoxin B_1, microbial load

INTRODUCTION

Rice (*Oryza sativa* L.), together with wheat and maize, is one of the most important cereal crops of the world and forms a large part of human diet, especially that of oriental people. In addition, processing of rice grains leaves considerable amounts of useful by-products, which are utilised as animal feeds (Morrison, 1975; Gohl, 1981). However, feedstuffs are not only a source of energy and nutrients (Coleman and Moore, 2003), but also can influence the quality of food in a variety of ways, through the presence of undesirable substances they may contain. Thus, safety of food of animal origin and feed safety are closely linked and interdependent (Flachowski and Danicke, 2005). After the food crises of the second half of the 90's, EU developed Regulation 178/2002 (EU, 2002a) which raised animal feed, in terms of safety, to the same level with that of food for humans. Among others, this Regulation, introduced the concept of traceability in food chain issues. In addition, EU Regulation on Feed Hygiene requires that feed manufacturers should plan, apply and maintain permanent procedures based on the principles of HACCP (Hazard Analysis and Critical Control Points) to meet quality standards (EU, 2005).

Rice is also an important crop for Greece. Twenty rice mills operate in Greek territory, four of them in western Greece, where the School's Laboratory is located. Due to initial claims against low performance of animals fed rations containing appreciable amounts of rice by-products as one of their ingredients, samples of broken rice and rice bran were taken from all four rice-mill establishments in the area. The aim was to investigate quality by analysing samples according to EU official methods, for nutrient content, presence of undesirable substances and hygienic parameters. This information will help to meet traceability needs and plan GMP (Good Manufacturing Practice) as prerequisite to the establishment of HACCP system for animal production sector in Greece but also other parts of the world.

MATERIALS AND METHODS

Feed ingredients: Broken rice and rice bran samples were taken from all 4 large-scale rice mill establishments in

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Western Greece (A, B, C and D). The rough rice (paddy) in the area, after being harvested and threshed is transported to rice-mills for processing into white (polished) rice through a series of operations that free it from the hull, germ and bran, which are sold as by-products. Finally, the mixture of whole and broken rice from the polishers is separated and the former is sold as food for humans, whereas the latter is sold as a by-product for animal feed. The average percentage of rice milling by-products in the area amounts up to 40% of the initial weight of paddy rice to be milled. Representative samples of broken rice and rice bran were obtained from the four rice-mill establishments according to official Community methods for sampling animal feed ingredients (EEC, 1976a).

Chemical analyses: Community methods of analysis were employed for the determination of constituents of broken rice and rice bran. Moisture was determined by heating samples at 105°C to constant weight, crude protein by the Kjeldahl method (NX6.25), fat by the Soxhlet technique and the concentration of P in solution, was determined spectrophotometrically as the coloured complex which is formed with molybdovanadate reagent (EEC, 1971a). Ash was determined by ignition at 550°C. To measure ash insoluble in HCl, the sample was ashed, the ash was boiled with HCl, the solution filtered and the residue ashed and weighed, whereas Ca was determined volumetrically (EEC, 1971b). For crude fibre determination, the sample was treated successively with boiling weak solutions of H_2SO_4 and KOH, whereas Mg and trace elements were measured by atomic absorption (EEC, 1978). Alfatoxin B_1 was determined by Thin Layer Chromatography (TLC) (EEC, 1976b). Heavy metals Pb and Cd were measured by atomic absorption method (ADAS, 1986) using a Perkin Elmer AAS Analyst 700 instrument.

Microbiological analyses: Counting of Total Bacteria (TBC) and yeast/moulds was carried out by the pour plate method (Busta *et al*., 1984) with the following culture media: TBC in Plate Count Agar (Oxoid) and yeast/moulds in Malt Extract Agar (Oxoid), with the Petri dishes incubated at 30 and 25°C for 3 and 5 days, respectively. Analyses for each constituent and sample were carried out in triplicate.

