A nutritional evaluation of *Geotrichum candidum* grown on an industrial effluent

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The nutritional value of *Geotrichum candidum*, produced on an acid effluent from Sasol, was determined. Analysis of *G. candidum* revealed (on an air-dry basis) a crude protein content of 53.4% and a true protein content of 38.8% with a favourable amino acid composition (1.63% available lysine and 1.14% available methionine and cystine). The relative nutritive value (RNV) was found to be 0.505 ± 0.038 and the true protein digestibility (PTD) was 0.851 ± 0.057. The true metabolizable energy for poultry was 12.74 ± 0.26 MJ/kg dry matter.

Die voedingswaarde van *Geotrichum candidum*, wat op 'n suuruitvloeisel van Sasol geproduseer is, is bepaal. Die *G. candidum* het (op 'n lugdroë-basis) 'n ruproteïeninhoud van 53.4% en 'n ware-proteïeninhoud van 38.8% met 'n gunstige aminosuursamestelling gehad (1.63% beskikbare lisien en 1.14% beskikbare metionien en sistien). Die relatiewe voedingswaarde (RNV) was 0.505 ± 0.038 en die ware-proteïenverteerbaarheid (PTD) was 0.851 ± 0.057. Die ware-metaboliseerbare-energie vir pluimvee was 12.74 ± 0.26 MJ/kg droë materiaal.

Keywords: chemical composition, *Geotrichum candidum*, poultry, rats, relative nutritive value, single cell protein, true metabolizable energy, true protein digestibility.

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Introduction

The South African Coal, Oil, and Gas Corporation (Sasol) produces oil from coal. Coal gasification followed by a variation of the Fisher–Tropsch synthesis process generates an effluent containing C_2 to C_5 monocarboxylic acids as well as small amounts of alcohols, ketones and hydrocarbons. Kühn & Pretorius (1989) produced a filamentous fungus, *Geotrichum candidum*, on this effluent. It is estimated that approximately 28 000 t *G. candidum* can be produced, in a commercial production plant, per annum.

The aim of this study was to determine the nutritional value of a small sample of *G. candidum* in terms of chemical composition as well as rat and chicken bioassays. Due to the small amount of test material available, it was not possible to do feeding trails with farm animals. It was therefore decided to determine protein quality and digestibility by means of the relative nutritive value and true protein digestibility technique, using rats. Bioavailable energy was determined with the true metabolizable energy technique, using adult cockerels.

Materials and Methods

Sample preparation

G. candidum was produced in a laboratory reactor (Kühn & Pretorius 1989). Excess effluent was removed by suction through a 50-μ sieve and the fibrous mats that remained were dried in a laboratory freeze-drier (Specht Scientific mod. SS-FD-5S). The dry mats were then milled in a rotary laboratory mill through a 1-mm sieve and stored at −15 °C.

Toxicity

A separate sample of *G. candidum* was used in a trial designed to determine the presence of acute toxicity. The experimental diet contained 21% *G. candidum* instead of the 18% fish-meal that was used to produce a control diet with a protein content of 20%. Male Wistar rats, 21 days of age, were divided into two groups of 10, the one group receiving the control diet and the other group the experimental diet *ad libitum*. Weight gain was determined after 21 days. The rats were killed and subjected to a pathological examination.

Chemical composition

The chemical composition of *G. candidum* was determined by the methods given below.

Dry matter: Harris (1970).

Ash: Harris (1970).

Ether extract: Tecator method using petroleum ether recommended by Harris (1970).

Crude fibre: Fibertec system (AOAC, 1984).

Phosphorus: AOAC (1984).

Calcium: Oxalate method (AOAC, 1984).

Crude protein: Kjeldahl procedure on a Büchi system (AOAC, 1984).

True protein nitrogen: Trichloracetic acid-precipitable nitrogen as a fraction of total nitrogen (Marais & Evenwell, 1983; Faichney & White, 1983).

Nucleic acid nitrogen: Tungstic acid-precipitable nitrogen subtracted from true protein nitrogen, as a fraction of total nitrogen (Faichney & White, 1983).

Amino acid composition: Beckman system 7300 high performance analyser after 22 h acid hydrolysis (6N HCl) at 110 °C according to AOAC (1984).

