

Full Length Research Paper

Nutritional studies with *Lentinus squarrosulus* (Mont.) Singer and *Psathyrella atroumbonata* Pegler: I. Animal assay

NWANZE PI^{1*}, KHAN AU², AMEH JB³ and UMOH VJ⁴

¹Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, P.M.B. 0006, Edo State, Nigeria.

²Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

³Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

⁴Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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Lentinus squarrosulus and *Psathyrella atroumbonata*, two edible indigenous mushroom species, were dried, ground and used in nutritional studies. According to the results *L. squarrosulus* and *P. atroumbonata* had significantly lower protein efficiency ratio (PER) values than the standard casein diet but gave rise to net protein retention (NPR) values which were at par, but slightly higher than that of the standard casein diet. In addition, the mean albumin and bilirubin levels were significant at $p < 0.01$ while the total serum protein level was significant at $p < 0.05$. The animals fed the protein free and *P. atroumbonata* diets produced comparable total serum protein levels that were significantly higher than the comparable total serum protein levels produced by the animals fed *L. squarrosulus* and the standard casein diet. The albumin levels produced by mice fed the protein free diet were at par with those of the *P. atroumbonata* diet, but significantly higher than the albumin levels of animals fed *L. squarrosulus* and the standard casein diets, which were similar.

Key words: *Lentinus squarrosulus*, *P. atroumbonata* protein efficiency ratio, net protein retention ratio, total serum protein, albumin, bilirubin.

INTRODUCTION

With the present high cost of meat and fish, many Nigerians are turning to mushrooms as an alternative source of protein. The proximate and amino acid composition as well as the mineral content of two indigenous mushroom species, *Lentinus squarrosulus* and *Psathyrella atroumbonata*, has been documented (Alofe, 1985; Fasidi and Kadiri, 1991; Aletor, 1995; Nwanze and Adamu, 2004a,b). The two species are highly nutritious and compare favourably with other foreign edible species.

Efforts to accurately measure protein nutritional quality started at the beginning of the 19th century. Two of the most commonly used techniques include net protein ratio (NPR) and protein efficiency ratio (PER), both of which are used to assess damage to proteins during processing and to rank foods according to their protein quality (Asiedu et al., 1994).

A limited amount of research has been carried out on the nutritive value of Nigerian mushrooms (Oke, 1966; Oso, 1977; Ogundana and Fagade, 1982; Ogbonda, 2000). Adewusi et al. (1993) however, expanded the scope of their work by using a larger number of mushrooms species as well as an increased number of parameters. Here, we evaluate the nutritional values of *L. squarrosulus* and *P. atroumbonata*.

*Corresponding authors E-mail: stonenwanze@yahoo.com.

Table 1. Composition of diets.

Components	% crude protein	Protein free diet	Casein diet	<i>P. atroumbonata</i> diet	<i>L. Squarrosulus</i> diet
Glucose	----	150.0	150.0	150.0	150.0
Corn starch	----	698.0	568.0	368.0	261.0
Casein	77.0	----	130.0	----	----
<i>P. atroumbonata</i>	30.3	----	----	330.0	----
<i>L. squarrosulus</i>	22.9	----	----	----	437.0
Rice bran	----	80.0	80.0	80.0	80.0
Vitamin mix	----	12.0	12.0	12.0	12.0
Ground nut oil	N.D.	60.0	60.0	60.0	60.0

Key: N.D.- not determined

MATERIALS AND METHODS

Feed formulation and evaluation

L. squarrosulus and *P. atroumbonata* were both dried at 45°C to a constant weight, homogenized using a Waring blender, and stored at room temperature until required. The protein quality of the two mushrooms was evaluated by the protein efficiency ratio (PER) and the net protein ratio (NPR) as described by Shlomai et al. (1992) and Eyo (2000).

