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# Grass biorefinery – effect of formic acid treatment, N fertilization and harvest time

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# Grass for biorefinery – Effects of N fertilization and harvest time on liquid yield and composition

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## Introduction

The concept of green biorefinery is based on the processing of green biomass into a range of innovative products. The development of biorefinery allows the utilisation of plants to feed both non-ruminants and ruminants due to the separation of liquid and solid fractions.

We hypothesized that the yield and quality of protein in liquid and fibre fraction from biorefinery may vary depending on N fertilizing level and time of harvesting of the green biomass.

## Materials and methods

A biorefinery experiment was carried out using pure Timothy grass from first and second cut.

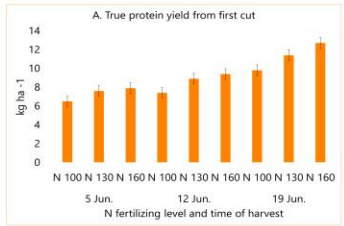

*Table 1. . Effect of N fertilizer level and harvest time of Timothy grass on grass composition and liquid yield*

| Harvest time in 2023 | N fertilizer, kg ha$^{-1}$ | Grass DM, g kg$^{-1}$ | Liquid yield, g kg$^{-1}$ | Liquid DM, g kg$^{-1}$ | Liquid ash, g kg$^{-1}$ DM | Liquid yield, kg ha$^{-1}$ | N yield in liquid, kg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|
| First cut | | | | | | | |
| June 5 | 100 | 299 | 528 | 184 | 88 | 3085 | 8.3 |
| | 130 | 290 | 539 | 178 | 86 | 3295 | 10.3 |
| | 160 | 289 | 546 | 171 | 98 | 3439 | 11.9 |
| June 12 | 100 | 314 | 491 | 189 | 97 | 3739 | 8.4 |
| | 130 | 297 | 503 | 175 | 101 | 4306 | 10.8 |
| | 160 | 294 | 516 | 176 | 99 | 4714 | 12.9 |
| June 19 | 100 | 265 | 553 | 135 | 116 | 6502 | 13.4 |
| | 130 | 262 | 561 | 128 | 124 | 6908 | 16.3 |
| | 160 | 250 | 567 | 118 | 143 | 7764 | 19.1 |
| Second cut | | | | | | | |
| July 25 | 110 | 244 | 555 | 96 | 201 | 7365 | 17.0 |
| | 140 | 224 | 576 | 91 | 185 | 8693 | 24.1 |
| July 31 | 110 | 206 | 631 | 82 | 171 | 12100 | 23.1 |
| | 140 | 198 | 635 | 73 | 190 | 13983 | 29.1 |
| August 7 | 110 | 225 | 578 | 78 | 165 | 12816 | 22.4 |
| | 140 | 218 | 603 | 70 | 190 | 13939 | 26.9 |
| | | P-value | | | | | |
| First cut | Harvest time | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| | N fertilizer | 0.002 | 0.036 | 0.006 | 0.003 | <0.001 | <0.001 |
| | Harvest time × N fertilizer | 0.470 | 0.975 | 0.801 | 0.036 | 0.158 | 0.640 |
| Second cut | Harvest time | 0.002 | <0.001 | <0.001 | 0.485 | <0.001 | 0.007 |
| | N fertilizer | 0.060 | 0.115 | <0.001 | 0.405 | 0.004 | <0.001 |

## Results

Both cuts had three harvest times at one-week intervals and fertilized with variable N kg ha-$^{1}$ with 3 replications per treatment.

