In vitro comparison of cellulolytic and hemicellulolytic enzyme activities from game herbivores

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An overview of talk

1. Introduction
2. Objectives
3. Experiments
4. Findings
5. Conclusion
6. Application

Introduction

- Livestock ~ 33 % the total protein in human diets
- High demand for meat as protein demand increases yearly
- Especially in Africa = plant protein sources scarce
- World's human population is about 6.8 billion
- World's Growth rate = 1.2%/yr = 81.9 million
- Africa contributing ~ 15% = 1.02 billion
- Africa growth rate = 2.2 %/yr = 22.5 million
- Europe growth rate = 0.3%/yr = 2.7 million

Introduction

- How can animal protein be increased to sustain the ever increasing demand with increasing human population ?

- Simple !!!, by increasing animal production.
- Simple???
- Lets ask the farmers both commercial and small scale.
- Daunting task of high feed cost and scarcity?
- Ask the beef farmers?
- In winter, most of them are broke, feed scarcity, high cost, sell most of their animals to avoid death
- Yet, hay, crop residues and agricultural by-products abundant !!!

- So what is stopping this animals from harnessing energy from these sources?
- Chemical structure these high energy polysaccharides (cellulose, hemicellulose and pectin)
- Herbivores depends on microbes to harnessed energy
- Researchers can do something about it

Introduction …

- Different studies that embarkes on enhancing fibre hydrolysis includes:

  - Single strain microbes, mixed microbial culture
  - Pure enzyme systems, components of cellulase enzymes
  - Mixed enzyme systems (synergism)
  - Ferment feed (silage)
  - Chemical treatment

Introduction …

- Focus of interest are cellulolytic enzymes
  - Feed supplementation did increase hydrolysis[5]
  - Limited information is available on
  - how much is being expressed
  - how active they are.

Objectives

1. Sample four game herbivores microbial ecosystems (**buffalo, impala, wildebeest llama**)

   Fishing for systems with;
   - High enzyme expression (concentrations)
   - High enzyme activity (activeness)

2. Does high expression imply high activity?

3. Investigate synergism among ecosystems

Material and Methods …

Sample collection

- Faces collection, *in situ*
- 50ml faeces + 100ml salivary buffer, mixed and squeezed through cheese cloth for faecal inoculum
  - 1 g (maize stover + Lucerne) + 67 ml salivary buffer + 33 ml Faecal inocula
  - Incubated for 72 hr at 39°C (anaerobic environment)

Material and Methods …

Sample collection

- 30 ml of 72 h culture was used for crude enzyme extraction
- Sonication of samples (break cells)
- Proteins precipitated using 60% ammonium sulfate.
- Protein concentration measured as described by Bradford.
- pH activity optimised using carboxymethyl cellulose as substrate

Material and Methods …

- Enzyme assay

  -**Exocellulase**-substrate crystalline cellulose

  **Specific activity (µg glucose/mg crude protein)

-**Endocellulase**- substrate carboxymethyl cellulose

-**Xylanase**-substrate xylan

*Specific activity (µg xylose/mg crude protein)

