

Mechanochemical Synthesis of a Urea-Proline Cocrystal as Dairy Cattle Feed and its Effect on Ruminal Degradation

Laura Contini,¹ Diptajyoti Gogoi,² Ana Carolina Fluck,³ Júlia Gomes Farias,⁴ Clinton Williams,⁴ Ranijt Thakuria,^{2,*} Jonas Baltrusaitis^{5,*} and Fabrizia Grepioni^{1,*}

¹Department of Chemistry “Giacomo Ciamician”, University of Bologna, Via Gobetti 85, 40129 Bologna, Italy

²Department of Chemistry, Gauhati University, Guwahati, Assam 781014, India

³Universidade Tecnológica Federal do Paraná, Estrada para Boa Esperança, s/n, Dois Vizinhos, PR, Brasil

⁴USDA-ARS, US Arid Land Agricultural Research Center, 21881 N. Cardon Ln, Maricopa, AZ 85138, USA

⁵Department of Chemical and Biomolecular Engineering, Lehigh University, B336 Iacocca Hall, 111 Research Drive, Bethlehem, PA 18015, USA

Supporting Information (10 pages)

1. Powder X-ray diffraction.....	Page 2
2. Crystallographic tables and Rietveld refinement.....	Page 4
3. DSC and TGA	Page 5
4. Variable Temperature X-ray diffraction.....	Page 6
5. Hot Stage Microscopy.....	Page 8
6. Raw digestibility data of urea·L-proline·H ₂ O (1)	Page 9
7. Solubility measurements.....	Page 10
8. Bibliography.....	Page 10

1. Powder X-ray diffraction

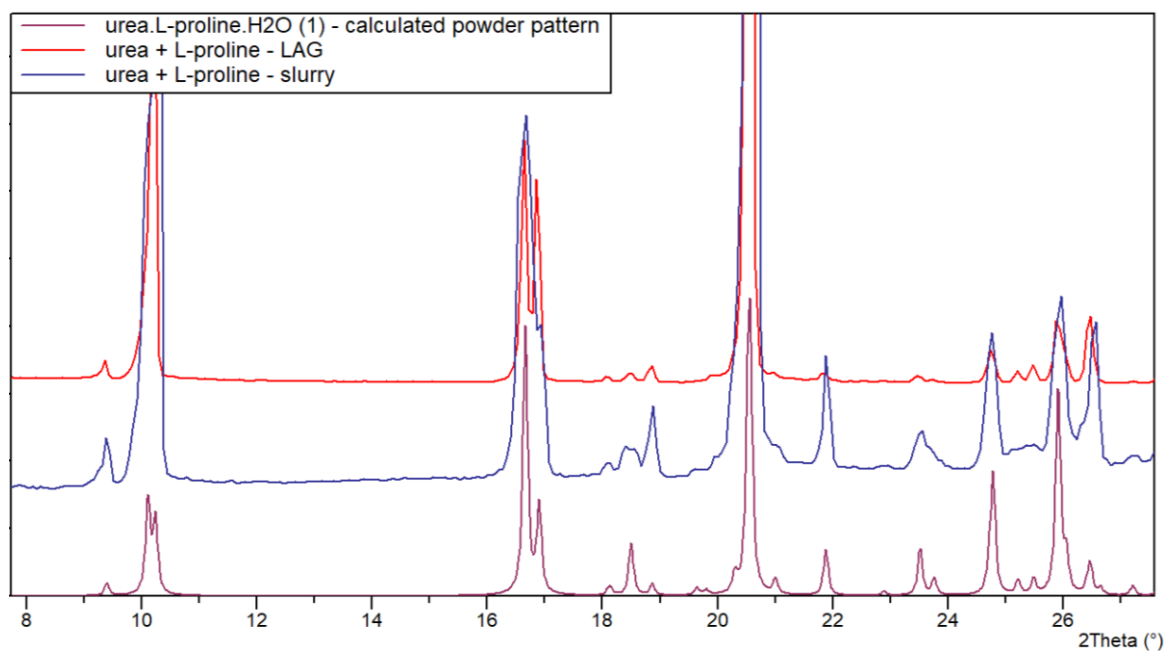


Figure SI-1. Comparison between the experimental PXRD patterns for urea·L-proline·H₂O (1) obtained via LAG (red) and slurry (blue) and the calculated one (purple).