The growing conditions were cool and dry for the first cut but humid and warm for the second cut. The grass was harvested and then frozen. Prior to the processing, the samples were allowed to melt. The biomass was separated into solid and liquid fractions using a laboratory scale twin screw Angel press



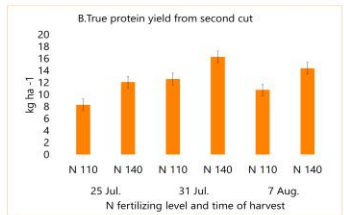




***Figure 1.** Effect of N fertilizer level and harvest time of Timothy grass on N yield as true protein in first (A) and second (B) cut.*

There is a great effect of agronomic factors in grass production on the total liquid, N and true protein yield per hectare. Optimizing the agronomy will play an important role for the success of the biorefinery process.

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*This work was conducted as part of GrassProtein project funded by the Finnish Ministry of Agriculture and Forestry / Makera (VN/7679/2021).*

# Effect of formic acid treatment of grass liquid fraction on protein separation efficiency

**Tomasz Stefański,** Nisola Ayanfe and Marketta Rinne, Natural Resources Institute Finland

## Introduction

Protein is one of the most expensive and most imported feed ingredients in European countries. Green forage can produce high protein yields per hectare with many environmental benefits for ecosystems. Through biorefinery, protein-rich liquid can be separated from the biomass. Low-cost method is needed to separate the protein from the liquid fraction.

## Hypothesis

We hypothesized that by addition of formic acid to the liquid fraction and by using natural sedimentation process, the soluble protein in liquid fraction can be concentrated in a small volume of sediment.

## Materials and methods

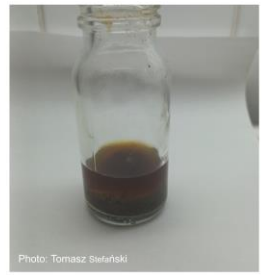
Liquid fraction from biorefining of pure Timothy grass from first cut was used to conduct this experiment. The study was conducted using 500 ml of liquid fraction with 3 replications for each treatment. The liquid fraction was treated with five levels of pure formic acid equivalent (L Mg$^{-1}$):

- 0
- 4
- 6
- 8
- 10


**Table 1:** Effect of formic acid treatment of grass liquid fraction on extraction rates of, dry matter, ash and crude protein into the sediment.

| | Formic acid in L Mg$^{-1}$ | | | | | SEM | P-values | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 8 | 10 | | Linear | Quadratic |
| Extraction rates (g kg$^{-1}$) of fresh liquid | | | | | | | | |
| Dry matter | 309 | 442 | 435 | 437 | 435 | 6.9 | <0.001 | <0.001 |
| Ash | 301 | 283 | 280 | 299 | 261 | 6.8 | 0.017 | 0.600 |
| Crude protein | 630 | 580 | 540 | 510 | 500 | 10.8 | <0.001 | 0.187 |
| True protein | 800 | 942 | 953 | 947 | 942 | 18.5 | <0.001 | 0.004 |

The bottles with formic acid treatment were allowed to stand in dark place with room temperature for 21 days and the bottles without formic acid (control) where opened after 6 days to reduce the effect of natural fermentation on the pH of the liquid.




When bottles were opened, the clear brown liquid at the top was removed using vacuum.

## Results

The sedimentation process could be observed already 2 h after the addition of the formic acid. Four days after the liquid fraction was treated with formic acid, no more progress in sedimentation process was visually observed in the formic acid treated vessels. In the bottles two clear layers could be observed: a transparent brown liquid layer on the top and a green non-transparent layer on the bottom of the bottles.

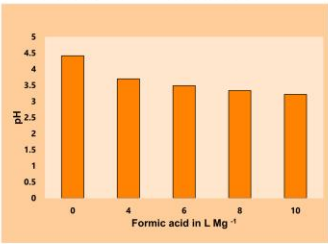



**Figure 1.** Effect of formic acid treatment of liquid fraction on pH of brown liquid after 6 days for control and 21 days for acid treatment.