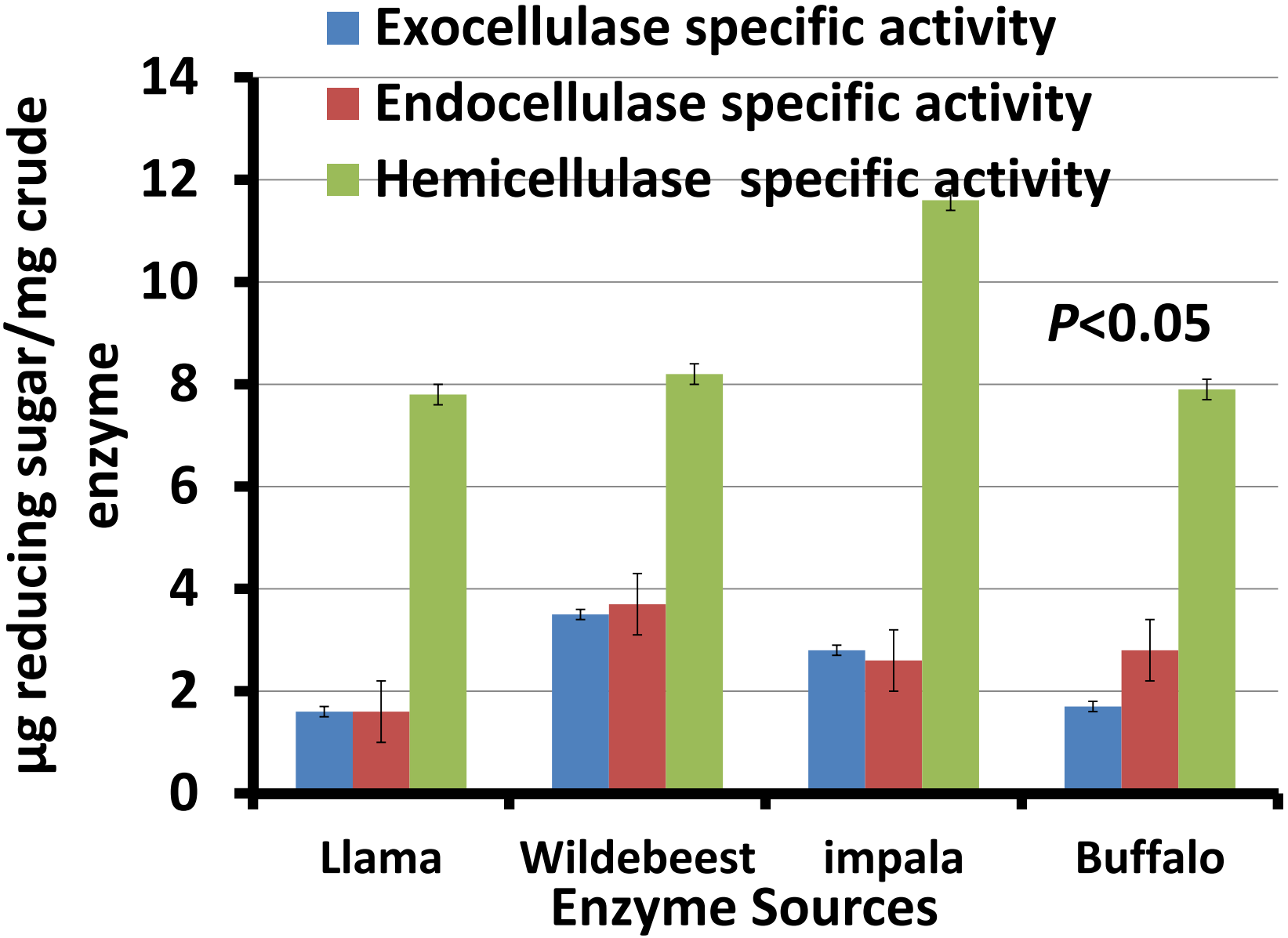
Material and Methods …

- Kinetic parameters were determined as described by Eisenthal and Cornish-Bowded (1974) using HYPER software programme (Easterby, 2003)
- Km = affinity of an enzyme to its substrate
- Vmax = rate of products formation/min from a substrate
- Kcat = Enzyme catalytic rate
- Kcat/Km = Catalytic efficiency of enzyme substrate pair