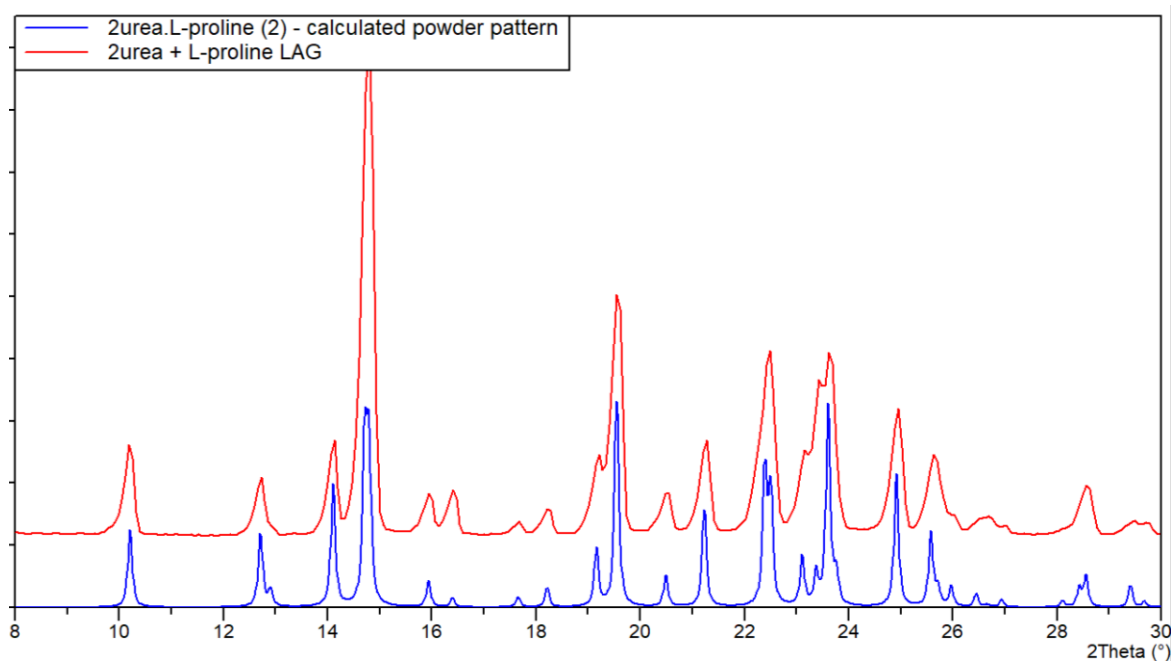


Figure SI-2. Comparison between the experimental PXRD patterns for urea₂·L-proline (2) obtained via LAG (red) and the calculated one (blue).

