

## Effect of nutritional status of faba bean on proximate composition, anti-nutritional factors and in vitro protein digestibility (IVPD)

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### Abstract

A field experiment was carried out to study the effect of moisture regime, vesicular arbuscular mycorrhiza (VAM) inoculation, phosphorus (P) and sulphur (S) fertilization on the proximate composition, in vitro protein digestibility (IVPD) and anti-nutritional factors of faba bean. The results indicated that the moisture content was significantly affected by the watering regime, VAM inoculation and P+S treatments. Ash content was significantly higher in the P+S and VAM+S for wet and dry samples, respectively. Sulphur and VAM+P+S were associated with higher levels of fat in the wet and dry samples, respectively. The protein content positively correlates with fertilizer and VAM treatments. Carbohydrate content was higher in the control samples. All treatments gave higher tannin contents. Phytic acid increased significantly in treated samples. Trypsin inhibitor activity was significantly higher for VAM+P+S and for S in wet and dry samples, respectively. Water stress and fertilizer treatments resulted in lower IVPD compared to the control. Reduced values for IVPD were associated with higher levels of tannin and phytic acid. © 1999 Published by Elsevier Science Ltd. All rights reserved.

### 1. Introduction

Faba bean (*Vicia faba*) production is widespread in temperate and subtropical regions of the world and it ranks as the fourth most important pulse crop in the world after dry beans, dry peas and chickpea. The crop is becoming increasingly important in Sudanese diets. Faba bean, like other leguminous crops, plays a unique role due to its high protein content and ability to fix atmospheric nitrogen.

Many factors affect seed quality, including cultivar, cultural practices and locality or environmental conditions (Elsheikh & Elzidany, 1997). Chemical, organic and biological fertilizers were reported to increase protein content of faba bean (Babiker, Elsheikh, Osman & El Tinay, 1995), groundnut (Elsheikh & Mohamedzein, 1998) and fenugreek (Abdelgani, Elsheikh & Mukhtar, 1999). The mycorrhizal symbiosis improved growth of crops, which was attributed to the better utilization of phosphorus by the mycorrhiza and enhanced nodulation by *Rhizobium* and better nitrogen nutrition (Elsheikh & Mohamedzein, 1998). Biofertilizers are very important for countries like Sudan, with a predominantly low-input

agricultural system of production and where chemical fertilizers, if available, may not be economically affordable (Mahadi, 1993).

Anti-nutritional factors, such as tannin, phytic acid and trypsin inhibitor, are undesirable and efforts are directed to minimize their contents in the seeds. Fertilization and breeding programmes could be used as useful tools to solve this problem. Hence, the objective of the present study was to investigate the relationship between faba bean nutritional status and its effect on proximate composition, in vitro protein digestibility and anti-nutritional factors.

### 2. Materials and methods

A field experiment was conducted during the 1995/1996 season in the Demonstration Farm of the Faculty of Agriculture at Shambat (latitude 15° 40' N, longitude 32° 32' E). The land was prepared by disc plough followed by ridging and the spacing between ridges and holes were 70 and 20 cm, respectively. The size of the subplot was 4×4 m consisting of five ridges of 3 m length. Between the main plots 1 m was left as guard area for water control. Sowing of faba bean cultivar, "Shambat 75", was done at a rate of two seeds/hole.

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The crop was irrigated every week during the first month. The experiment was arranged in split-plot design with four replications. The main plots were allotted to the watering intervals: (a) wet = plants were irrigated every week, and (b) dry = plants were irrigated every two weeks, and the subplots to the following treatments:

1. Untreated control plants.
2. Plants inoculated with vesicular arbuscular mycorrhiza (VAM) (*Glomus* sp.) as previously described with Mahadi and Atabani (1992).
3. Plants fertilized by 200 kg/ha P<sub>2</sub>O<sub>5</sub> superphosphate (at sowing).
4. Plants fertilized with 50 kg/ha sulphur (at sowing)
5. Plants treated with both mycorrhiza and phosphorus (treatments 2 and 3).
6. Plants treated with both mycorrhiza and sulphur (treatments 2 and 4).
7. Plants treated with both phosphorus and sulphur (treatments 3 and 4).
8. Plants treated with mycorrhiza, phosphorus and sulphur (treatments 2, 3 and 4).

