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Evaluation of Protein Quality of Unfermented and Fermented Blends of Cereal Based Complementary Food Using Rats

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Abstract: Weanling male rats of 45-55 g were used to compare the protein quality of the unfermented and fermented blends of cereal based complementary food. Paddy rice, parboiled rice, soybean and crayfish were obtained from Jos main market, Nigeria. The paddy rice malted for 72 h and all the foodstuffs were processed into flours. Parboiled rice and soybean mix was formulated in a standard ratio of 70:30 g (PR:DSB_O). A modified standard formulation of parboiled rice, soybean, malted rice and crayfish mix in the ratio of 65:25:5:5 g (PR:DSB:MR_{72}:CFo) was made. From the formulation fermentation of different blends at varying periods 24, 48, 72, 96 and 120 h was carried out. Protein content of the unfermented and fermented blends was determined by the standard assay technique. Seven different diets were fed. Five rats were assigned to each diet for 28-days growth studies and a 7-day N balance period. The rats fed the unfermented modified standard (PR:DSB:MR_{72}:CFo) blend had the highest food intake, weight gain, N intake, retained N, BV, and NPU values (69 g, 27 g, 2.96 g, 2.23 g, 83.5 and 75.47, respectively) than the rest of the groups. The result appeared to suggest that fermentation affected the protein quality of the fermented blends negatively making the protein quality of the unfermented modified standard (PR:DSB:MR_{72}:CFo) blend the best.

Key words: Fermentation, malting, rats complementary

INTRODUCTION

Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods (Parveen and Hafiz, 2003). In opinion of many consumers, fermentation of foods makes the nutrients more readily available than the unfermented. There is quite a lot of evidence suggesting that fermentation enhanced micronutrients and amino acids (Odumodu, 2007; Odumodu, 2008, respectively) than the unfermented foods. However, there are almost no published papers on the animal feeding trials comparing nutrients content of unfermented and fermented foods.

The major objective of this study was to determine the protein quality of the unfermented and fermented blends of the cereal based complementary food.

MATERIALS AND METHODS

A 28 day study was conducted. It consisted of a 21 day growth period, and a 7 day Nitrogen (N) balance period.

Animal and housing: Thirty five male albino weanling rats (supplied by the Animal House, University of Jos), (45-55 g) were divided into seven groups of five rats each on the basis of body weight. The rats were weighed prior to access to the test diets and at weekly intervals to determine weight gain. The animals were housed in individual metabolism cages and fed the diets and tap water ad libitum for 28 days. The cages were of the stainless steel and screen-bottom type equipped to separate urine and feces (Waham ann Laboratory Animal Cages, Timonium, MD 21093, USA).

Diets: Paddy and parboiled rice (*Oryza-sativa)*, soybean (*Glycine max L*) and crayfish (*Astacus fluviatilis)* were purchased from Jos Main Market. The grains and crayfish were manually cleaned to remove foreign materials.

Production of Amylase Rich Flour (ARF): Amylase rich flour was produced by washing 1 kg raw rice grain in 5% (w/v) sodium chloride to prevent growth of mould. It was soaked in tap water in a ratio of 1:3 (w/v), grains to water for 12 h. The grains were spread on a wet jute bag in a basket, covered with a moist muslin cloth and allowed to germinate for 72 h at room temperature (30±3°C). The grains were watered at regular intervals of 12 h. The germinated grains were dried at 80°C for 24 h, devegetated by rubbing between palms, winnowed and dehulled mechanically. The malt was milled in a laboratory hammer mill to fine flour (300 µm mesh screen) and packaged in a low density name labeled polyethylene bag. It was placed in a plastic bucket with a lid and stored in a deep freezer (-18°C) for subsequent use.

Production of soybean, parboiled rice and crayfish: One kilogram of raw soybean was placed in 20 litres of boiling water containing 50.0 g sodium bicarbonate. The soybean was boiled for 10 min and the water drained off.

It was dried in the oven at 80°C for 24 h and dehulled mechanically using laboratory hammer mill.

Five kilograms parboiled rice was washed in tap water and allowed to drain water. It was dried in the oven at 80°C for 24 h. One kilogram of crayfish was measured, dried in an oven at 95°C for 50 min.

Each of the foodstuffs was separately milled in a laboratory hammer mill into a fine flour (300 micrometer mesh screen) and packaged separately in a low density name labeled polyethylene bag. The bags were placed in plastic buckets with covers and stored in a deep freezer at -18°C for analysis.

Formulation of rice-soybean mix 70:30 g ratio (FAO/WHO/UNU, 1985): Rice-soybean mix (70:30 g w/w) was formulated and thoroughly mixed using a laboratory hammer mill to ensure evenness. It was packed in a low density name labeled polyethylene bag and stored in a deep freezer (-18°C) for analysis.

