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Effects of a fibrolytic enzyme cocktail on *in sacco* and *in vitro* digestibility of forages or feed

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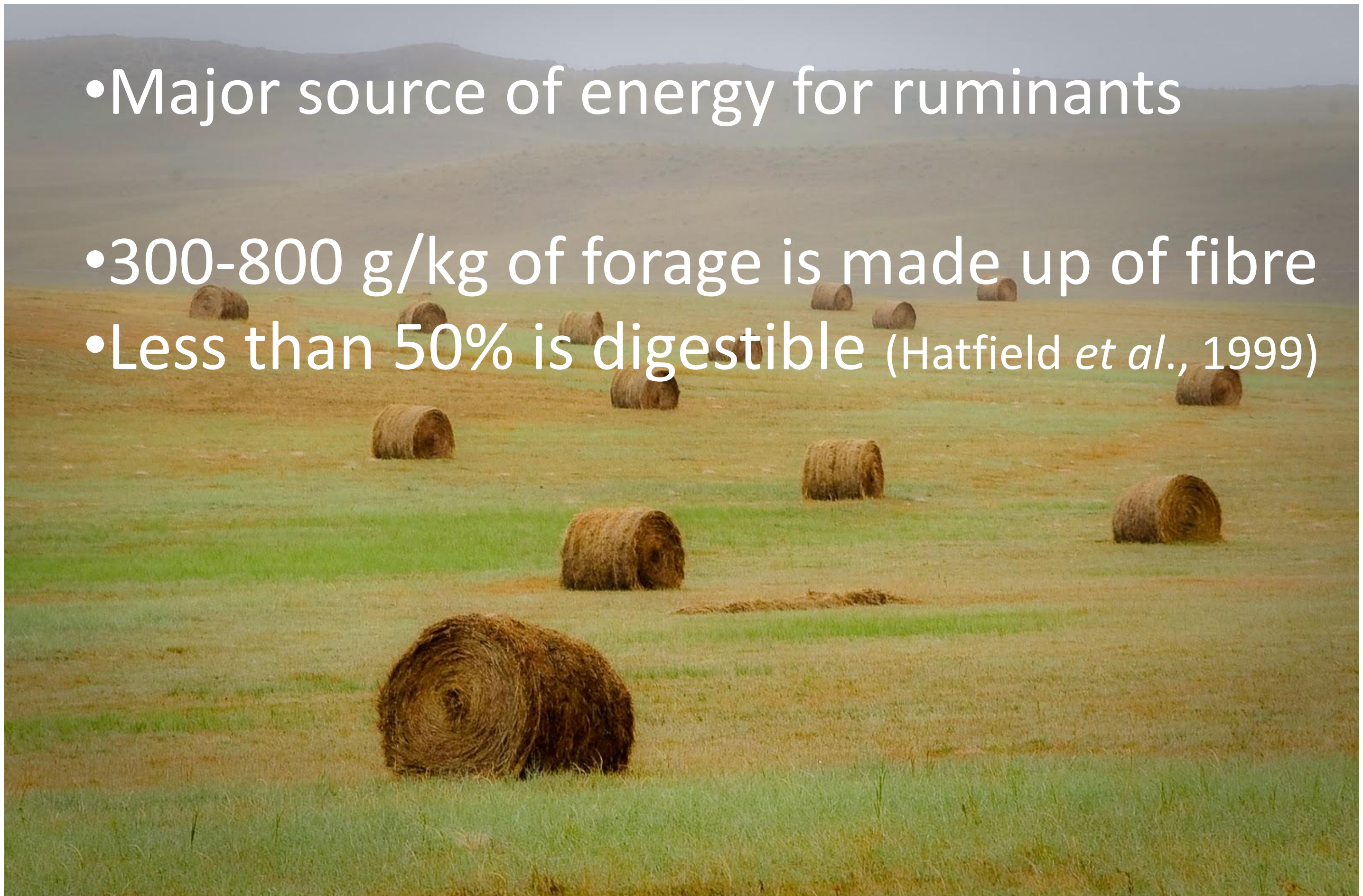
Introduction

Where do exogenous enzymes originate from?