At harvest, the seeds were carefully cleaned, then ground to pass through a 0.4 mm screen, for proximate analysis, on a dry weight basis. AOAC (1975) methods were followed in the determination of moisture (7.003),

petroleum ether extracts (7.048), crude fibre (7.057), ash (14.006), and crude protein (7.016). Carbohydrate was calculated by difference. IVPD (pepsin digestion) was determined according to the method of Maliwal (1983) as modified by Manjula and John (1991) whereas, tannin was determined according to AOAC. The phytic acid content was determined by the method described by Wheeler and Ferrel (1971), whereas the trypsin inhibitor assay of seed extracts was determined according to Roy and Rao (1971). Each sample was analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA). The Duncan multiple range test was used to separate means. Significance was accepted at  $P \leq 0.05$ .

### 3. Results and discussion

#### 3.1. Moisture content

The moisture content of faba bean was significantly affected by moisture regime but there were no significant differences with regard to fertilizer treatment, except for mycorrhiza treatment, which resulted in a significant increase in moisture content compared to control (Tables 1 and 2). The VAM treatment significantly

Table 1  
Effect of mycorrhiza, phosphorous and/or sulphur on the proximate composition (%) of faba bean irrigated every week (wet treatment)

Treatments	Moisture	Ash	Fibre	Fat	Protein	Carbohydrates
Control	7.07 ( $\pm 0.05$ )a <sup>a</sup>	3.09 ( $\pm 0.05$ )a	8.79 ( $\pm 0.05$ )ab	1.50 ( $\pm 0.02$ )a	31.8 ( $\pm 0.50$ )a	47.7 ( $\pm 0.84$ )e
VAM	7.62 ( $\pm 0.05$ )b	3.21 ( $\pm 0.09$ )ab	8.95 ( $\pm 0.03$ )ab	1.76 ( $\pm 0.02$ )bc	35.7 ( $\pm 0.37$ )f	42.7 ( $\pm 0.23$ )a
Phosphorous	7.29 ( $\pm 0.07$ )ab	3.21 ( $\pm 0.04$ )ab	8.76 ( $\pm 0.03$ )ab	2.06 ( $\pm 0.05$ )c	32.8 ( $\pm 0.09$ )bc	45.9 ( $\pm 0.45$ )cd
Sulphur	7.39 ( $\pm 0.05$ )ab	3.23 ( $\pm 0.03$ )abc	8.74 ( $\pm 0.02$ )a	2.12 ( $\pm 0.05$ )c	33.1 ( $\pm 0.37$ )c	45.3 ( $\pm 0.45$ )abc
VAM + phosphorous	7.24 ( $\pm 0.05$ )ab	3.30 ( $\pm 0.01$ )abc	9.78 ( $\pm 0.03$ )c	1.65 ( $\pm 0.02$ )b	33.8 ( $\pm 1.21$ )d	44.2 ( $\pm 0.90$ )abc
VAM + sulphur	7.29 ( $\pm 0.07$ )ab	3.37 ( $\pm 0.01$ )abc	9.35 ( $\pm 0.02$ )bc	1.70 ( $\pm 0.05$ )b	39.7 ( $\pm 0.27$ )b	45.6 ( $\pm 0.06$ )bcd
Phosphorous + sulphur	7.21 ( $\pm 0.07$ )ab	3.62 ( $\pm 0.07$ )c	9.02 ( $\pm 0.05$ )ab	1.76 ( $\pm 0.03$ )bc	34.6 ( $\pm 0.31$ )e	43.8 ( $\pm 0.45$ )ab
VAM + phosphorous + sulphur	7.36 ( $\pm 0.07$ )ab	3.57 ( $\pm 0.06$ )bc	8.81 ( $\pm 0.03$ )ab	1.65 ( $\pm 0.05$ )b	32.8 ( $\pm 0.16$ )bc	46.3 ( $\pm 0.14$ )de
Mean	7.31	3.33	9.02	1.78	33.4	45.2

<sup>a</sup> Means not sharing a common letter in a column are significantly different at  $P \leq 0.05$ .