Dough preparation using parboiled rice, dehulled soybean, malted rice and crayfish mix (65:25:5:5 g w/w ratio) fermented at varying periods: A blend of parboiled rice (65 g), soybean (25 g), malted rice (5 g) and crayfish (5 g) flour was prepared and divided into six equal parts. Five parts were fermented for 24, 48, 72, 96 and 120 h, respectively, after mixing with tap water to form a dough by bringing the moisture content to 50%. The other part served as a control. At the end of each fermentation time the blend was taken out and dried at 80°C in the oven for 24 h. The dried blend was remilled in a laboratory mill to fine flour and packaged in low density name labeled polyethylene bag. The bags were placed in plastic buckets with covers and stored in the deep freezer (-18°C) for analysis.

Laboratory analysis: Carmine red was fed on the morning of day 21 and day 28. Coloured feces appeared beginning on days 22 and 29. The coloured feces excreted on day 22 were included in the pool fecal sample and those excreted on day 29 were excluded. Urine was collected from 7.00am of day 22 through the morning of day 29 (7days) and food consumption was recorded for the same 7 day period. Hydrochloric acid (0.1 N) (0.5 ml) was used as a preservative to the pooled urine samples for each group. The urine samples were made to a volume of 200 ml with distilled water and refrigerated until analyzed for nitrogen. Individual fecal collection was dried at 85°C for 3 h, weighed and ground into a fine powder.

Statistical analysis: All data collected were statistically analyzed using analysis of variance and Duncan's new multiple range test as described by Steel and Torrie (1960). Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

The crude protein contents of the blends used in this study as sources of N were as follows; PR:DSBo 20.81 g; PR:DSB:MR_{72}:CFo 21.78 g; PR:DSB:MR_{72}:CF_{24} 22.42 g; PR:DSB:MR_{72}:CF_{48} 23.23 g; PR:DSB:MR_{72}:CF_{72} 22.95 g; PR:DSB:MR_{72}:CF_{96} 22.96 g and PR:DSB:MR_{72}:CF_{120} 23.48 g. Values obtained for food intake, weight grain, N intake, fecal N, urinary N, retained N, Biological Value (BV) and Net Protein Utilization (NPU) of the rats are presented in Table 1. The PR:DSBo and the PR:DSB:MR_{72}:CFo were the unfermented standard and modified standard blends, respectively. The food intake, weight gain and N intake of the group of rats fed the PR:DSB:MR_{72}:CFo blend were highest (69.00, 27.00 and 2.96g, respectively) and that of rats fed the PR:DSB:MR_{72}:CF_{120} blend were the lowest (24.00, 6.00 and 0.90 g, respectively). There were significant differences ($p<0.05$) in food intake, weight gain and N intake values between the groups of rats fed the standard and modified standard blends. The differences could be attributed to effects of the malted rice and crayfish supplements which improved the flavour, aroma and palatability of the PR:DSB:MR_{72}:CFo blend as well as improved the essential amino acid pattern of the blend. The improved pattern of the essential amino acids was utilized by the rats for the synthesis of tissue protein. There were significant differences ($p<0.05$) in food intake, weight gain and N intake values between the group of rats fed the PR:DSB:MR_{72}:CFo blend and all the groups fed the fermented blends except for N intake values for the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{96} blends that were insignificantly different ($p>0.05$). The differences could be as a result of (a) effect of fermentation which reduced the flavour, aroma and palatability of the blends therefore decreasing the food intake of the rats. Odumodu (2008) reported that prolonged fermentation reduced the sweetness of the blends to sourness. (b) leaching of amino acids into the fermentation media as well as utilization of some of the amino acids by the micro-organisms for metabolic activities which resulted in low weight gain of the rats.

The group of rats fed the modified standard (PR:DSB:MR_{72}:CFo) blend had similar fecal nitrogen, higher urinary and retained nitrogen (0.26, 0.47 and 2.23 g, respectively) than the group fed the standard (PR:DSBo) blend (0.22, 0.34 and 0.57 g respectively). This could be as result of higher protein content (21.78 g) of the blend and food intake of the rats (69 g) which resulted in higher nitrogen retention (2.23 g). On the other hand, the groups fed the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{120} blends had similar fecal and urinary nitrogen values except that the latter had negative nitrogen value (-0.11 g). The negative nitrogen value could be due to lower food intake (24.0 g).