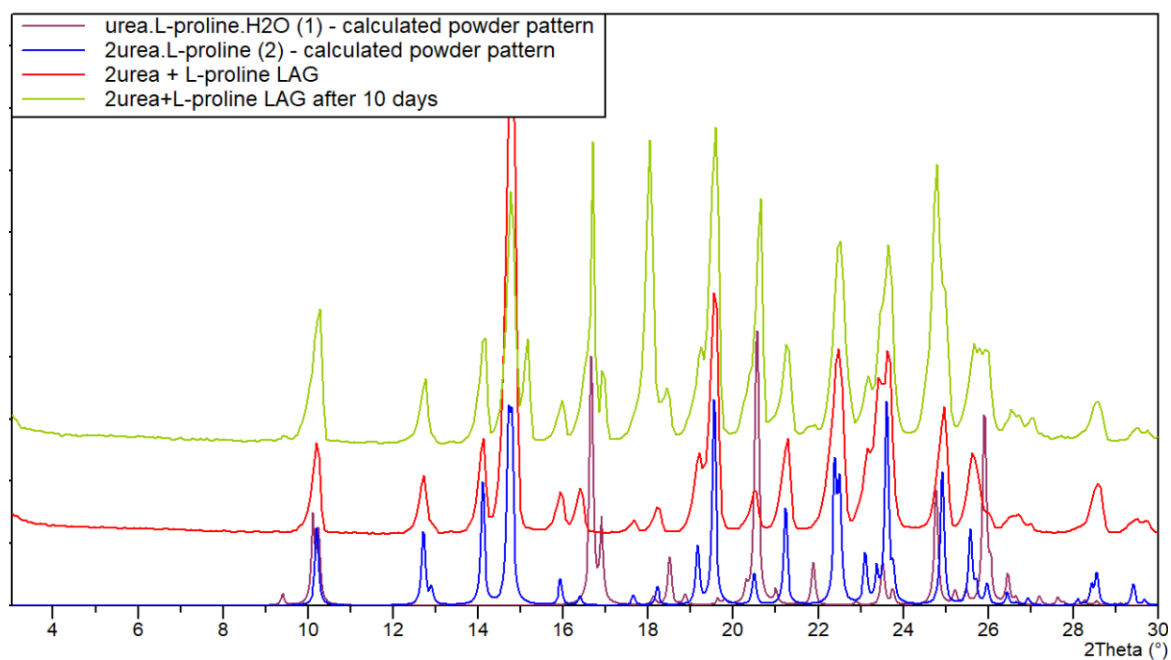


Figure SI-3. Partial conversion of urea₂·L-proline (2) obtained via LAG (red) in urea·L-proline·H₂O (1) over the course of 10 days (green). Calculated powder pattern of 1 (purple) and 2 (blue) are also reported.

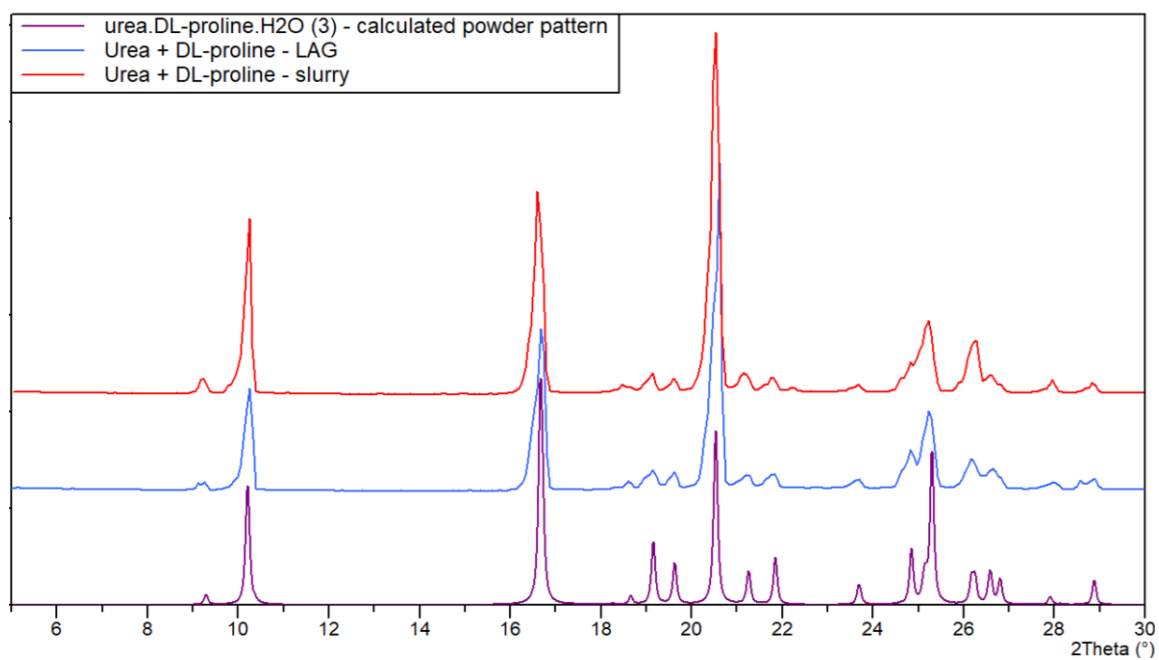


Figure SI-4. Comparison between the experimental PXRD patterns for urea·DL-proline·H₂O (3) obtained via LAG (blue) and slurry (red) and the calculated one (purple).

2. Crystallographic tables and Rietveld refinement

Table SI-1. Crystallographic data and details of measurement for urea·L-proline·H₂O (1), urea₂·L-proline (2) and urea·DL-proline·H₂O (3)