Table 2  
Effect of mycorrhiza, phosphorous and/or sulphur on the proximate composition (%) of faba bean irrigated every 2 weeks (dry treatment)

Treatments	Moisture	Ash	Fibre	Fat	Protein	Carbohydrates
Control	6.34 ( $\pm 0.15$ )b <sup>a</sup>	3.03 ( $\pm 0.05$ )a	9.03 ( $\pm 0.23$ )a	1.08 ( $\pm 0.03$ )a	31.9 ( $\pm 0.04$ ) <sup>a</sup>	48.3 ( $\pm 0.26$ ) <sup>c</sup>
VAM	6.75 ( $\pm 0.12$ )c	3.44 ( $\pm 0.06$ )bc	9.62 ( $\pm 0.38$ )ab	1.27 ( $\pm 0.08$ )b	33.4 ( $\pm 0.01$ )d	45.5 ( $\pm 0.07$ )a
Phosphorous	6.45 ( $\pm 0.36$ )bc	3.32 ( $\pm 0.03$ )ab	9.21 ( $\pm 0.27$ )a	1.25 ( $\pm 0.07$ )b	32.4 ( $\pm 0.88$ )bc	47.4 ( $\pm 0.99$ )ab
Sulphur	6.69 ( $\pm 0.17$ )bc	3.27 ( $\pm 0.08$ )ab	9.57 ( $\pm 0.18$ )ab	1.25 ( $\pm 0.03$ )b	32.7 ( $\pm 0.91$ )c	46.5 ( $\pm 0.75$ )a
VAM + phosphorous	6.54 ( $\pm 0.29$ )bc	3.38 ( $\pm 0.07$ )abc	9.47 ( $\pm 0.26$ )ab	1.46 ( $\pm 0.04$ )bc	32.6 ( $\pm 0.29$ )c	46.5 ( $\pm 0.75$ )a
VAM + sulphur	6.46 ( $\pm 0.11$ )bc	3.59 ( $\pm 0.06$ )c	9.91 ( $\pm 0.12$ )b	1.43 ( $\pm 0.08$ )bc	32.2 ( $\pm 0.88$ )bc	46.2 ( $\pm 0.15$ )a
Phosphorous + sulphur	6.73 ( $\pm 0.24$ )c	3.34 ( $\pm 0.01$ )abc	9.18 ( $\pm 0.04$ )a	1.64 ( $\pm 0.01$ )cd	32.1 ( $\pm 0.53$ )bc	47.0 ( $\pm 0.02$ )ab
VAM + phosphorous + sulphur	6.51 ( $\pm 0.11$ )bc	3.31 ( $\pm 0.07$ )abc	9.24 ( $\pm 0.15$ )a	1.81 ( $\pm 0.03$ )d	32.4 ( $\pm 0.88$ )bc	46.7 ( $\pm 0.87$ )a
Mean	6.57	3.32	9.40	1.40	32.5	46.8

<sup>a</sup> Means not sharing a common letter in a column are significantly different at  $P \leq 0.05$ .

increased the moisture content (7.6%) compared to the control in the wet samples, while it was greater for the P+S treatment (6.7%) in dry samples. These results accorded with data reported by Elsheikh (1998), but were higher than those reported by El Tinay, Mahgoub, Mohamed and Hamed (1989). Moisture content is generally affected by the relative humidity of the surrounding atmosphere at growing and harvesting and during storage.

### 3.2. Ash content

The ash content was significantly increased (3.6%) in the P+S treatments in the wet samples while it was highest in the VAM+S treatment (3.6%) in dry samples (Tables 1 and 2). Means obtained in this study were in close agreement with the results obtained by Elsheikh and Elzidany (1997), but were higher than those reported by El Tinay et al. (1989). Statistical analysis showed no significant differences among fertilizer treatments or water interval treatments except for VAM, P+S and VAM+P+S compared to the control in wet samples, and mycorrhiza and P+S in the dry treatments compared to the control.

### 3.3. Crude fibre content

Crude fibre content of faba bean, in this investigation, (Tables 1 and 2) was higher than that obtained by El Tinay et al. (1989), but in agreement with ranges given by Elsheikh (1998). No significant differences were observed within water interval treatments within fertilizer treatments, except for VAM+P and VAM+S which were significantly higher in crude fibre compared to the control in the wet treatment. This is not true for dry samples in which crude fibre content insignificantly varied.