**Formic acid-assisted natural sedimentation method could be very useful in concentrating the fresh liquid from biorefinery for further treatment or for direct use as a semi-liquid feed. Many commercial silage additives are based on formic acid so treatment with formic acid is also preserving the sediment as well as the brown liquid.**

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# Effects of N fertilization and harvest time on liquid yield and composition

The growing conditions were cool and dry for the first cut but humid and warm for the second cut

| Harvest time in 2023 | N fertilizer, kg ha$^{-1}$ |
|---|---|
| **First cut** | |
| **June 5** | 100 |
| | 130 |
| | 160 |
| **June 12** | 100 |
| | 130 |
| | 160 |
| **June 19** | 100 |
| | 130 |
| | 160 |
| **Second cut** | |
| **July 25** | 110 |
| | 140 |
| **July 31** | 110 |
| | 140 |
| **August 7** | 110 |
| | 140 |
| | |

# *Effect of N fertilizer level and harvest time of Timothy grass on grass composition and liquid yield*

| Harvest time in 2023 | N fertilizer, kg ha$^{-1}$ | Grass DM, g kg$^{-1}$ | Liquid yield, g kg$^{-1}$ | Liquid DM, g kg$^{-1}$ | Liquid ash, g kg$^{-1}$ DM | Liquid yield, kg ha$^{-1}$ | N yield in liquid, kg ha$^{-1}$ |
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| **First cut** | Harvest time | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
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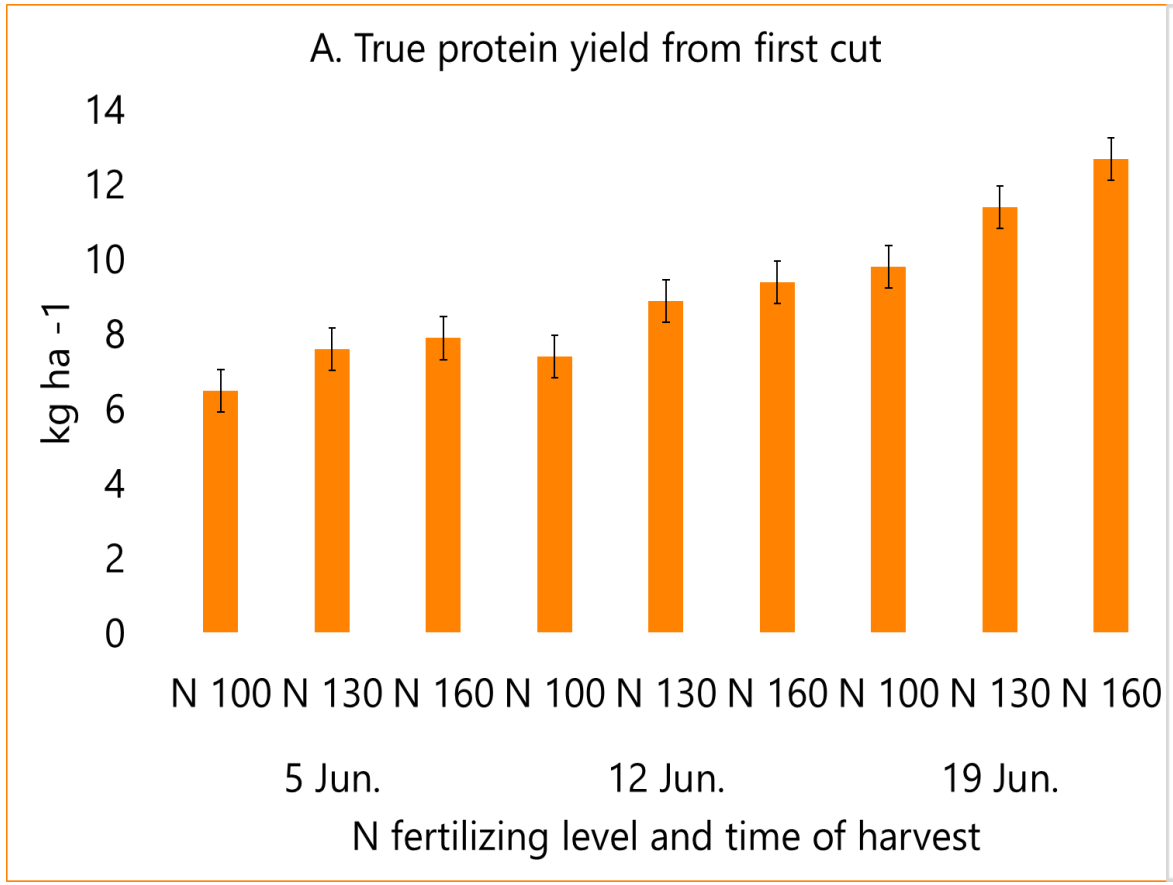
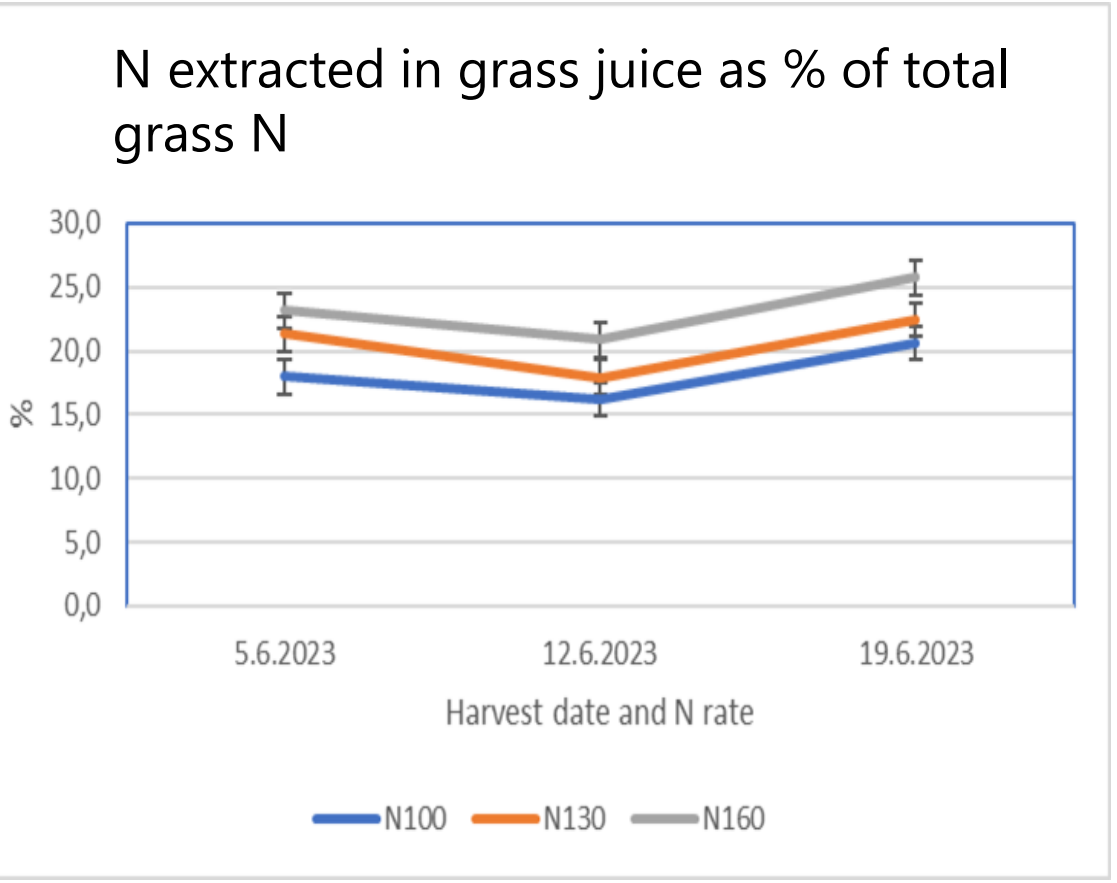
| | Harvest time × N fertilizer | 0.470 | 0.975 | 0.801 | 0.036 | 0.158 | 0.640 |
| **Second cut** | Harvest time | 0.002 | <0.001 | <0.001 | 0.485 | <0.001 | 0.007 |
| | N fertilizer | 0.060 | 0.115 | <0.001 | 0.405 | 0.004 | <0.001 |