Table 1: Food intake, weight gain, nitrogen balance of rats fed the blends

Blend treatment	Food intake (g)	Weight gain (g)	Nitrogen intake (g)	Fecal nitrogen (g)
PR:DSBo 70:30	34.0^{d}±3.5	14.8^{d}±1.0	1.13^{c}±0.18	0.22^{a}±0.03
PR:DSB:MR_{72}:CFo 65:25:5:5	69.0^{a}±5.8	27.0^{a}±1.30	2.96^{a}±0.3	0.26^{a}±0.06
PR:DSB:MR_{72}:CF_{24} 65:25:5:5	56.2^{b}±4.1	22.1^{b}±2.4	2.66^{a}±0.2	0.29^{a}±0.04
PR:DSB:MR_{72}:CF_{48} 65:25:5:5	50.2^{c}±43.6	18.0^{c}±0.9	1.87^{b}±0.2	0.30^{a}±0.03
PR:DSB:MR_{72}:CF_{72} 65:25:5:5	29.0^{e}±2.3	10.4^{e}±0.8	1.05^{c}±0.13	0.16^{b}±0.05
PR:DSB:MR_{72}:CF_{96} 65:25:5:5	55.8^{b}±5.6	14.2^{d}±0.73	2.05^{a}±0.1	2.28^{a}±0.04
PR:DSB:MR_{72}:CF_{120} 65:25:5:5	24.0^{f}±2.7	6.0^{f}±1.5	0.90^{d}±0.2	0.35^{a}±0.06
LSD	1.2	1.56	0.23	0.17
Blend treatment	Urinary nitrogen (g)	Retained nitrogen (g)	Apparent biological value	Net protein utilization
PR:DSBo 70:30	0.34^{c}±0.08	0.57^{d}±0.18	69.61^{e}±7.0	50^{d}.44±7.3
PR:DSB:MR_{72}:CFo 65:25:5:5	0.47^{b}±0.19	2.23^{a}±0.031	83.5^{a}±10.0	75.47^{a}±6.0
PR:DSB:MR_{72}:CF_{24} 65:25:5:5	0.62^{a}±0.07	1.75^{b}±0.19	67.38^{b}±8.3	65.79^{b}±5.0
PR:DSB:MR_{72}:CF_{48} 65:25:5:5	0.36^{c}±0.01	1.21^{c}±0.18	80.35^{b}±2.7	64.11^{c}±7.1
PR:DSB:MR_{72}:CF_{72} 65:25:5:5	0.29^{d}±0.05	0.60^{d}±0.11	72.33^{d}±10.7	57.14^{c}±4.2
PR:DSB:MR_{72}:CF_{96} 65:25:5:5	0.41^{b}±0.15	1.36^{b}±0.16	76.61^{c}±15.1	66.34^{b}±3.2
PR:DSB:MR_{72}:CF_{120} 65:25:5:5	0.66^{a}±0.05	-0.11^{e}±0.14	25.46^{g}±5.0	-12.22^{c}±0.1
LSD	0.14	0.47	2.15	2.21

Values with the same superscript in the column are not significantly difference (p>0.05). Values are means±standard deviations of triplicate determinations. LSD = Least Significant Difference.

PR:DSBo 70:30 = Parboiled rice 70% and 30% dehulled soybean (unfermented)

PR:DSB:MR_{72}:CFo = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (unfermented)

PR:DSB:MR_{72}:CF_{24} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 24 h)

PR:DSB:MR_{72}:CF_{48} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 48 h)

PR:DSB:MR_{72}:CF_{72} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 72 h)

PR:DSB:MR_{72}:CF_{96} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 96 h)

PR:DSB:MR_{72}:CF_{120} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 120 h)

The BV value for the group fed the modified standard (PR:DSB:MR_{72}:CFo) blend was higher (83.5) than the value for the standard (PR:DSBo) blend (69.61). This revealed the protein of the blend to be superior than the standard blend. In the same vein the group fed the PR:DSB:MR_{72}:CF_{24} had higher BV value (80.35) than the rest of the groups fed the fermented blends. This also suggested the PR:DSB:MR_{72}:CF_{24} blend to be more superior than the rest of the fermented blends.

The group of rats fed the modified standard (PR:DSB:MR_{72}:CFo) blend had the highest NPU value (75.47). The groups fed the PR:DSB:MR_{72}:CF_{24}, the PR:DSB:MR_{72}:CF_{48} and the PR:DSB:MR_{72}:CF_{96} blends had similar NPU values (65.79, vs 64.11 vs 66.34) while the group fed the PR:DSB:MR_{72}:CF_{120} blend had a negative value (-12.22).

The higher NPU value for the group fed the PR:DSB:MR_{72}:CFo could be due to lower fecal and urinary N excretion and higher N retention. The similar NPU values for the groups fed the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{96} blends could be attributed to their comparable nitrogen retention values (1.75 vs 1.36). The negative NPU value for the group fed the PR:DSB:MR_{72}:CF_{120} blends could be as a result of low food intake and nitrogen retention.

Conclusion: The group of rats fed the PR:DSB:MR_{72}:CFo blend had higher food intake, weight gain and N intake than the rest of the groups. The higher retained N, BV and NPU values for the same group revealed the blend to have the best protein quality than the rest of the blends. However the PR:DSB:MR_{72}:CF_{24} blend fermented for 24 h was the second best.

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