Nitrogen content of rats: The entire rat was placed whole in a 2 l Erlenmeyer flask with a wide top and dried at 105 °C for 48 h, after which concentrated H_2SO_4 (800 ml) was added to the flask. The flask was heated carefully to avoid excessive foaming, while the sides were continuously washed down with concentrated H_2SO_4. Once boiling occurred without foaming, the flask was heated for another 6 h to form a colloidal suspension. Three representative aliquots of 5 ml were weighed into digestion tubes. Nitrogen content was determined according to the Technicon Auto Analyser II Industrial Method.

Protein quality

The protein quality was determined by means of a multi-point slope ratio assay as developed by Hegsted *et al.* (1968) and applied by Siebrits *et al.* (1986). Fifty-eight male Wistar rats, 27 days of age, were fasted for 24 h and divided into 10 groups of equal mass. Before the start of the experiment, a group of 10 rats (the initial slaughter group) was asphyxiated, and their protein content was determined. One group (n = 8) received a protein-free diet whilst the remaining eight groups (n = 5) were randomly allocated to the other treatments, namely diets with 2, 4, 6 or 8% protein with either lactalbumin (Sigma) or *G. candidum* as protein sources. The experimental diets (Table 1) were fed *ad libitum* from 28 to 49 days of age, whereafter the rats were fasted again for 24 h before being killed and treated similarly to the initial slaughter group. Hegsted *et al.* (1968) stated that the measurement of body protein is usually presumed to be the measurement of choice. Therefore, body nitrogen gain was chosen as the measurement of response to dietary nitrogen intake. The initial body nitrogen content of the final slaughter groups was calculated from a linear regression equation that was fitted to the data of the initial slaughter group. The response was estimated as the gain of body nitrogen by subtracting initial body nitrogen from final body nitrogen.

The rats were individually housed in metabolism cages at 22 °C and subjected to a 12-h light-dark cycle. Spillage was collected daily. Faeces were accumulated in 10% H_2SO_4 for subsequent analysis.

The experimental diets are described in Table 1. Maize starch was replaced by the experimental protein sources while each diet contained 5% sunflower oil and 5% of a vitamin and mineral mixture.

The efficiency of nitrogen utilization for growth and maintenance was estimated as the coefficient (b) of the linear regression ($y = a + bx$) of body nitrogen gain (y) on nitrogen intake (x). Data from rats fed the protein-free diet were included in the regression calculation. A common intercept was fitted for the two protein sources in order to transfer all variation to the slopes. Relative nutritive value (RNV) is the ratio of the regression coefficient (b) for the test protein to that for the reference protein, lactalbumin (Hegsted *et al.*, 1968; Siebrits *et al.*, 1986).

Protein digestibility

Protein true digestibility (PTD) was also estimated by a regression procedure (Siebrits *et al.*, 1986). The faeces were digested with boiling, concentrated H_2SO_4 and a representative aliquot of the suspension was analysed for nitrogen. Total faecal nitrogen was related to nitrogen intake according to the same technique employed to calculate the efficiency of protein use, and the coefficient of the relationship, which defined indigestibility, was subtracted from 1.00.

The regression coefficients were statistically analysed by analysis of covariance (Snedecor & Cochran, 1980).

Bioavailable energy

Digestible energy (DE) for pigs was determined with the mobile nylon bag technique (MNBT) as described by Sauer & Ozimek (1985).

The true metabolizable energy (TME) method for poultry, originally described by Sibbald (1976) and adapted by McNab & Fisher (1984), was used to determine the metabolizable energy content of *G. candidum* and other protein sources. Four adult cockerels were used for each protein source. Corrections for nitrogen retention were made on the TME values, as proposed by Wolynetz & Sibbald (1984), to determine TME_n. Amino acid content of the excreta of the cockerels was analysed to determine available amino acids (AA) for poultry by the method of Likuski & Dorrell (1978), adapted by McNab & Fisher (1984).

Results and Discussion

Toxicity

There was no significant difference ($P > 0.05$) in weight gain between the groups fed diets containing *G. candidum* and fishmeal. No macroscopic lesions were found post mortem and there were no histopathological lesions in the liver and kidney tissue. It was concluded that *G. candidum* did not cause acute toxicity.