- Industrial applications (Bhat, 2000)
  - Food and beverage industry
  - Laundry and textile industry
  - Paper biotechnology
- Novel enzyme production
  - Fungal strains grown on wheat straw (deep bed fermentation)
- Why the interest in enzymes for the animal feed industry?

Fibre!

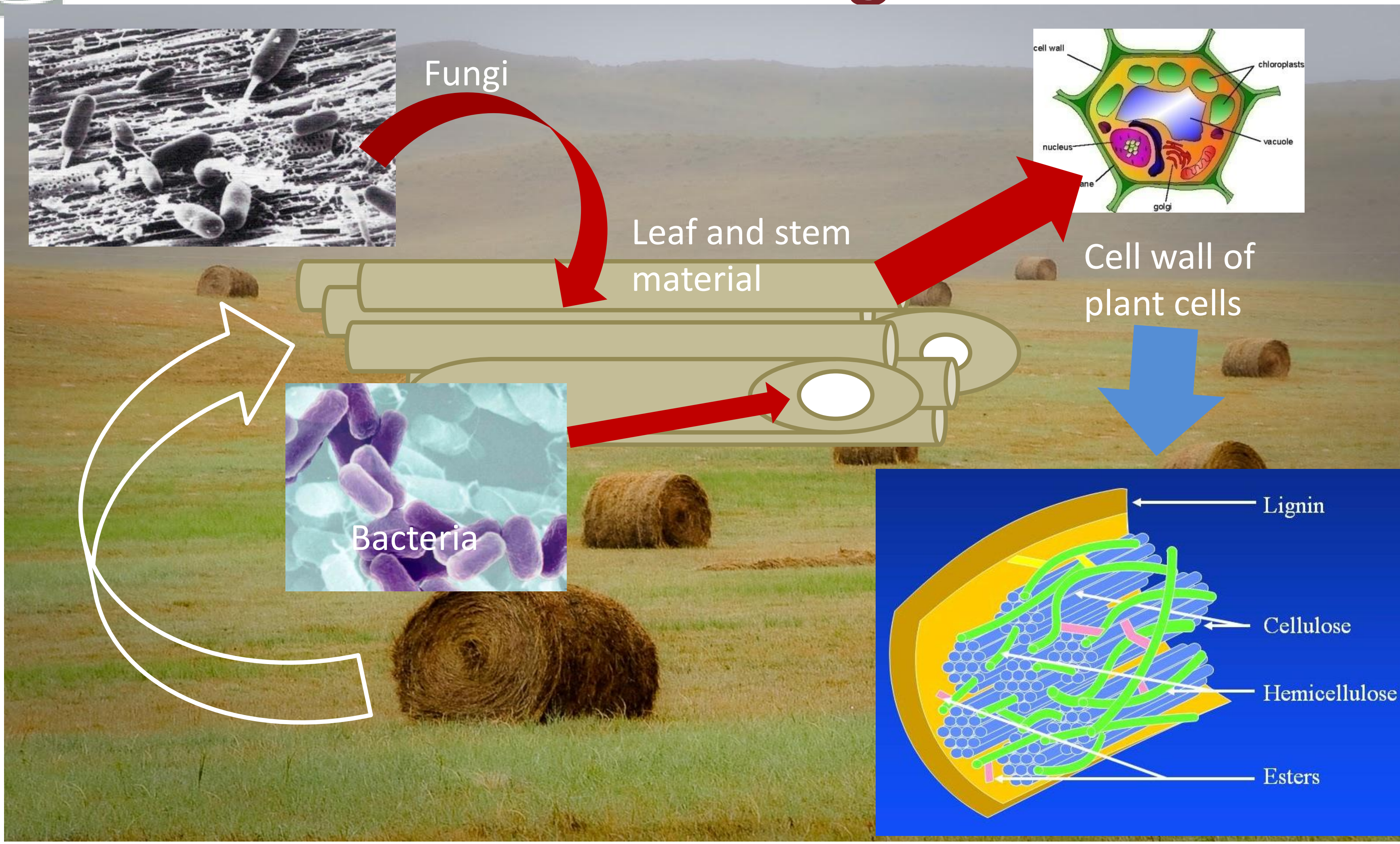


- Major source of energy for ruminants

- 300-800 g/kg of forage is made up of fibre
- Less than 50% is digestible (Hatfield *et al.*, 1999)

http://cowtowncarrie.blogspot.com/

Limitations to digestion



Boon *et al.* (2005)