Weanling male albino mice of the same age, size and weight were divided into four groups of eight animals each and housed in individual wire cages in a room at 25 ± 2°C, with light and dark periods of 12 h each (Sinha et al., 2000; Sur et al., 2001). The mice were fed a diet composed of 3 parts each of groundnut cake, soybeans and corn chaff and 1 part cassava flour for 24 h during which the animals became acclimatized to the cages. The above feed is used in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria to feed all their laboratory rats and mice. The mice were starved for 24 h and then placed on the individual diets (Table 1), which were formulated to contain the same amount of protein (100g kg⁻¹) derived from casein. The animals were provided food and water *ad libitum*. Throughout the study period, each animal was observed at least once daily for clinical signs of toxicity such as clonic movements (e.g. convulsions, tremors and muscle fasciculations), piloerection, gait abnormalities, overt respiratory abnormalities, lethargy and abnormal behaviour/appearance (rough coat) (Cifuentes et al., 2001; Tiwari et al., 2001). The amount of food consumed and the weight gained or lost by the animals were recorded at two-day intervals for a period of 10 days (Dewanji and Matai, 1996).

Analytic methods

At the end of a ten-day feeding period sixteen of the mice, four from each diet group, were anesthetized prior to decapitation (Nguyen et al., 2001; Zhu et al., 2001). Blood samples were collected in sterile vacutainer serum separation tubes and the serum samples were obtained by allowing the blood to clot prior to centrifugation (5,000 rpm x 10 min) (Swash, 1995; Male et al., 2001). The samples were analyzed for total serum protein, albumin and bilirubin as described by Halfman et al. (1976) and Gorinstein et al. (1998).

Statistics

The experimental layout was a completely randomized design (Ruiz et al., 2000). All the values obtained for the above parameters were

subjected to analysis of variance (ANOVA) and Duncan's multiple range test (Snedecor and Cochran, 1987). In addition, protein efficiency ratio and net protein ratio values were calculated and expressed as means ± standard deviation (Hwang et al., 1998).

Table 2. Results of animal assay.

Diet	PER	NPR
Casein standard	2.27±0.56	2.94±0.48
<i>L. squarrosulus</i>	-1.92 ±0.54	3.30±0.91
<i>P. atroumbonata</i>	-0.56±1.47	3.03±1.54

Data presented as means (±SD) of four determinations.

RESULTS

Results of the feeding trials are shown in Table 2. The results reveal that *L. squarrosulus* and *P. atroumbonata* had significantly lower protein efficiency ratio (PER) values than the standard casein diet. Both mushroom species gave rise to net protein retention (NPR) values which were at par, but slightly higher than that of the standard casein diet.

The results of the plasma analysis are presented in Table 3. According to the analyzed data the mean albumin and bilirubin levels were highly significant ($p < 0.01$) while the total serum protein level was significant at $p < 0.05$. The animals fed the protein free and *P. atroumbonata* diets produced comparable total serum protein levels that were significantly higher than the total serum protein produced by the animals fed *L. squarrosulus* and the standard casein diet. The albumin levels produced by mice fed the protein free diet, though at par with the *P. atroumbonata* diet, was significantly higher than the albumin levels of animals fed *L. squarrosulus* and the standard casein diets, which were similar. Animals fed with the *P. atroumbonata* diet produced significantly higher levels of bilirubin than the ones fed casein, protein free and *L. squarrosulus* diets, respectively.

Table 3. Effect of various protein diets on the plasma levels of total serum protein, albumin and bilirubin.

Diets	Total serum protein (g dl ⁻¹)	Albumin (g dl ⁻¹)	Bilirubin (mg dl ⁻¹)
casein	5.66b	3.28c	0.16b
protein free	6.36a	3.93a	0.12c
<i>L. squarrosulus</i>	5.76b	3.55bc	0.04d
<i>P. atroumbonata</i>	6.34a	3.90ab	0.23a
SE±	0.16	0.12	0.01
Significance	*	**	**

Means followed by the same letter(s) within a treatment group are not significantly different statistically at 5% level of probability using DMRT.

* and ** = significant at 5% and 1% levels, respectively.

NS = not significant.