### 3.4. Fat content

The fat content was significantly increased (2.1%) by S treatment in the wet samples while it was highest in the VAM+P+S treatment (1.8%) in dry samples (Tables 1 and 2) and is in agreement with the value obtained by El Tinay et al. (1989) and Elsheikh (1998). Statistical analysis of fat content showed significant differences ( $P \leq 0.05$ ) with regard to water interval treatments. All fertilizer treatments increased fat content of faba bean. For S and P, it gave significantly higher fat contents compared to the control. Significant differences were observed for the interaction between fertilizer treatment and water interval treatments except for VAM and VAM+P.

### 3.5. Protein content

The protein content was significantly increased (39.8%) in the VAM+S treatments in the wet samples while it was greatest for VAM treatment (33.4%) in dry samples (Tables 1 and 2) which is in close agreement with

values obtained by El Tinay et al. (1989), Elsheikh and Elzidany (1997) and Babiker et al. (1995). Statistical analysis revealed that fertilizer treatments significantly increased protein content. Mycorrhiza+S, or S+P and the combination between them were found to be effective. Helen, Martha & Mirsol (1991) reported that protein content increased with improved plant nutritional status. Inoculation has been reported to increase protein content of faba bean (Elsheikh & Elzidany, 1997). Water interval treatments have no effect on the protein content.

### 3.6. Carbohydrate content

Carbohydrate content was higher in the control samples for both wet and dry regimes (47.8 and 45.3%, respectively) (Tables 1 and 2), which is in close agreement with the mean carbohydrate content for raw legumes (27–64%) reported by El Tinay et al. (1989), but lower than those obtained by Elsheikh and Elzidany (1997). Statistical analysis revealed negative significant differences within all fertilizer treatments.

### 3.7. Tannin content

Tannin content was higher in the P+S treatment (0.46%) for wet samples while it was greatest in the VAM treatment (0.46%) in dry samples (Tables 3 and 4). Elsheikh and Elzidany (1997) and Babiker et al. (1995) reported lower values. Tannin content of faba bean significantly increased for all treatments. Water stress has no significant effect on tannin content except for the combination of VAM+S and P+S. Although all treatments improved seed quality, their only drawback was the increment in tannin content. Babiker et al. (1995) reported similar results. Many factors affect tannin content, such as genotype, time of harvest and temperature.

### 3.8. Phytic acid content

Phytic acid increased significantly compared to the control; it was highest in the VAM+P+S treatment (0.27%) for wet samples while it increased to 0.43% for the VAM+S treatment for dry samples (Tables 3 and 4). El Tinay et al. (1989) reported higher values while El Mubarak, Salih, Abdl-Galiel and Ghorashi (1988) reported lower ranges compared to those obtained in this study. Fertilizer treatments, except VAM+P in the wet sample, caused an increase in phytic acid content in comparison with the control. Fertilizer treatments have a significant effect on phytic acid content. However, watering has no significant effect on phytic acid content.

### 3.9. Trypsin inhibitor activity

Trypsin inhibitor activity was highest (12.1 TUI) for the VAM+P+S treatment in wet samples while it

Table 3

Effect of mycorrhiza, phosphorus and/or sulphur on the IVPD, tannin, phytic and trypsin inhibitor of faba bean irrigated every week (wet treatment)

Treatments	IVPD (%)	Tannin (%)	Phytic acid (%)	Trypsin (TUI)
Control	80.1 ( $\pm 0.05$ )d <sup>a</sup>	0.20 ( $\pm 0.05$ )a	0.119 ( $\pm 0.05$ )a	7.50 ( $\pm 0.22$ )a
VAM	66.2 ( $\pm 0.25$ )a	0.34 ( $\pm 0.19$ )bc	0.221 ( $\pm 0.30$ )bc	9.69 ( $\pm 0.28$ )bcd
Phosphorus	71.6 ( $\pm 0.07$ )bc	0.44 ( $\pm 0.24$ )cd	0.238 ( $\pm 0.29$ )bc	7.81 ( $\pm 0.25$ )ab
Sulphur	72.2 ( $\pm 0.45$ )bc	0.42 ( $\pm 0.13$ )cd	0.204 ( $\pm 0.40$ )bc	9.06 ( $\pm 0.55$ )bc
VAM + phosphorus	74.2 ( $\pm 0.25$ )c	0.34 ( $\pm 0.11$ )bc	0.119 ( $\pm 0.08$ )a	8.75 ( $\pm 0.18$ )bc
VAM + sulphur	71.3 ( $\pm 0.17$ )bc	0.42 ( $\pm 0.10$ )cd	0.180 ( $\pm 0.42$ )b	10.6 ( $\pm 0.15$ )cde
Phosphorus + sulphur	69.1 ( $\pm 0.07$ )ab	0.46 ( $\pm 0.27$ )d	0.180 ( $\pm 0.25$ )b	10.9 ( $\pm 0.23$ )de
VAM + phosphorus + sulphur	71.4 ( $\pm 0.07$ )bc	0.42 ( $\pm 0.26$ )cd	0.272 ( $\pm 0.09$ )c	12.1 ( $\pm 0.25$ )e
Mean	72.0	0.38	0.234	9.65