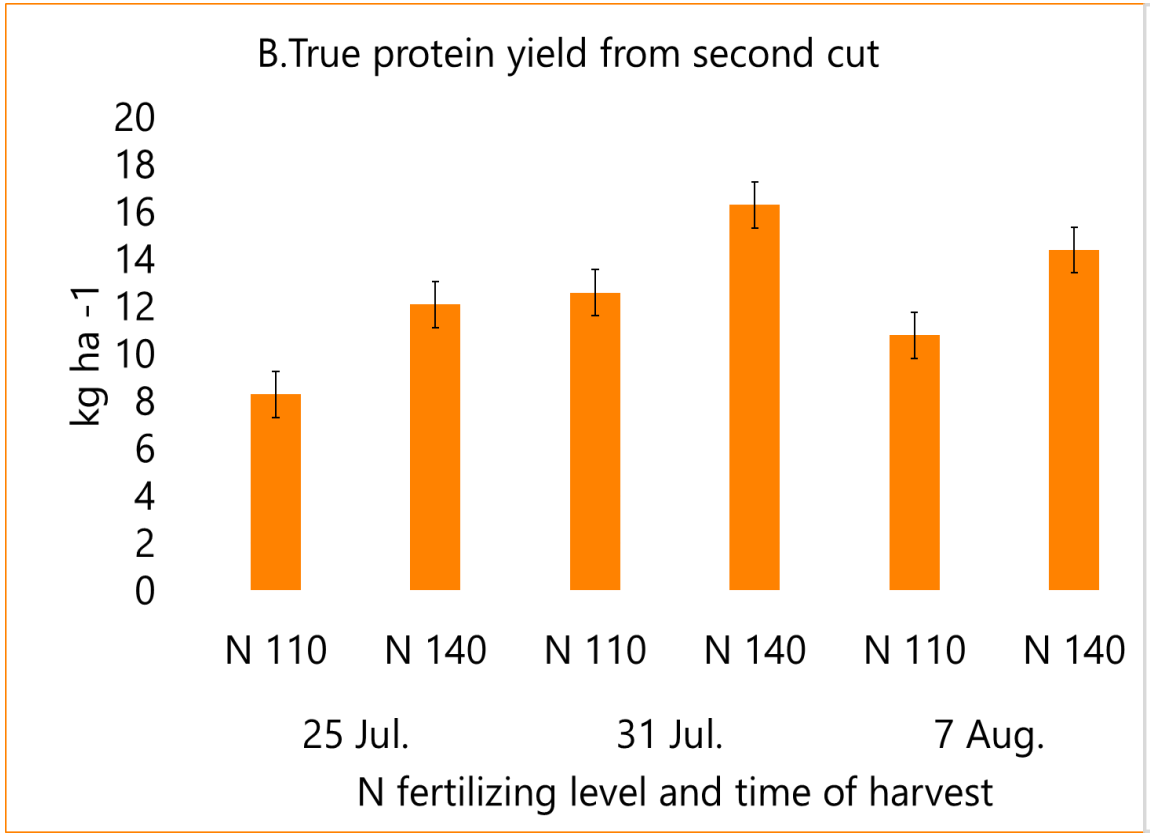
# Effect of N fertilizer level and harvest time of Timothy grass on N and true protein yield in first cut.