DISCUSSION

The PER for the casein standard diet was slightly lower than that reported by Adewusi et al. (1993). The negative PER observed for *L. squarrosulus* and *P. atroumbonata* were similar to the negative PER values reported for *T. robustus* and *V. esculenta* also by Adewusi et al. (1993). However, these authors reported a positive PER value for *P. atroumbonata*. This disparity could be explained by the fact that Adewusi et al. (1993) used *P. atroumbonata* to supply 10% of the dietary protein while the present study used it to supply 100% of the dietary protein. The resultant feed which was very pungent may have caused an aversion to the olfactory organ of the animals, thus resulting in a decreased feed intake with the resulting loss of body weight (Fromentin and Nicolaidis, 1996). Since the *P. atroumbonata* had a better amino acid profile (Nwanze and Adamu, 2004a,b), the animals should have preferred it, but its smell and palatability may have taken precedence (Fromentin and Nicolaidis, 1996). It is thus apparent that the significant differences in the PER values were due to the fact that the animals fed the experimental diets lost weight while the controls (casein diet) gained weight (Goudie et al., 2002).

Both *L. squarrosulus* and *P. atroumbonata* contain phytates (Aletor, 1995). Phytic acid has an adverse effect of reducing mineral bioavailability in human and animal nutrition and also reacts directly with proteins and starch reducing their solubility and digestibility (Thompson, 1994; Oomah, et al., 1996). This could reduce the nutritional value of the proteins and minerals found in the two mushroom species. It could also explain the lower PER value obtained for *L. squarrosulus* since it contains less proteins and minerals than *P. atroumbonata* (Nwanze and Adamu, 2004a,b). However, phytic acid also has hypocholesterolemic, oxidative, anti-carcinomic and hypolipidemic effects (Thompson, 1993). As far as human consumption is concerned, however, it may be inferred that the local methods of food processing used in Nigeria minimize the concerns posed by metal chelation and protein-binding action

brought about by the phytate present in mushrooms (Osagie, 1998). In addition, the quantities observed in edible mushrooms are very minimal (Aletor, 1995).

Tannins and alkaloids were detected in both mushrooms (Alofe, 1985). Alkaloids are known to decrease food consumption in rats, thus decreasing PER while there is an inverse relationship between high tannin level and palatability, voluntary intake, digestibility and nitrogen retention in mammals (Osuntogun et al., 1987; Friedman, 1996; Silanikove et al., 1996).

The NPR values obtained for *L. squarrosulus* and *P. atroumbonata* were slightly higher than the values observed for the casein diet, suggesting that the two mushrooms could provide enough protein for maintenance. This same trend was observed by Adewusi et al. (1993) for *C. Molybditis*. However, the NPR values observed in the present study for the two mushroom species were higher than those reported for *V. esculenta*, *T. striatus* and *T. robustus*, but lower than that of *C. molybditis* and *T. robustus* (Adewusi et al., 1993). In addition, the NPR value presently reported for *P. atroumbonata* was slightly higher than previously reported by Adewusi et al. (1993).

The protein free and *P. atroumbonata* diets produced a significantly higher total serum protein level than the standard casein and *L. squarrosulus* diets. This could have been as a result of the breakdown of muscle protein through gluconeogenesis (Talwar et al., 1989; Bray et al., 1999).

The main parameters known to indicate liver disorders are the plasma levels of albumin and bilirubin (Schiff and Schiff, 1993; Guyton and Hall, 2000). The albumin levels obtained for the casein diet was slightly lower than the value reported by Shlomai et al. (1991). However, the albumin levels of the protein free, *L. squarrosulus* and *P. atroumbonata* diets were similar to the results reported for *P. blakesleeanus* by Shlomai et al. (1991).

L. squarrosulus diet produced animals with the lowest PER and bilirubin values, respectively. The low PER infers that this group of animals was the most nutritionally compromised while the significantly low levels of bilirubin observed in this group of experimental

animals may imply that an enormous number of red blood cells had already been destroyed by the time that the animals were sacrificed.

In conclusion, the amino acid, protein profile and NPR values obtained for both mushroom species is quite encouraging and suggests that they provide enough protein for maintenance. However, the low PER values obtained in this present work as well as other related works here in Nigeria might be due to problems of low palatability.

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