Table 1 Composition of the experimental diets on an air-dry basis (%)

Component	Control	Lactalbumin				*Geotrichum candidum*			
Crude protein	0	2	4	6	8	2	4	6	8
Maize starch	90.0	87.5	85.0	82.5	80.0	86.1	82.1	78.2	74.3
Sunflower oil	5	5	5	5	5	5	5	5	5
Mineral & vitamin premix[a]	5	5	5	5	5	5	5	5	5
Lactalbumin	–	2.5	5.0	7.5	10.0	–	–	–	–
G. candidum	–	–	–	–	–	3.9	7.9	11.8	15.7

[a] Supplied per kilogram feed: Vitamin A, 2.0 IU; Vitamin D, 1 000 IU; Vitamin E, 35 mg; Vitamin K, 50 µg; Thiamin hydrochloride, 1.25 mg; Riboflavin, 2.5 mg; Vitamin B12, 5 µg; Calcium pantothenate, 8 mg; Niacin, 15 mg; Choline chloride, 750 mg; Cu, 5 mg; Mn, 50 mg; Zn, 12 mg; I, 0.15 mg; Fe, 35 mg; Se, 0.04 mg; Mg, 0.4 g; P, 4.0 g; K, 1.8 g; Na, 0.5 g; Ca, 5.0 g.

Chemical composition

The chemical composition of the *G. candidum* used in this study is presented in Table 2. According to Kühn & Pretorius (1989), the composition will vary depending on production conditions (e.g. temperature and mean cell residence time). During production trials, the crude protein varied between 44.2% and 65.8% (Kühn, A.L., 1991, personal communication). This variation was mainly due to unstable production conditions which resulted in excessive bacterial growth. However, the commercial production of *G. candidum* will be under stable conditions which will minimize bacterial growth and variation (Kühn, A.L., 1991, personal communication).

Table 2 Chemical composition of *G. candidum* on an air-dry basis

Component	Total (%)	Amino acid	Total (%)	Avail.[a] (%)
Dry matter	95.4	Lysine	1.91	1.63
Ash	8.2	Methionine	0.65	0.62
Ether extract	2.2	Cystine	0.65	0.52
Crude fibre	<0.5	Threonine	1.69	1.38
Phosphorus	1.60	Isoleucine	1.10	0.96
Calcium	0.02	Leucine	2.57	2.38
Crude protein		Tyrosine	1.16	–
(N × 6.25)	53.4	Phenylalanine	1.05	–
True protein				
nitrogen[b]	72.7	Histidine	0.51	0.44
Nucleic acid		Arginine	1.43	1.37
nitrogen[b]	8.6	Glycine	1.51	1.28

[a] Available amino acid.

[b] Percentage of total nitrogen.

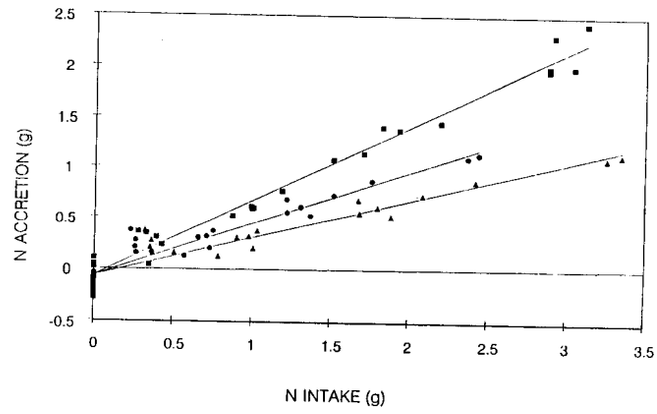

Figure 1 Slope ratio response curves, of nitrogen intake against nitrogen accretion, for lactalbumin (■) and *Geotrichum candidum* as response to true protein intake (●) and to total nitrogen intake (▲).

Table 3 The relative nutritive value of *Geotrichum candidum* with body nitrogen accretion as response to total nitrogen intake and to true protein nitrogen intake[1]

Protein source	Slope ± SD	RNV ± SD
Lactalbumin	0.740 ± 0.014[a]	1.000
G. candidum[2]	0.374 ± 0.017[b]	0.505 ± 0.038
G. candidum[3]	0.514 ± 0.024[c]	0.695 ± 0.056

[1] Intercept = -0.193.

[2] Response to total nitrogen intake.

[3] Response to true protein nitrogen intake.

[a–c] Slopes with different superscripts differ significantly ($P \leq 0.01$).

Protein quality

The response curves of nitrogen intake against nitrogen accretion are presented in Figure 1, while the slopes of the lines and the RNV values are given in Table 3.