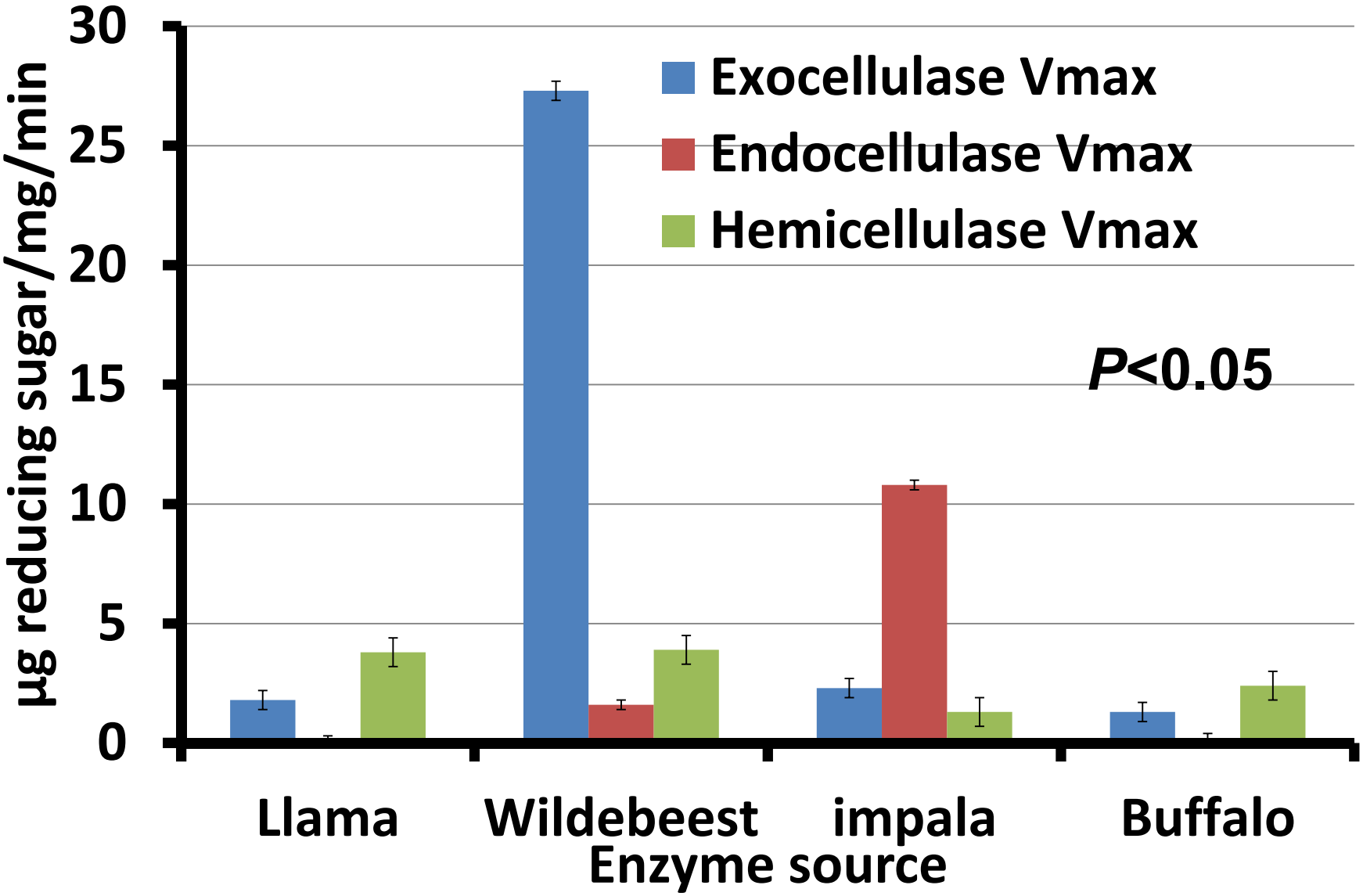
Statistical analysis

•Enzyme activities and kinetic parameters were subjected to analysis of variance (ANOVA) using the general linear model of SAS.