RESULTS AND DISCUSSION

Values for nutrient content of broken rice and rice bran are given in Table 1 and 2, respectively. In appears that, in general, our results for broken rice are consistent with already published values (Morrison, 1975; McDonald *et al*., 2002). On the contrary, mean values for crude protein (152), crude fibre (703), ash (82.7) and especially fat (155 g kg^{-1} on dry matter basis) revealed that, what was assumed to be rice bran, was not actually, either the brown rice bran or the white rice bran described in EU legislation for feed materials (EU, 1996), with typical composition (Allen, 1989) but it should be characterized as rice pollards, i.e., a mixture of rice bran and polishing (Gohl, 1981). One should take into consideration the high fat content of rice bran in this study (155 g kg^{-1}), because such rice bran often turns rancid in storage conditions. Obtained values for ash insoluble in HCl were low. Ash insoluble in HCl is not a nutrient and its presence implies detection of silica due to the mixing of silica-rich rice hulls

Table 1: Values for major nutrients, trace element content in broken rice (on dry matter basis)

	Rice-mill establishment					
Nutrients	A	B	C	D	Mean	SD
Major (g kg^{-1})						
Dry matter	869	878	862	877	872	7.51
Crude protein	73.3	64.8	74.6	86.9	74.9	9.10
Crude fibre	5.1	10.7	5.8	6.8	7.10	2.50
Fat	5.1	3.4	6.0	7.8	5.58	1.83
Ash	5.8	4.3	4.6	7.8	5.63	1.59
Ca	0.1	0.1	0.1	0.06	0.09	0.02
P	1.5	1.1	1.1	1.5	1.30	0.23
Mg	1.9	1.5	1.4	2.7	1.90	0.59
Trace elements (mg kg^{-1})						
Fe	66.7	27.9	11.0	205	77.7	88.0
Zn	57.5	62.1	52.2	55.3	56.8	41.6
Mn	43.2	36.4	33.1	59.3	43.0	11.7
Cu	38.6	22.2	16.2	182	64.8	78.7
Co*	ND+	ND	ND	ND	-	-

*Detection limit for Co: 0.03 mg kg^{-1}, +ND: Not Detected

Table 2: Major nutrients, trace elements and ash insoluble in HCl content in rice bran (on dry matter basis)

	Rice-mill establishment					
Nutrients	A	B	C	D	Mean	SD
Major (g kg^{-1})						
Dry matter	895	896	884	896	893	5.85
Crude protein	147	156	143	160	152	7.85
Crude fibre	73.7	71.4	61.1	74.8	70.3	6.26
Fat	185	132	156	145	155	22.6
Ash	90.4	68.6	87.1	84.8	82.7	9.69
Ash insoluble in HCl*	0.9	3.6	3.7	3.1	2.83	1.31
Ca	0.84	0.06	1.1	1.3	0.83	0.54
P	22.3	11.2	20.5	20.1	18.5	4.98
Mg	48.3	36.9	40.7	38.4	41.1	5.06
Trace elements (mg kg^{-1})						
Fe	384	373	398	444	400	31.2
Zn	203	189	160	196	187	18.9
Mn	542	792	583	630	637	110
Cu	21.2	42.4	50.3	17.3	32.8	16.1
Co+	ND‡	ND	ND	ND	-	-

*Ash insoluble in HCl is not a nutrient. Its presence may implies fraud in terms of purity of the by-product, + Detection limit for Co: 0.03 mg kg^{-1}, ‡ND: Not Detected with rice bran; the former is not included in the so called positive list, i.e. a Community catalogue with approved feed materials to be used in compound feed manufacturing (EU, 1996). Although, EU standards for ash insoluble in HCl for rice by-products do not exist, caution should be taken because rice bran varies considerably in fibre content, depending on the proportion of hulls present and sometimes it is adulterated with hulls.

As regards presence of toxic constituents in broken rice and rice bran, only contaminants included in the EU legislation for undesirable substances were dealt with (EU, 2002b). The topic of presence of mycotoxins in foods and feeds has been extensively studied (Sangare-Tigori *et al.*, 2006; Silva *et al.*, 2007; Lenng *et al.*, 2006), for rice (Rustoun, 1997) and bran (Sassahara *et al.*, 2005) in particular. The scientific field of risk of contamination of foods with toxic substances present in animal feeds, including heavy metals, mycotoxins and pesticide residues has been recently reviewed (Kan and Meijer, 2007). Presence of Pb, Cd and aflatoxin B_1 was not detected in the present investigation, either in broken rice or in rice bran samples, since all these contaminants were below the detection limits of the methods employed. The detection limits of the quantitative assessment techniques employed in this study were 0.05, 0.01 and 0.01 mg kg^{-1} for Pb, Cd and aflatoxin B_1, respectively. In addition, Community legislation maximum permitted levels are 30, 1 and 0.02 mg kg^{-1} for Pb, Cd and aflatoxin B_1, respectively. It has been reported (Vlachou *et al.*, 2004) that, in general, aflatoxin B_1 does not seem to constitute a problem for animal feeds in Greek territory.