<sup>a</sup> Means not sharing a common letter in a column are significantly different at  $P \leq 0.05$ .

Table 4

Effect of mycorrhiza, phosphorous and/or sulphur on the IVPD, tannin, phytic and trypsin inhibitor of faba bean irrigated every 2 weeks (dry treatment)

Treatments	IVPD (%)	Tanin (%)	Phytic acid (%)	Trypsin (TUI)
Control	79.0 ( $\pm 0.15$ )d <sup>a</sup>	0.23 ( $\pm 0.15$ )a	0.180 ( $\pm 0.23$ )a	14.1 ( $\pm 0.08$ )a
VAM	71.5 ( $\pm 0.12$ )a	0.46 ( $\pm 0.26$ )c	0.238 ( $\pm 0.38$ )bc	29.8 ( $\pm 0.27$ )e
Phosphorous	71.9 ( $\pm 0.36$ )bc	0.44 ( $\pm 0.20$ )bc	0.225 ( $\pm 0.27$ )bc	23.1 ( $\pm 0.17$ )c
Sulphur	73.1 ( $\pm 0.17$ )bc	0.38 ( $\pm 0.08$ )bc	0.221 ( $\pm 0.18$ )bc	31.3 ( $\pm 0.13$ )e
VAM + phosphorous	73.6 ( $\pm 0.29$ )c	0.36 ( $\pm 0.07$ )bc	0.221 ( $\pm 0.36$ )bc	18.1 ( $\pm 0.38$ )b
VAM + sulphur	71.9 ( $\pm 0.11$ )bc	0.34 ( $\pm 0.36$ )ab	0.425 ( $\pm 0.12$ )d	15.9 ( $\pm 0.08$ )ab
Phosphorous + sulphur	74.0 ( $\pm 0.24$ )ab	0.34 ( $\pm 0.11$ )ab	0.170 ( $\pm 0.04$ )b	18.0 ( $\pm 0.21$ )b
VAM + phosphorous + sulphur	71.4 ( $\pm 0.11$ )bc	0.40 ( $\pm 0.17$ )bc	0.272 ( $\pm 0.15$ )c	25.3 ( $\pm 0.10$ )d
Mean	73.3	0.38	0.248	22.0

<sup>a</sup> Means not sharing a common letter in a column are significantly different at  $P \leq 0.05$ .

was 31.3 TUI for S treatment in dry samples (Tables 3 and 4). Statistical analysis of the results illustrated that fertilizer treatment has a highly significant effect on trypsin inhibitor units compared with the control except for the phosphorus treatment in wet samples. Water stress (dry) significantly increased trypsin inhibitor units compared with wet samples. This indicates that trypsin inhibition is affected by fertilizer treatment as well as watering of the plant and the interaction between them.

### 3.10. *In vitro* protein digestibility

The *in vitro* protein digestibility (IVPD) was significantly higher for the controls for both wet and dry samples (Tables 3 and 4) and is in agreement with results obtained by Babiker et al. (1995) but were lower than those obtained by Elsheikh and Elzidany (1997). Statistical analysis showed that all fertilizer treatments negatively affected the *in vitro* protein digestibility. Fertilizer treatment and water interval treatments and their combination significantly affected digestibility of protein. The *in vitro* protein digestibility has been reported to be affected by many factors such as genotype and tannin content (Babiker et al., 1995).

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