Overcoming these limitations

- Mastication (Wilson *et al.*, 1990)
- Optimal conditions within the rumen
  - Colonization of particles
  - Successive microbial adhesion and digestion
- Plant breeding
- Mechanical processing
- Manipulation of rumen microorganisms
- **Exogenous fibrolytic enzyme treatment** (numerous researchers)

Exogenous fibrolytic enzymes (**EFE**)

- Commercial products from other industries
- Novel production of fungal strains
  - Department of Microbiology, SU
  - Production of ABO 374 from South African soil
  - Supernatant contains fibrolytic activities
    - **Xylanases (296 ± 0.07 U/mg protein)**
    - Cellulases (1.44 ± 0.39 U/mg protein)
    - Mannanases (1.10 ± 0.37 U/mg protein)
      - Cruywagen & van Zyl (2008)

Does it work?

- Beef cattle performance
  - Improved daily gains
  - Increased feed intake
  - Improved FCR
- Dairy cattle
  - Increased feed intake
  - Increased milk production
- Sheep and goats
  - Improved FCR
  - Improved body weight gains
  - Increased DMI
- Monogastric animals
  - Alleviate anti-nutritional feed factors
    - Fibre
    - Phytase
      - Phosphorous

Where does it work?

- Animal responses are related to:
  - Improvement in feed digestibility
  - Improvement in fibre digestibility
  - Improvement in protein digestibility
  - Improvement in the rate of digestion of nutrients
  - Decreased lag times for digestion

  - *In vivo, in vitro* and *in situ*

Does it work?

- Inconsistent results
  - Feed digestibility not consistently affected
  - No or even negative effects have been reported
- **Clarification of the Mode-of-action and application method is of importance**

Experimental design

Two studies:

1. The effect of EFE on *in vitro* gas production and digestibility of different forages
2. The effect of EFE on *in sacco* and *in vitro* digestibility of a complete feed for sheep

1. *In vitro* gas production and digestibility of forages

Hypothesis:

- Can EFE improve the *in vitro* gas production and digestibility of forages
  - Lucerne hay *(Medicago sativa*)
  - Weeping love grass hay *(Eragrostis curvula*)
  - Kikuyu hay (*Pennisetum clandestinum*)

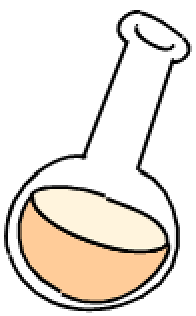
1. Methodology

Kikuyu
Weeping love grass
Lucerne

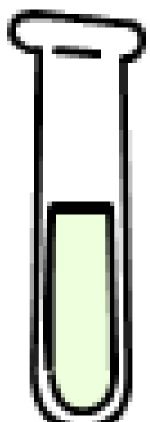
Rumen fluid

1. *In vitro* gas production
→ manual measurement

2. *In vitro* true digestibility
ANKOM Daisy fermentor



Exogenous
fibrolytic enzyme



Anaerobic incubation,
$39^{o}C$

1. $\mathbf{Y = b(1-e^{-c(t-L)})}$
   $\mathbf{Y = b(1-e^{-ct})}$

Y = cumulative GP
b = asymptotic GP
c = rate of GP (per h)
t = incubation time, h
L = lag time, h

2. IVTD according to ANKOM procedure

In vitro fibre digestibility

Statistical analysis

Randomized design with triplicate repetitions within two runs

- Fractional GP and IVTD were analyzed using Main effects ANOVA
- Total GP were analyzed using Repeated measures ANOVA
- GP kinetic values were analyzed using a factorial ANOVA
- Statistica 8.1

Results

Table 1: Gas production fermentation kinetics of lucerne hay or kikuyu treated with EFE and incubated in buffered rumen fluid for 72 h