Although the response curves appear to deviate from linearity (Figure 1), regression analysis revealed that these were best described by linear regression. According to Hegsted *et al.* (1968), animals fed a protein-free diet may respond in an atypical manner and there is not always a linear response from the zero dose to higher doses. Siebrits *et al.* (1986) stated that some caution should be exercised in relying upon the usual measure of net protein use (NPU). By omitting the data on the protein-free rats, somewhat smaller slopes were recorded but the relative nutritive values remained the same. This suggests that the slope ratio technique is insensitive to changes in absolute slope and that relative values remain constant. The procedure of using a common intercept ensures that all the variation between treatments, even in the event of a slight curvature, is expressed by the difference between slopes. In a recent review of protein evaluation methods, Sarwar & McDonough (1990) stated that the RNV method is probably the best assay for predicting protein quality.

Relative nutritive value is usually calculated as a response to total nitrogen intake (Hegsted *et al.*, 1968). The RNV value of *G. candidum* (0.505), calculated as response to total nitrogen intake, was significantly ($P < 0.01$) lower than that of lactalbumin. This value is lower than that of other protein sources such as cottonseed flour (0.61), fish flour (0.60) and full fat soya flour (0.65) (Hegsted *et al.*, 1968). The presence of a high percentage (27.3%) non-protein nitrogen in the nitrogen fraction of the product could be the main reason for the lower RNV for *G. candidum* when calculated as response to total nitrogen intake. However, when the RNV of *G. candidum* is calculated as a response to true protein nitrogen intake, the RNV improves with 19 percentage units to 0.695. This value is even higher than that for fish flour (Hegsted *et al.*, 1968). This suggests that the true protein fraction of *G. candidum* is of high quality. The value of 0.741 found for the slope of lactalbumin is relatively low compared to the value of 0.854 found by Siebrits *et al.* (1986), but this does not affect the RNV since RNV is based on the ratio of the test protein slope to that of the reference protein.

Protein digestibility

The true protein digestibility (PTD) values together with the slopes of the equations used to calculate PTD are listed in Table 4.

The PTD of *G. candidum* did not differ significantly ($P > 0.05$) from the PTD of lactalbumin, which indicates that the protein of *G. candidum* is highly digestible. The digestibility values of *G. candidum* in the present study is better than the values found by Dabrowski *et al.* (1980). When they replaced

Table 4 True protein digestibility (PTD) of the experimental diets containing lactalbumin and *Geotrichum candidum*

Protein source	Slope ± SD	PTD ± SD
Lactalbumin	0.097 ± 0.019	0.903 ± 0.046
Geotrichum candidum	0.149 ± 0.021	0.851 ± 0.057

75% of a diet for rainbow trout with *G. candidum*, the apparent digestibility declined from 64.7% to 37.5%. The higher PTD found in the present study is probably due to the fineness to which the product was ground. The PTD for *G. candidum* compares favourably to the PTD of lucerne leaf protein concentrate (80.9%), determined according to the same technique by Siebrits *et al.* (1986).

Bioavailable energy

The TME and TME_n values for poultry of *G. candidum* and other protein sources determined according to the same technique are listed in Table 5.

Table 5 True metabolizable energy for poultry of *Geotrichum candidum* and other protein sources (MJ/kg dry matter)

Protein source	n	TME ± SD	TME_n ± SD
G. candidum	4	12.74 ± 0.46	11.76 ± 0.26
Fish-meal	4	17.09 ± 0.12	15.90 ± 0.12
Blood meal	4	15.34 ± 0.98	14.09 ± 0.98
Cowpea meal	4	13.54 ± 0.46	12.45 ± 0.45
Sunflower oilcake	4	10.35 ± 0.32	9.06 ± 0.29

The determination of DE for pigs by means of the mobile nylon bag technique was unsuccessful. The loss of *G. candidum* through the 53-μ pores of the nylon bags was abnormally high and resulted in unrealistically high DE values. DE for pigs was calculated from TME as 13.8 MJ/kg dry matter by means of a linear equation (Sibbald *et al.*, 1983).

Conclusion

G. candidum showed no signs of acute toxicity. The fungus was found to have a crude protein content of 53.4% and a true protein content of 38.8%, with a favourable amino acid composition and protein quality. Furthermore the amount of bioavailable energy for poultry was found to be reasonable. *G. candidum* can, therefore, be regarded as a potentially good source of protein for monogastric animals. Provided that the product can be produced at an acceptable price, *G. candidum* can be used as a source of protein in the diets of monogastric animals. More research is, however, needed to determine growth and inclusion levels in diets of target species such as poultry and pigs.

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