	urea·L-proline·H ₂ O (1)	urea ₂ ·L-proline ^a (2)	urea·DL-proline·H ₂ O (3)
Chemical formula	C ₁₂ H ₃₀ N ₆ O ₈	C ₇ H ₁₇ N ₅ O ₄	C ₁₂ H ₃₀ N ₆ O ₈
Formula weight	386.42	235.24	386.42
Temperature/K	293	293	293
Crystal system	triclinic	orthorhombic	monoclinic
Space group	P1	P2 ₁ 2 ₁ 2 ₁	P2 ₁ /n
a / Å	5.3188(4)	11.970(3)	9.7167(7)
b / Å	9.8293(11)	12.549(4)	5.3018(3)
c / Å	10.6800(15)	8.364(2)	19.0119(11)
α / °	62.794(13)	90	90
β / °	80.482(9)	90	90.166(6)
γ / °	85.192(8)	90	90
Volume / Å ³	489.72(11)	1256.4(6)	979.41(11)
Z	2	4	4
d _{calc} / g cm ⁻³	1.310	1.244	1.310
μ / mm ⁻¹	0.109	0.866	0.109
Refls. measd./unique	7221/4388	-	7190/2335
R _{int}	0.0552	-	0.0234
Goodness-of-fit on F ²	1.078	8.57566	1.136
R ₁ [I > 2σ (I)]	R ₁ = 0.0852	R ₁ = 0.08332	R ₁ = 0.0645
wR ₂ [all data]	0.2076	0.10871	0.1348
R _{WP}	-	10.871	-

^a Structural solution from powder data. CCDC 2422986-2422988 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

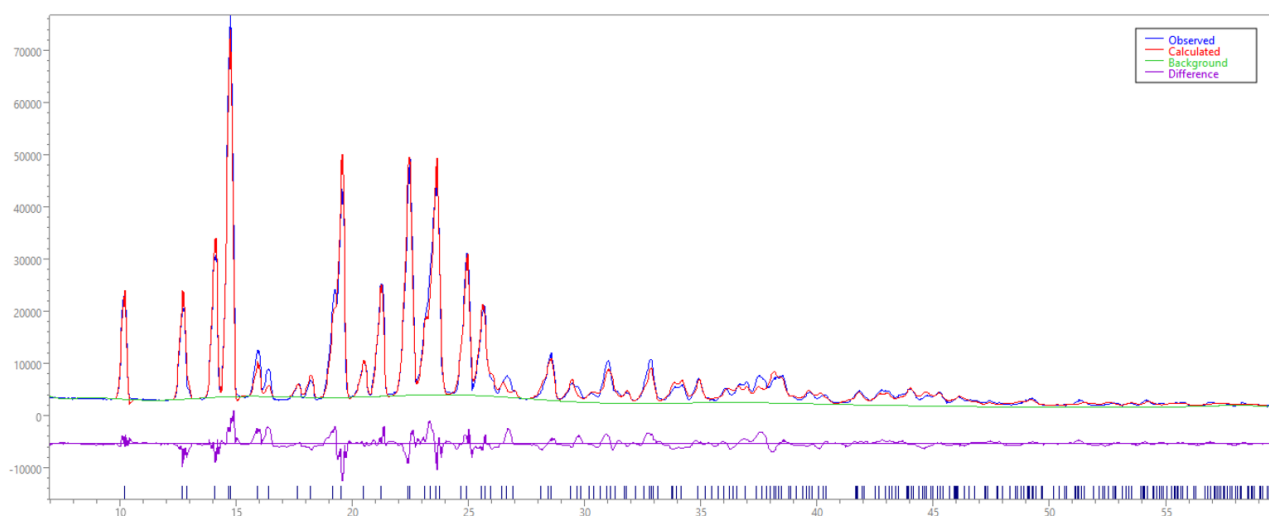


Figure SI-5. Rietveld refinement for urea₂·L-proline (2).

3. DSC and TGA

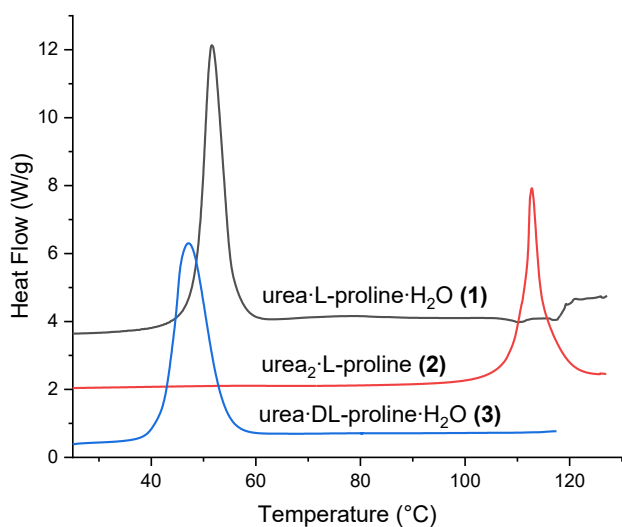


Figure SI-6. DSC traces of **1** (black), **2** (red) and **3** (blue).