Lucerne hay	Control	EFE cocktail	SEM	*P*
		Model with lag		
b	108.87	103.86	1.16	0.013
c	0.049	0.054	0.00089	0.0046
Lag	0.373	0.307	0.039	0.26
		Model without lag		
b	109.84	104.56	1.15	0.0086
c	0.047	0.052	0.00082	0.0022
Dried kikuyu	**Control**	**EFE cocktail**	**SEM**	***P***
		Model with lag		
b	156.32	165.94	2.87	0.039
c	0.016	0.019	0.00029	0.0011
Lag	1.40	1.40	0.027	0.99
		Model without lag		
b	175.43	183.41	3.74	0.16
c	0.013	0.015	0.00030	0.0021

b: asymptotic total gas production, c: rate of gas production (per h), lag: lag time (h)

Results

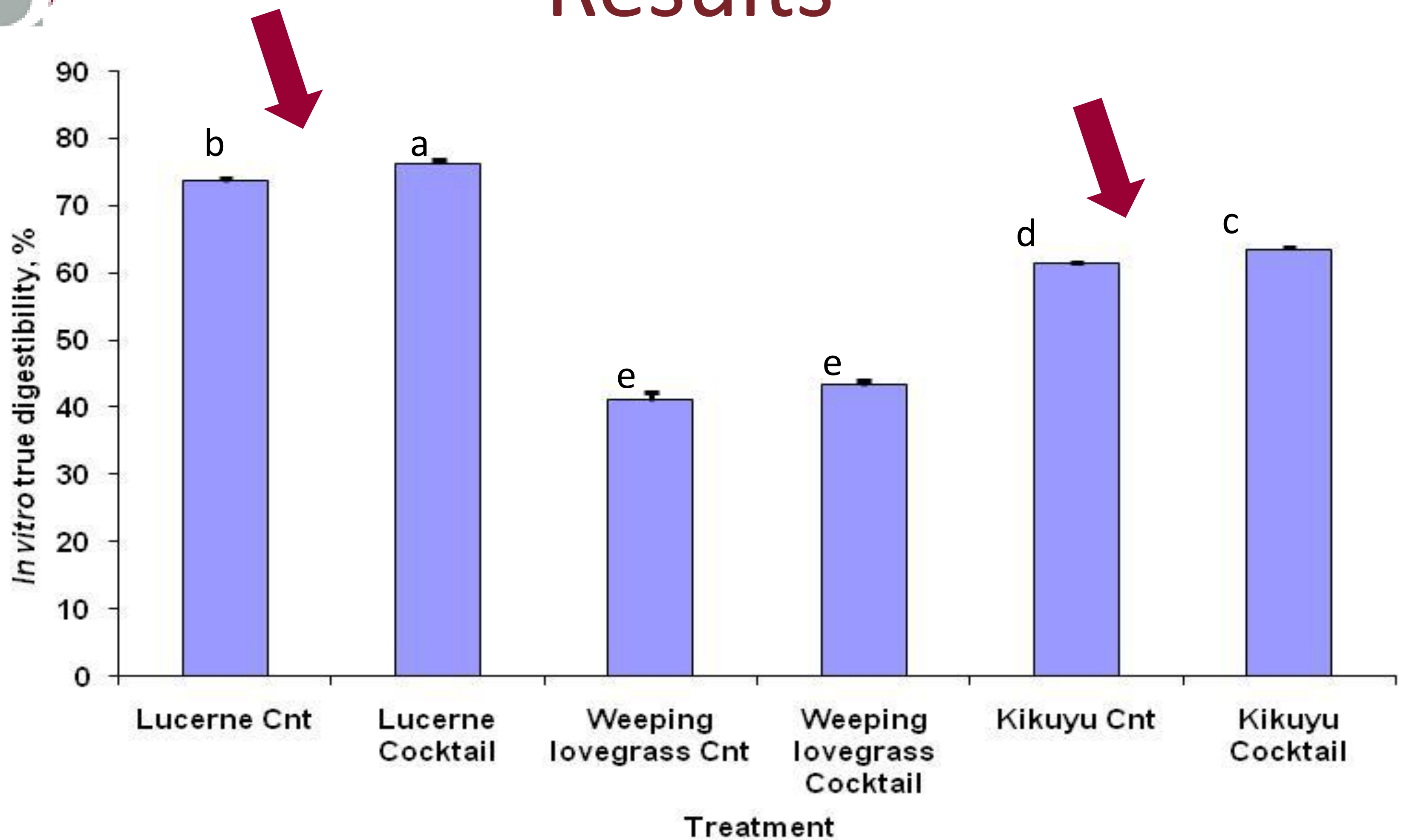


Figure 1. *In vitro* true digestibility of three forages treated with EFE after 24h incubation in buffered rumen fluid.