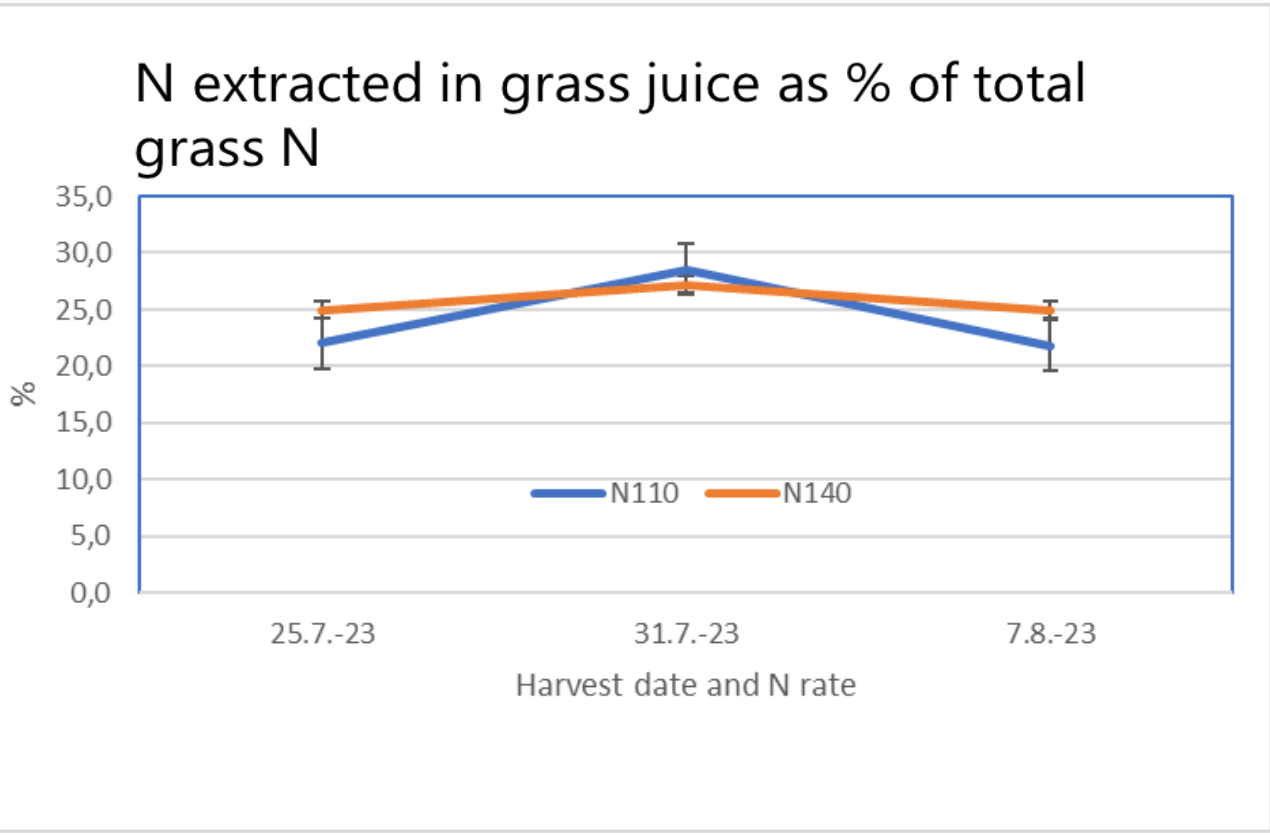


# Effect of N fertilizer level and harvest time of Timothy grass on N and true protein yields in second cut.



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# Effect of formic acid treatment of grass liquid fraction on protein separation efficiency



## Liquid fraction – green juice

- Very low nutrience density
- Ease to spoil
- High potassium level

Photo: Marketta Rinne / Luke

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# New low-cost methods of extracting-condensing green protein from fresh green juice

- Treatment of juice from fresh material with organic acid and assist with natural sedimentation
- 500 g green juice
  - Control
  - 4 l/t of formic acid
  - 6 l/t of formic acid
  - 8 l/t of formic acid
  - 10 l/t of formic acid
- 21 days sedimentation
- (after 7 days there was no
- progress in sedimentation)