Counts for TBC and yeasts/moulds are shown in Table 3 and 4 for broken rice and rice bran, respectively. The implications on livestock of feed contaminated with bacteria and fungi have been recently reviewed (Maciorowski *et al.*, 2007). Counts of Table 3 and 4 were expressed in $\log_{10}$ numbers to enable us to include standard deviations as well. Although, EU standards for TBC and yeasts/moulds counts do not exist, there are informal standards that are used as working guidelines for routine agricultural practice in Greece. According to these guidelines, TBC and yeasts/moulds counts of rice mill by-products should not exceed 3×10^6 and 4×10^4 cfu gr^{-1} or if one transforms to $\log_{10}$ numbers 6.5 and 4.6 log cfu g^{-1}, respectively. All values concerning broken rice, either for TBC or for yeasts/moulds counts were below the above standards. However, the situation differed as regards yeasts/moulds counts for rice bran (Table 4). Specifically, only one factory (not the same) out of the 4, for each parameter studied, was well below the standards, 2 factories for both variables were marginally below standards, whereas one factory (the same for both cases), was higher than the standards for both variables, the situation being worse for yeasts/ moulds load. Therefore, particular care should be taken for the hygiene conditions in the rice-mill establishments, since it has been reported that *Aspergillus* Section *Flavi* has been found in dusts generated by agricultural processing facilities, including rice-mills in the Philippines (Sales and Yoshizawa, 2006). Also care should be taken in storing conditions of rice mill by-product, rice bran in particular.

Table 3: Microbial load in broken rice (log cfu g^{-1})‡

	Rice-mill establishment					
Microorganism	A	B	C	D	Mean	SD
TBC*,+	5.30	2.75	5.08	5.46	4.65	1.27
Yeasts and mould	2.20	1.78	2.36	4.59	2.73	1.26

* TBC: Total Bacterial Count (aerobic-mesophilic), +EU maximum permitted levels do not exist; informal standards as working guidelines for this feed material in Greece were set at 6.5 and 4.6 log cfu g^{-1} for TBC and yeasts/moulds, respectively, ‡cfu: colony forming units

Table 4: Microbial load in rice bran (log cfu g^{-1})‡

	Rice-mill establishment					
Microorganism	A	B	C	D	Mean	SD
TBC*,+	6.11	3.75	6.28	6.54	5.67	1.29
Yeasts and mould	3.15	4.23	4.04	5.08	4.13	0.79

*TBC: Total Bacterial Count (aerobic-mesophilic), + EU maximum permitted levels do not exist; informal standards as working guidelines for this feed material in Greece were set at 6.5 and 4.6 log cfu g^{-1} for TBC and yeasts/moulds, respectively, ‡ cfu: colony forming units

CONCLUSION

The approach employed in this investigation for the characterization of rice industry by-products in Western Greece, revealed that, although broken rice had a typical composition, the product assumed to be rice bran, was in fact rice pollards, in other words, a mixture of rice bran with rice polishing. Samples from all factories and for both by-products were free of heavy metals and aflatoxin B_1. Finally, the bacteria and yeasts/moulds count study, revealed that, although samples of broken rice taken from all four establishments were below standards for both variables measured, on the other hand, the situation was rather worrying for rice bran, as regards TBC and especially yeasts and moulds. Special care should be taken for a sound hygienic situation, including dust, within the rice-mill establishment, as well as the storing conditions of rice mill by-products, rice bran in particular. Regarding feed hygiene, the information from the present investigation could contribute to appropriate measures taken to develop traceability procedures and to establish a HACCP system as well as GMP programme in Greece and elsewhere.

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