Error bars represent the SEM.

Different superscripts (a, b, c, d or e) indicate significant differences ($P \leq 0.05$).

Discussion

1. Enzyme effects are exerted during the 1st 6-12h of incubation (kikuyu, weeping love grass)
2. EFE resulted in increased total GP (b) (kikuyu, ~~lucerne~~)
3. EFE resulted in higher rate of GP (c) (kikuyu, lucerne)
4. GP results were substantiated by improved IVTD (kikuyu, lucerne)

2. The effect of EFE on *in sacco* and *in vitro* digestibility of a **complete feed for sheep**

Hypothesis:

- Can EFE improve the *in sacco* and *in vitro* digestibility of a composite sheep feed?

Formulation and chemical composition (DM basis) of a composite feed for sheep

Raw material	Inclusion, g/kg
Maize meal	450
Wheat	150
Lucerne meal	120
Oat hay	200
Cotton seed oilcake meal	50
MuttonGainer premix	30
TOTAL	1000
Chemical composition	**g/kg**
Moisture	132
DM	868
Crude protein	166
NDF	349
Ether extract	11.3
Ash	46.5

2. Methodology

Composite feed

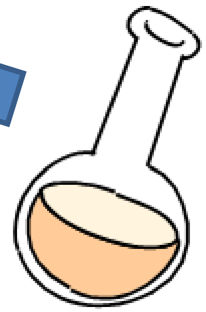
1

ANKOM polyester bags

***In sacco* nutrient disappearance**

$Y = a + b(1-exp^{-c(t-L)})$

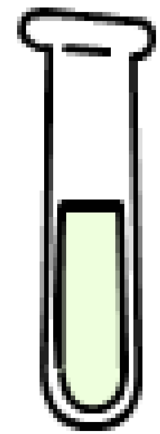
a = readily degradable fraction
b = potentially degradable fraction
c = degradation rate of fraction b
t = incubation time
L = Lag time



EFE
12h pre
incubation

2

Rumen fluid



Anaerobic incubation, 39^{o}C

IVTD according to ANKOM procedure

In vitro fibre digestibility

Statistical analysis

- *In sacco* study: Cross over design with six cannulated sheep receiving the Control or EFE treated feed
  - Kinetic values and Effective degradability were subjected to a Main effects ANOVA
- IVTD study: Three replications per treatment and performed parallel to the *in sacco* study
  - Data were subjected to a Repeated Measures ANOVA
- Statistica 9.0

In sacco digestibility results DM and CP degradation fermentation kinetic values of a composite feed