Photo: Tomasz Stefański

- Treatment of juice from fresh material with organic  acid preserve the juice preventing of spoilage

# Effect of formic acid treatment of grass liquid fraction on extraction rates of, dry matter, ash and crude protein into the sediment.

| | Formic acid in L $Mg^{-1}$ | | | | | SEM | P-values | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 8 | 10 | | Linear | Quadratic |
| Extraction rates (g $kg^{-1}$) of fresh liquid | | | | | | | | |
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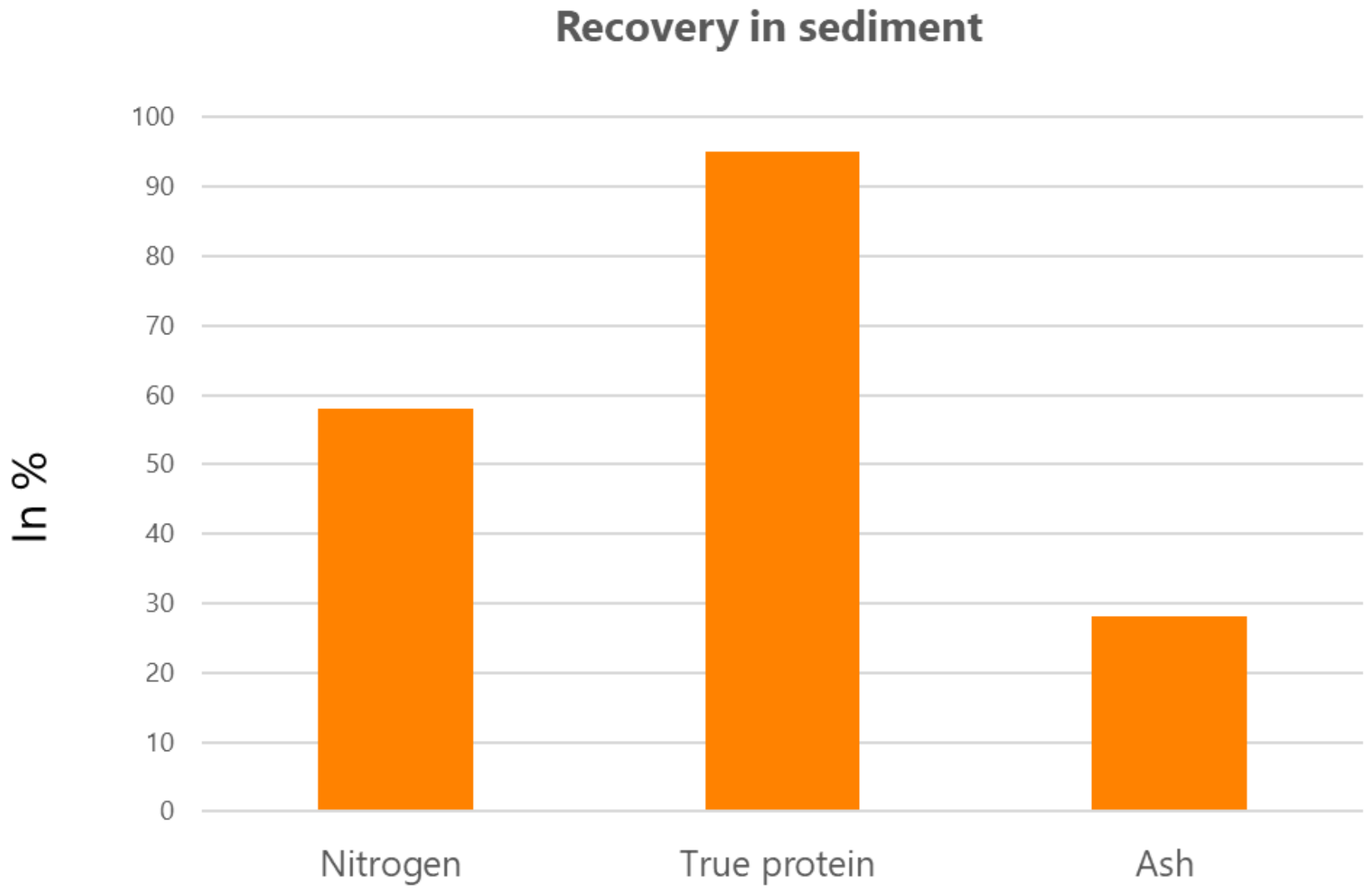
Recovery in sediment
In %
100
90
80
70
60
50
40
30
20
10
0
Nitrogen
True protein
Ash

