Deuterium labelling of sorghum and black beans for dual tracer approach to measure digestibility

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Background

- Intrinsically ^{2}H-labelled protein is needed for dual tracer approach
- Previous studies have labelled various plant species:
  - Lowest mean level: 251 ppme for finger millet
  - Highest mean level: 1607 ppme for maize

Objective

Cultivate plants with sufficient ^{2}H-enrichment to be used in a clinical trial to determine their indispensable amino acid digestibility:

- Sorghum seeds (*Sorghum bicolor* (L.) Moench)
- Black beans (*Phaseolus vulgaris* L.)

Greenhouse

2 plants/pot

Sorghum
235 pots
March - July 2022

Black beans
175 pots
October 2022 - January 2023

Table 1. Greenhouse data for temperature (in °C) and humidity (in %)

Temperature & Humidity	Sorghum	Black beans
☀	25.5±1.4°C 53.4±11.2%	21.5±0.8°C 67.6±12.2%
☾	20.6±0.6°C 65.9±11.8%	19.4±0.6°C 67.9±11.9%

Labelling protocol

Initiation:

- Sorghum: beginning of flowering (~80 days after sowing)
- Black beans: start of the pod formation (~40 days after sowing)

Watering protocol:

Table 2. Labelling protocol used for sorghum and black bean pots

Watering	Days	Dilution	$^{2}H_2O$/pot (mL) for sorghum	$^{2}H_2O$/pot (mL) for black beans
1	0	25%	100	75
2	2	5%	20	15
3	4	5%	20	15
4	6	5%	20	15
5	8	5%	20	15
Total			180	135

- Ground products were analyzed for nutrient content.
- ^{2}H-enrichment was determined using gas chromatography coupled to a pyrolysis furnace and an isotope ratio mass spectrometer.

Results

For both plants:

- ^{2}H-enrichment for indispensable amino acids was >4000ppme
- Threonine was more enriched compared to other amino acids

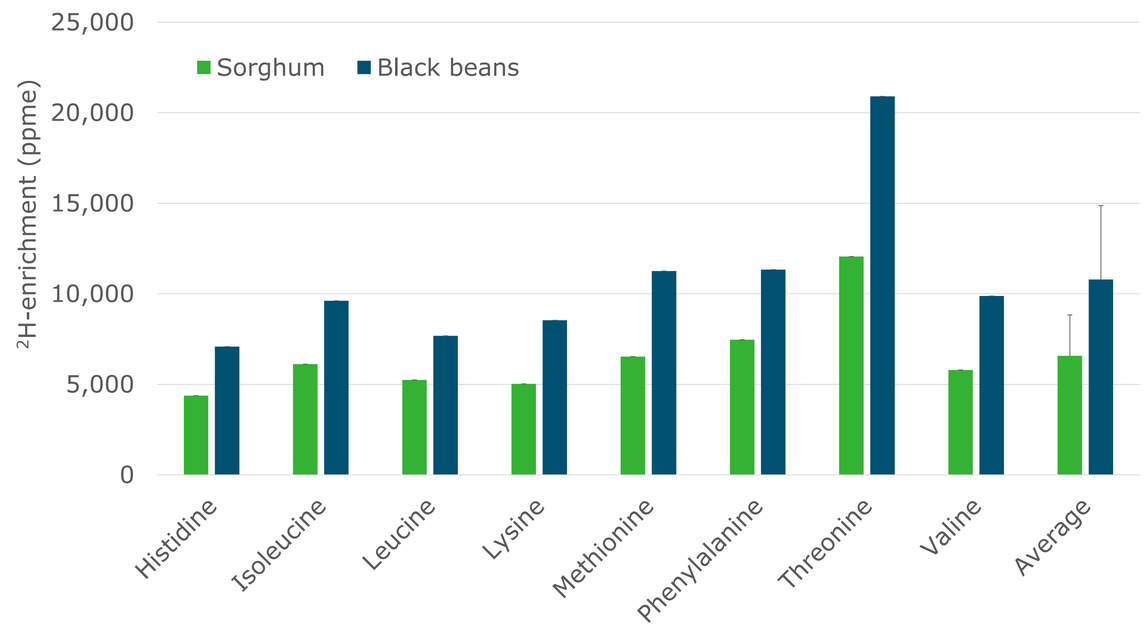

Figure 1. Average isotopic ^{2}H-enrichment in sorghum and black beans indispensable amino acids

Table 3. Macronutrient content of harvested sorghum seeds and black beans

Amount (g/100g of product)	Sorghum	Black beans
Protein (N*5.4)	9.32	22.20
Fat	4.30	1.90
Carbohydrates	72.54	57.89

Table 4. Amino acid content of harvested sorghum seeds and black beans

Amount (g/100g of protein)	Sorghum	Black beans
Histidine	2.79	3.36
Isoleucine	4.83	5.47
Leucine	16.48	9.68
Lysine	2.36	8.33
Methionine	2.20	1.26
Phenylalanine	6.17	6.89
Threonine	3.76	4.77
Valine	6.01	6.15

Conclusions

- Successful ^{2}H-enrichment of indispensable amino acids in two types of plants
- Higher enrichment level than reported earlier
- Sufficient to determine digestibility with the dual-tracer method
- For future labelling experiments: lower $^{2}H_2O$ dose to save costs

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https://www.wur.nl/en/project/Quantifying-differences-in-bioavailability-of-different-dietary-proteins-in-older-adults.htm

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