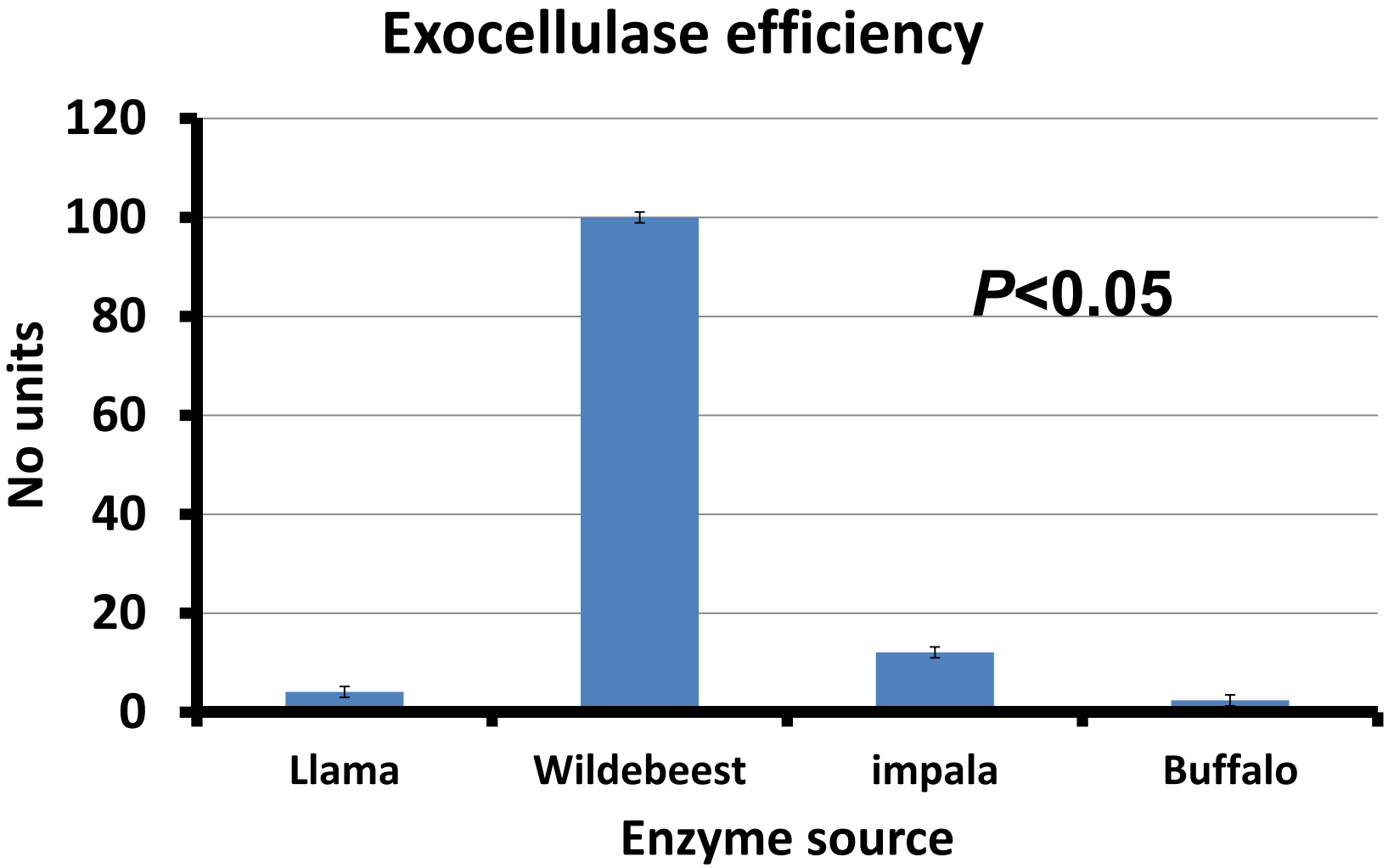
Results …


Exocellulase specific activity
Endocellulase specific activity
Hemicellulase specific activity
µg reducing sugar/mg crude enzyme
14
12
10
8
6
4
2
0
P<0.05
Llama
Wildebeest
impala
Buffalo
Enzyme Sources

