Application of the Indicator Amino Acid Oxidation Technique to Study the Bioavailability of Lysine in Wheat

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"We created pairs, that you may reflect." (The Holy Quran, 51:50)

ABSTRACT

The protein quality (PQ) of wheat, limiting in Lysine (Lys), is understudied in humans. Allah says in the Holy Quran: "*And of everything have We created **pairs**, that you may reflect.*" (51:50). Wheat and pulse mixed diet (MD) improves PQ, but data on combination ratio is lacking. Thus, we aimed to:

1) determine the PQ of wheat (bread) by assessing the metabolic availability (MA) of Lys, and

2) determine the effect of bread and lentil stew as a MD on the combined PQ of the meal to reflect if they make up a complete pair.

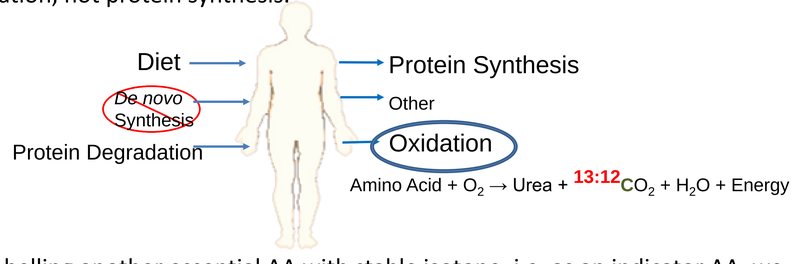
For this, we will use the indicator amino acid oxidation method as a repeated measures design in 5 healthy adult males.
Based on the slope-ratio principles, L-[1-13C] phenylalanine oxidation to $^{13}CO_2$ in response to graded Lys intakes fed as crystalline amino acid (reference protein), wheat (test protein), and wheat complemented with lentil (MD) provided the MA and MD effectiveness.
Despite being of limiting concentration, the MA of Lys in yeast-leavened bread is 90%. Complementation with lentils reduced Phe^{ox} i.e. augmented MD Lys content. We recommend a **combination ratio** of 2:1 **wheat to lentil** protein.

INTRODUCTION

- Majority of the world's hungry survive primarily on vegetarian fare [1].
- Wheat is the most-consumed cereal crop [2].
- However, lysine is a limiting essential amino acid (AA) in wheat (24mg lysine /g protein) compared to the FAO amino acid scoring pattern (45mg lysine/g protein) [3].
- Additionally, treatment of wheat during processing and cooking further renders the amino acid unavailable for anabolic needs [4].

PREMISE OF IAAO TECHNIQUE

If one essential AA is in limited quantity, then rest of the AAs become in excess at the assembly line of polypeptide chain. Excess AAs are partitioned towards oxidation, not protein synthesis.



By labelling another essential AA with stable isotope, i.e. as an indicator AA, we can then measure changes in oxidation of the **indicator AA** in response to the degree of graded intake levels of **test AA.**

A change in ^{13}C expired as carbon dioxide will reflect the change in oxidation.

OBJECTIVES

1. **Determine the metabolic availability of the most limiting AA in wheat; lysine.**
2. **Determine the effectiveness of complementation of wheat + lentils.**

METHODS

- **No. of participants =** 5 healthy adult males.
- **Baseline :** Individual caloric needs were estimated using indirect calorimetry.

EXPERIMENTAL DIETS

✓ **4 reference experiments : lysine fed as crystalline AA**

(Aim: achieve a reference slope of oxidation in the sample population by feeding free crystalline lysine, which is 100% bioavailable)

✓ **3 wheat experiments : lysine fed as crystalline AA + wheat**

(Aim: Obtain a 2nd oxidation slope, whereby, part of lysine comes from wheat, thus the change in oxidation slope from reference will be due to wheat lysine).