# Some ideas for use of the condense liquid green protein



- Feed for pigs
- Piglets are very **sensitive** to soybeans ( it need to be specially processed)
  - The liquid protein concentrate could replace soya in piglet feeds
  - Source of formic acid for pig feeds

Photo: Improve Gut Health in Piglets – MSP[RS] Resistant Starch (mspresistantstarch.com)

- Centrifugated and dry to produce

  dry protein feed for poultry and pigs

Photo: Broiler Photos, Download The BEST Free Broiler Stock Photos & HD Images (pexels.com)/

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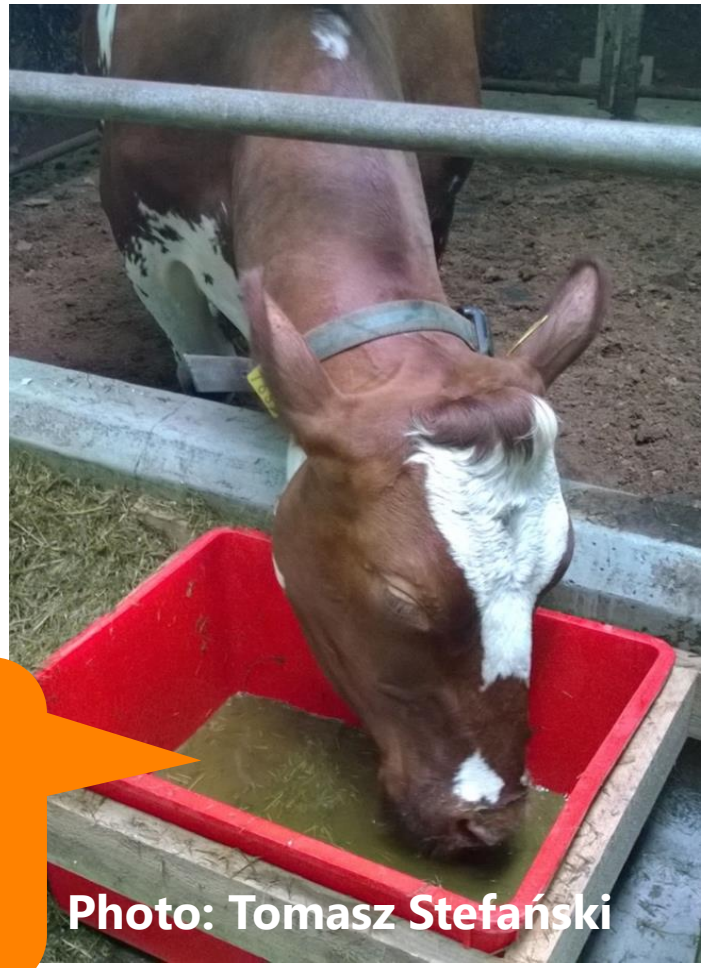
# Condense liquid green protein for ruminants

- Could provide a new protein source
- By-pass of protein from rumen?
- Lower fill value
- Increase the proportion of 'forage' in dairy cows diet




Condense liquid green protein could be offered as such or mixed in TMR

# Thank you!

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