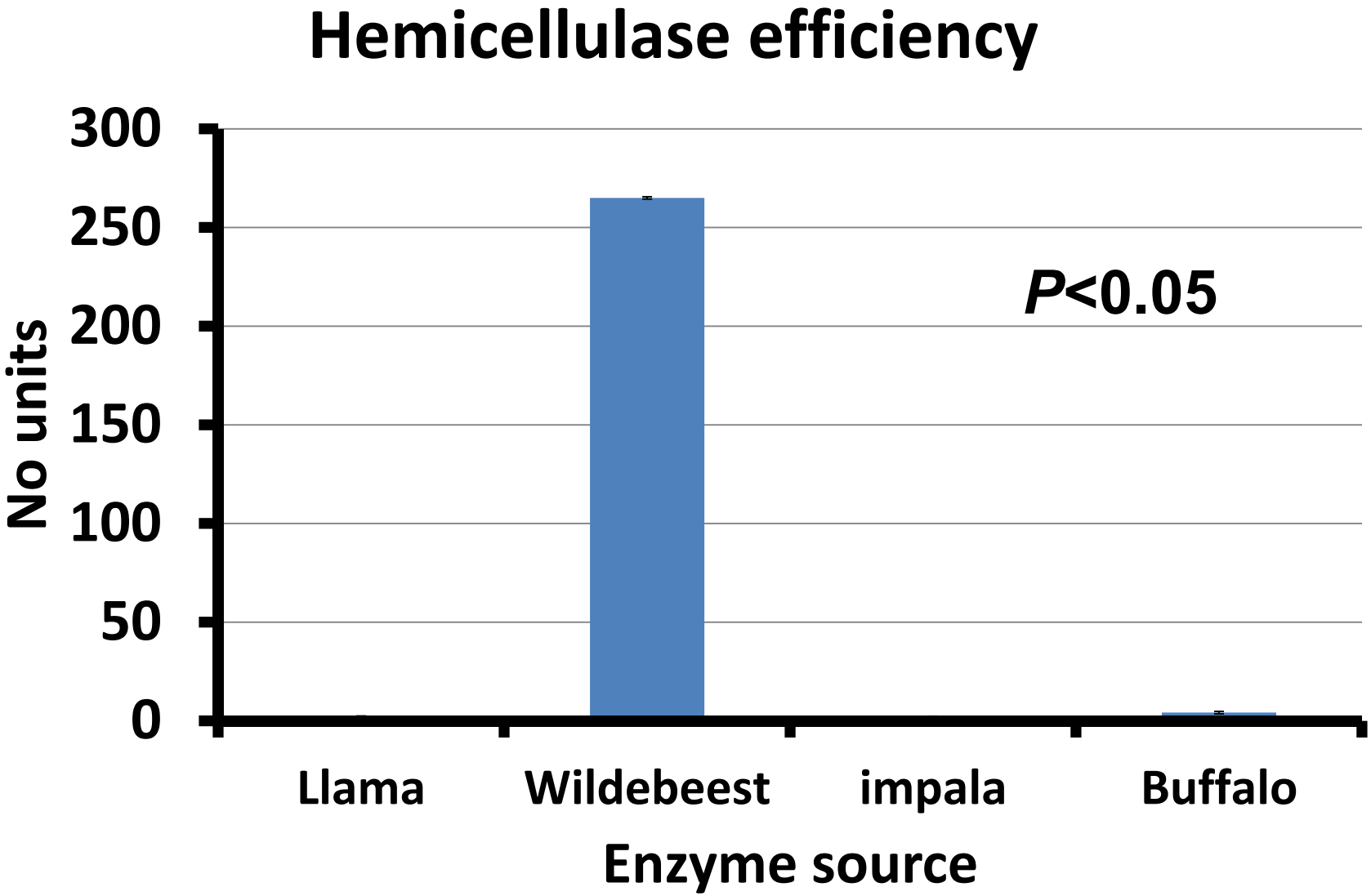
Results …


30
25
20
15
10
5
0
µg reducing sugar/mg/min
Exocellulase Vmax
Endocellulase Vmax
Hemicellulase Vmax
P<0.05
Llama
Wildebeest
impala
Buffalo
Enzyme source