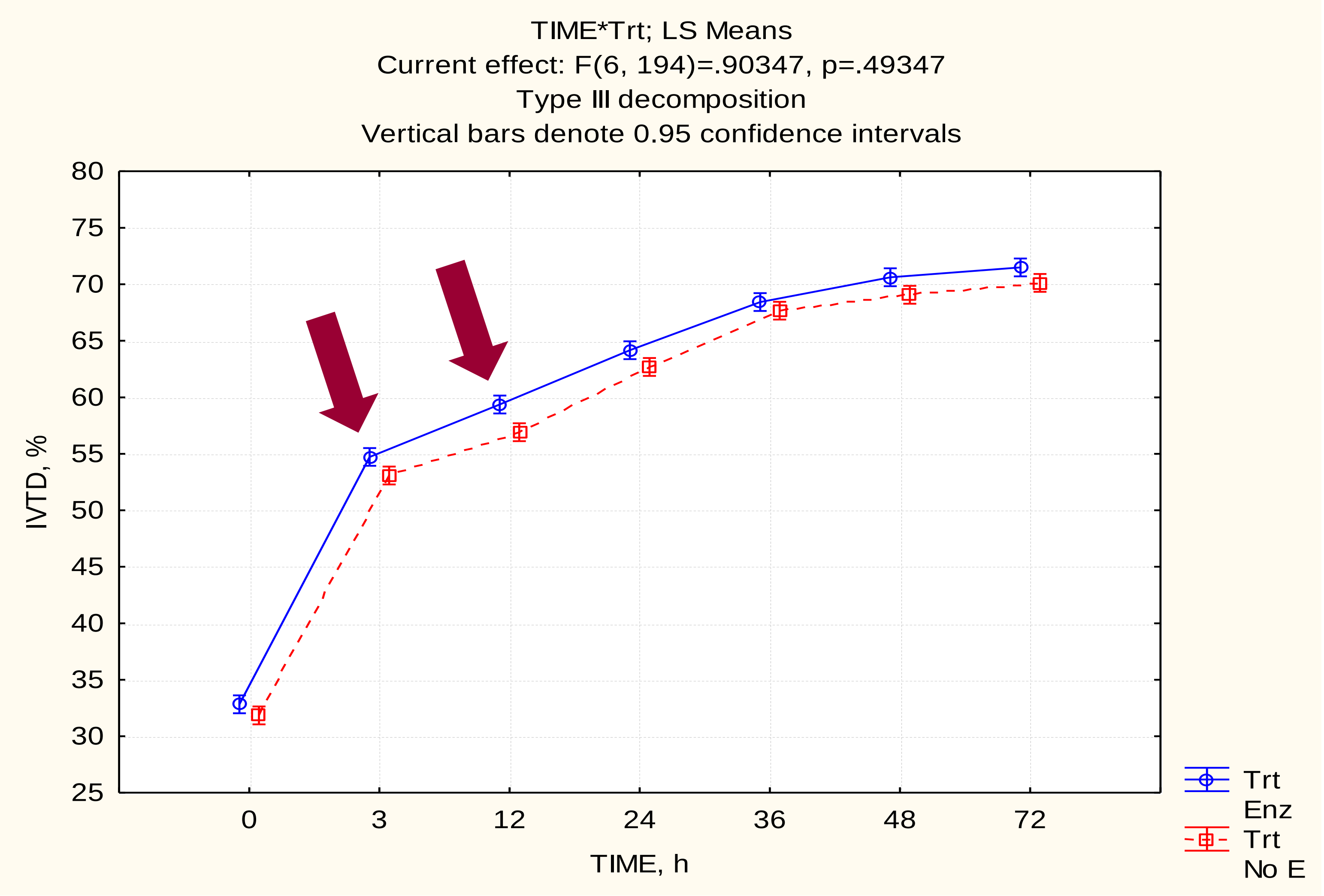
DMD	Control	EFE	SEM	P
a, %	22.06	21.64	0.47	0.54
b, %	60.97	62.89	0.84	0.14
c, per h	**0.042**	**0.062**	**0.002**	**0.0002**
Lag	0.086	0.085	0.079	0.90
Eff. dg. %	**49.89**	**57.40**	**1.04**	**0.0006**

CP dg	Control	EFE	SEM	P
a, %	30.20	29.47	0.49	0.32
b, %	**38.31**	**41.13**	**0.59**	**0.008**
c, per h	**0.061**	**0.091**	**0.007**	**0.015**
Lag	0.14	0.14	0.011	0.93
Eff. dg. %	**50.88**	**55.91**	**0.91**	**0.0037**

In sacco digestibility results NDF disappearance fermentation kinetic values of a composite feed

NDF disapp.	Control	EFE	SEM	P
a, %	**19.30**	**16.39**	**0.61**	**0.0082**
b, %	61.12	60.60	4.33	0.93
c, per h	**0.019**	**0.040**	**0.0037**	**0.0031**
Lag	**0.27**	**0.10**	**0.022**	**0.0004**
Eff. dg. %	**35.17**	**42.82**	**0.74**	**0.0005**

Results
IVTD of a completely balanced sheep feed



Discussion & Conclusion

1. EFE resulted in increased rate of degradation (c) *in situ* for DM, CP and NDF
2. EFE resulted in improved Effective degradability of DM, CP and NDF
3. Results were again substantiated by improved IVTD of the composite feed
4. Of interest is the positive results of the fibrolytic enzyme treatment on the protein of the diet
   1. Corresponds with literature
   2. Protein is made available for digestion by EFE

Fibrolytic enzymes: General conclusion

- Improvement of the digestibility of poor quality roughages is yet to be achieved
  - Clear characterization of enzyme products needed
- Increased focus on the architecture of plant material in addition to its chemical composition

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