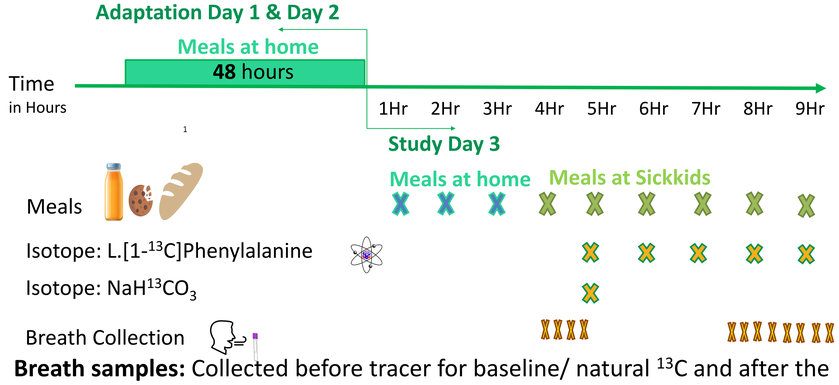
✓ **2 complementation experiments : lysine fed as crystalline AA + wheat + lentil**

(Aim: Achieve oxidation ≥ the reference oxidation slope; ie. balance the lack of lysine from wheat by supplementing with lentil, reflecting the adequacy of mixed diet).

- **Protein intake** = 1.0g.kg^{-1}.d^{-1}
- **Lysine test levels**= 5, 8, 12, 15 mg.kg^{-1}.d^{-1}(<40% lysine EAR of 37 mg.kg^{-1}.d^{-1})[5].
- **Balance of calories** were provided from protein-free cookies and formula (made using protein-free powder, fresh-plus crystals, grapeseed oil, water)

PROTOCOL FOR EACH EXPERIMENT

FIGURE 1. Typical experimental protocol for each study.



Breath samples: Collected before tracer for baseline/ natural ^{13}C and after the tracer to calculate ^{13}C enrichment above the baseline.

ANALYSIS

- $^{13}CO_2$ enrichment in breath was analyzed by continuous flow-IRMS
- **Linear regression model** was applied for statistical analysis (sas ver. 9.3).

Results

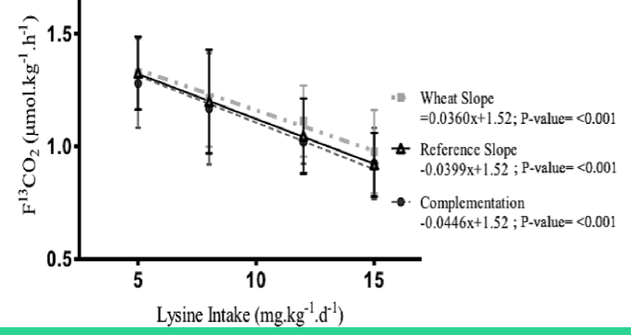
SUBJECT CHARCTERISTICS

Study population	n = 5
Age (y)[1]	32.8 ± 5.8
Weight (kg)	69.5 ± 5.5
Height (cm)	175.1 ± 6.9
BMI (kg/m^2)	22.8 ± 1.8
Waist-to-Hip ratio	0.87 ± 0.06
Fat Free Mass-SK[2] (kg)	51.35 ± 6.78
% Fat –SK[2]	26.31 ± 4.88
REE[3] (kcal/d)	1523 ± 198

[1] All values are means ± SD.
[2] Determined by skin fold (SF) Analysis
[3] Determined by open-circuit indirect calorimetry

RESULTS

Metabolic Availability of Lysine = $\frac{Wheat\ oxidation\ slope}{Reference\ oxidation\ slope}$ x 100% = 90%



CONCLUSION

- Metabolic availability of lysine was 90% from wheat protein to meet anabolic needs when wheat is baked as a light golden colored bread.
- Complementing lentils with bread resulted in a reduction in oxidation, reflecting an improved protein synthesis as mixed meal.

SIGNIFICANCE

- This is the first time to study protein quality of wheat directly in humans.
- This study provides evidence that foods also follow the principle given in the Holy Quran that "*We created pairs, that you may reflect.*" (51:50) as applied to wheat and lentils, i.e wheat protein and lentil protein combined as 2:1 make up a well-balanced protein, a complete pair.

ACKNOWLEDGEMENTS

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