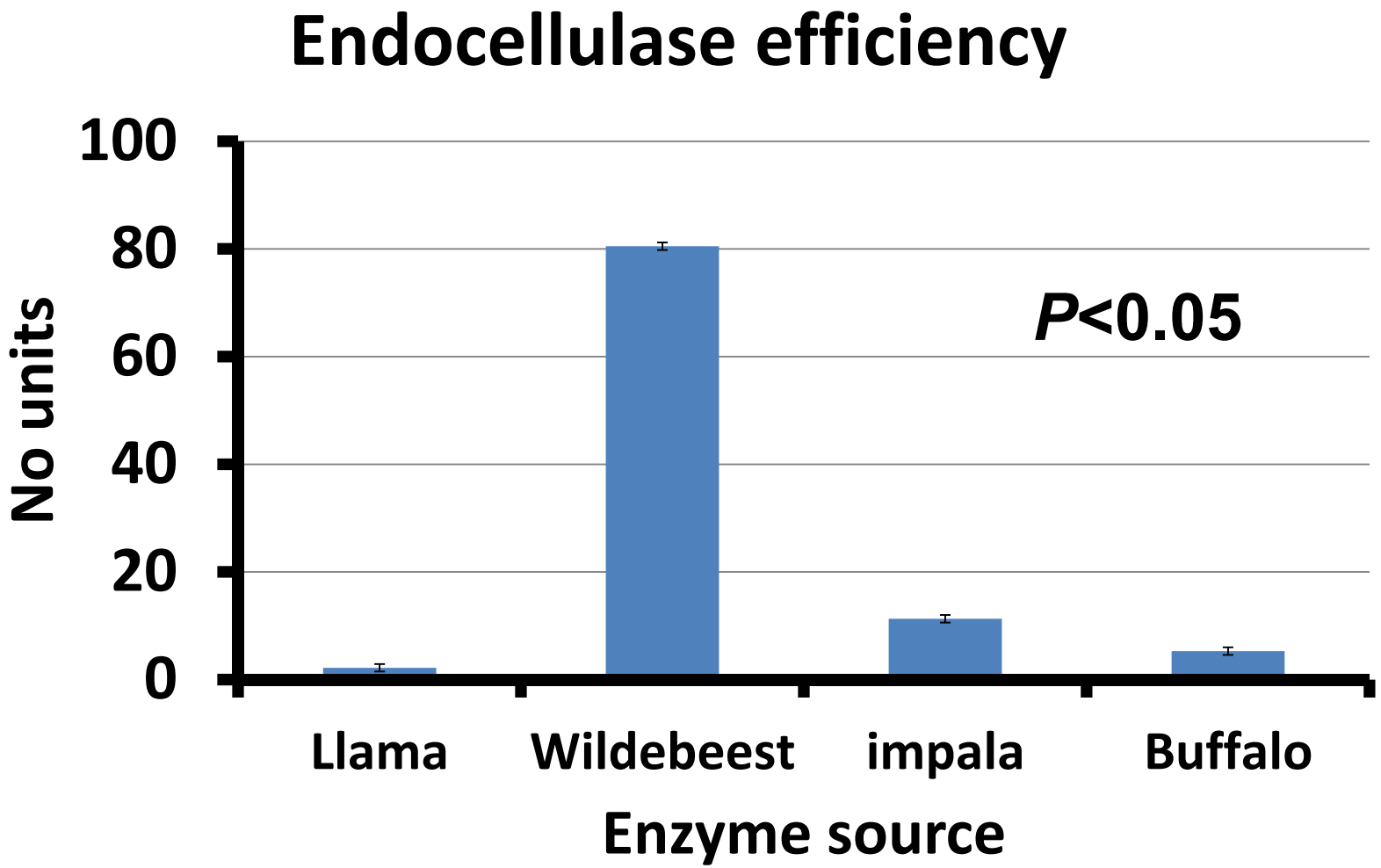
Results …


Exocellulase efficiency
120
100
80
60
40
20
0
No units
P<0.05
Llama
Wildebeest
impala
Buffalo
Enzyme source

Results …


Hemicellulase efficiency
300
250
200
150
100
50
0
No units
P<0.05
Llama
Wildebeest
impala
Buffalo
Enzyme source

Results …


Endocellulase efficiency
100
80
60
40
20
0
No units
P<0.05
Llama
Wildebeest
impala
Buffalo
Enzyme source

Findings

- Enzymes were successfully Isolated
- Both cellulase and hemicellulase crude enzyme mixtures were active
- Wildebeest and impala showed the greatest potential in hydrolyzing cellulose, endocellulose and hemicellulase

conclusion

- **Wildebeest and impala showed the greatest potential in hydrolyzing cellulose and hemicellulose than llama and buffalo grazing of same field**
- **<u>Further studies</u>**
- Harbour more cellulolytic microbes ?
- Microbes that have evolved?
- Purification of enzymes may answer these questions
- Application as feed supplements !!!
- Synergism among microbial ecosystems

Thanks for thinking about us.

At least we can spend less time grazing