***In Vitro* Fermentation of High-Fibre Sunflower Meal: A Potential Protein Source for Poultry Feed**

**Application**

Fermenting high-fibre sunflower meal with *Bacillus subtilis* offers a sustainable, cost-effective protein alternative to soybean meal in poultry feed.

**Introduction**

Feed costs represent 60–70% of poultry production expenses **(Adesehinwa, 2007)**, with soybean meal (SBM) being a key protein source. However, SBM is environmentally unsustainable due to issues like deforestation and high water use **(Ferreira et al., 2016)**, necessitating viable alternatives. Sunflower meal (SFM) offers a cost-effective option **(Ciurescu et al., 2019)**, but its high fibre content limits digestibility and may impair poultry performance **(Villamide & San Juan, 1998)**. Fermentation using microorganisms such as bacteria and yeast has emerged as a promising method to enhance SFM’s nutritional profile by breaking down fibre and improving digestibility. This study explored the in vitro fermentation of high-fibre SFM with microorganisms, aiming to develop it as a sustainable alternative protein source for poultry.

**Materials and methods**

This experiment followed a 4x2x2 factorial design to investigate the effects of three microorganisms (*Bacillus subtilis*, *Cellulomonas sp.*, and *Saccharomyces cerevisiae* strain K5-5A) compared with a negative control. Two moisture levels (80% and 100%) were applied during *in vitro* fermentation of SFM, with fermentation durations of 4 and 8 days. Microbial slope cultures (obtained from the National Centre for Biotechnology Education, University of Reading). Samples in triplicate were inoculated with 10% (v/w) active yeast/bacteria; controls were inoculated with sterilised water. Flasks were incubated at 30ºC. Samples were collected on days 4 and 8, freeze dried for 65 h, and stored at -20°C for further analysis. Crude protein (CP) content was determined using reference method AOAC 2001.11 **(AOAC, 2005)**, while phytic acid and total dietary fiber (TDF) were determined using a commercially available kit (K-PHYT and K-RINTDF, Megazyme, Ireland). Neutral detergent fiber (NDF) was measured using an Ankom 200 Fiber Analyzer. Amino acid content was analysed after hydrolysis with 6M HCl in an atmosphere of nitrogen, followed by separation using Shimadzu LCMS-8050 triple quadrupole mass spectrometer with Nexera UPLC based on their mass-to-charge ratio (m/z) without derivatisation. Data were analysed using a three-way ANOVA within the GLM procedure in Minitab version 22.1 with fixed effects of microorganisms, moisture levels, and fermentation durations, with Tukey's test for post hoc comparisons. The independent variables were treated as fixed factors. Differences were considered significant if *p* < 0.05.

**Table 1**

Effect of fermentation conditions (microorganism, moisture level and period) on chemical composition of *in vitro*-fermented high fibre sunflower meal (g/kg DM).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Microorganisms | | | | | |  | Moisture level | | | |  | Fermentation period | | | |
| Composition | Control | *B. subtilis1* | *S. cerevisiae2* | *C. sp.3* | SEM | *P-values* |  | 80% | 100% | SEM | *P-values* |  | 4 days | 8 days | SEM | *P-values* |
| CP | 237b | 243a | 239ab | 238b | 1.19 | 0.014 |  | 240 | 239 | 0.84 | 0.298 |  | 250 | 229 | 0.84 | <0.001 |
| TDF | 659a | 645b | 649ab | 650ab | 2.63 | 0.004 |  | 652 | 650 | 1.86 | 0.468 |  | 628 | 674 | 1.86 | <0.001 |
| aNDF | 490 | 483 | 484 | 487 | 3.52 | 0.441 |  | 482 | 490 | 2.49 | 0.020 |  | 470 | 502 | 2.49 | <0.001 |
| PA | 30.7a | 30.4ab | 27.1c | 29.5b | 0.27 | <0.001 |  | 29.6 | 29.2 | 0.19 | 0.164 |  | 28.7 | 30.2 | 0.19 | <0.001 |

Data were analyzed using a three-way ANOVA within the GLM procedure in Minitab version 22.1. Different letters within the within each of micro-organisms, moisture level, fermentation period significant differences at p < .05. 1 *Bacillus subtilis*. 2 *Saccharomyces cerevisiae* Strain K5-5A. 3 *Cellulomonas sp*. CP: crude protein, TDF: total dietary fibre, aNDF: neutral detergent fibre corrected for ash, PA: phytic acid.

**Table 2**

Effect of interactions between fermentation conditions (microorganism and period) on selected amino acid contents of *in vitro*-fermented high-fibre sunflower meal (g/kg DM).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Interaction: Microorganisms × Fermentation period* | | | | | | | | | | |
|  | Control | | *B. subtilis1* | | *S. cerevisiae2* | | *C. sp3* | | SEM | *P-values* |
|  | 4 d | 8 d | 4 d | 8 d | 4 d | 8 d | 4 d | 8 d |  |  |
| Lysine | 7.2a | 5.9bc | 7.8a | 5.8bc | 7.4a | 6.2b | 6.1b | 5.4c | 0.12 | 0.002 |
| Arginine | 7.9a | 4.8de | 7.6a | 5.7c | 7.9a | 5.1d | 6.7b | 4.4e | 0.10 | <0.001 |
| Valine | 10.1a | 6.2ef | 9.2bc | 7.2d | 9.7ab | 6.9de | 8.8c | 5.9f | 0.15 | <0.001 |
| Histidine | 4.3a | 2.9d | 4.2a | 3.3c | 4.4a | 3.3c | 3.8b | 2.8d | 0.06 | 0.004 |

Data were analyzed using a three-way ANOVA within the GLM procedure in Minitab version 22.1. Different letters within the same row indicate significant differences at p < .05. Tukey's test was used for post hoc comparisons. **Con.** (control: no probiotics added)**, A:** *Bacillus subtilis.* **B**: *Saccharomyces cerevisiae* Strain K5-5A. **C:** *Cellulomonas sp.*

**Results**

Fermenting sunflower meal (SFM) with *Bacillus subtilis* significantly increased crude protein and reduced total dietary fibre compared to unfermented SFM, while fermentation with *Saccharomyces cerevisiae* significantly reduced phytic acid content (P<0.05, Table 1). Total dietary fibre (TDF), neutral detergent fibre (aNDF), and phytic acid (PA) increased with 8 days of fermentation. No significant interactions were observed between microorganism, moisture level, and fermentation period for chemical composition. However, interactions between treatments influenced specific amino acids (Table 2). Four-day fermentation with *B. subtilis* or *S. cerevisiae* significantly enhanced lysine, arginine, and histidine content compared to fermentation with *Cellulomonas sp.* (P<0.05). Essential amino acid levels decreased after 8 days of fermentation compared to 4 days (P<0.05). Fermentation with *B. subtilis* at both moisture levels yielded significantly higher leucine, isoleucine, and phenylalanine compared to other microorganisms or unfermented SFM (P<0.05).

**Conclusion**

Fermenting high-fiber SFM with *Bacillus subtilis* enhances crude protein and reduces dietary fiber, while *Saccharomyces cerevisiae* decreases phytic acid levels. Fermentation for 8 days significantly increased TDF, aNDF, and PA. This might be attributed to reduced crude protein after 8 days, altering nutrient concentrations, as TDF was adjusted for ash and protein, while aNDF was corrected for ash. Four-day fermentation boosts essential amino acids like lysine and arginine, particularly with *B. subtilis*, making it a promising approach to improve SFM's nutritional value as a poultry protein source.

**References**

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