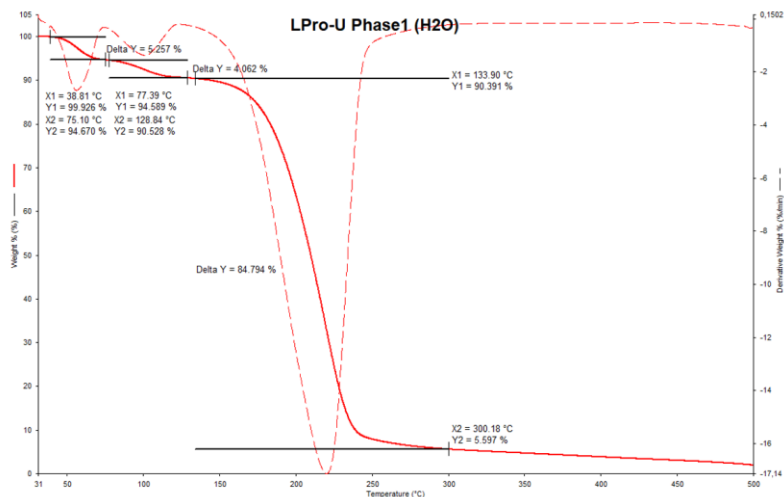


Figure SI-7. TGA trace for urea·L-proline·H₂O (**1**).

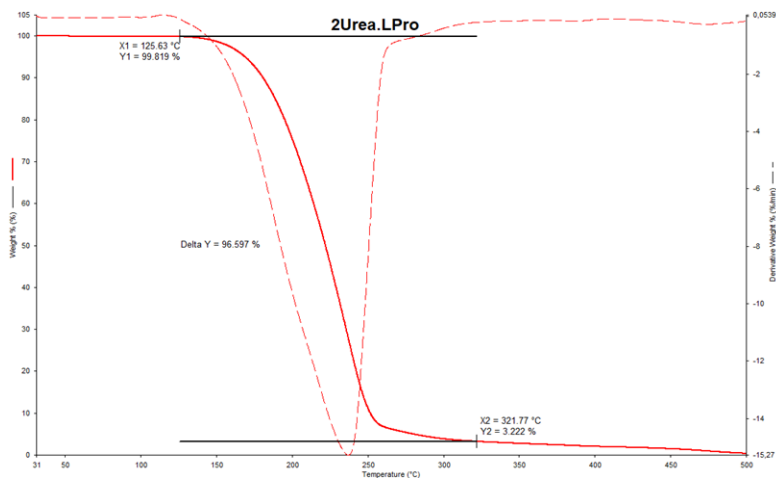


Figure SI-8. TGA trace for urea₂·L-proline (**2**).

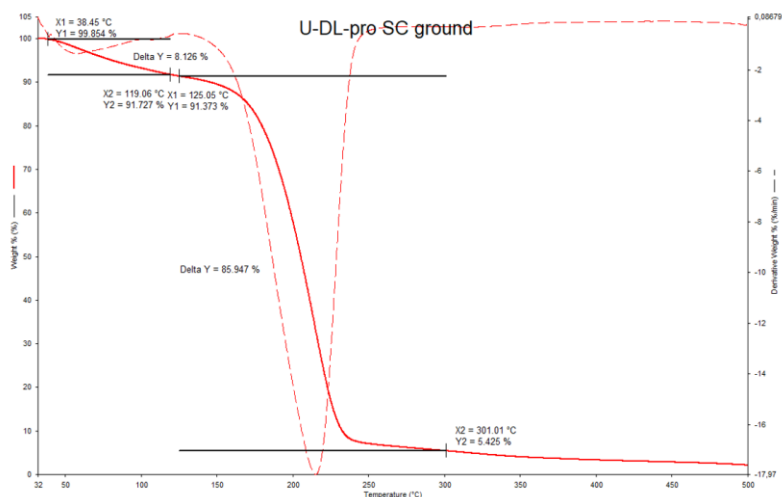


Figure SI-9. TGA trace for urea·DL-proline·H₂O (3).

4. Variable Temperature X-ray diffraction (VT-XRD)

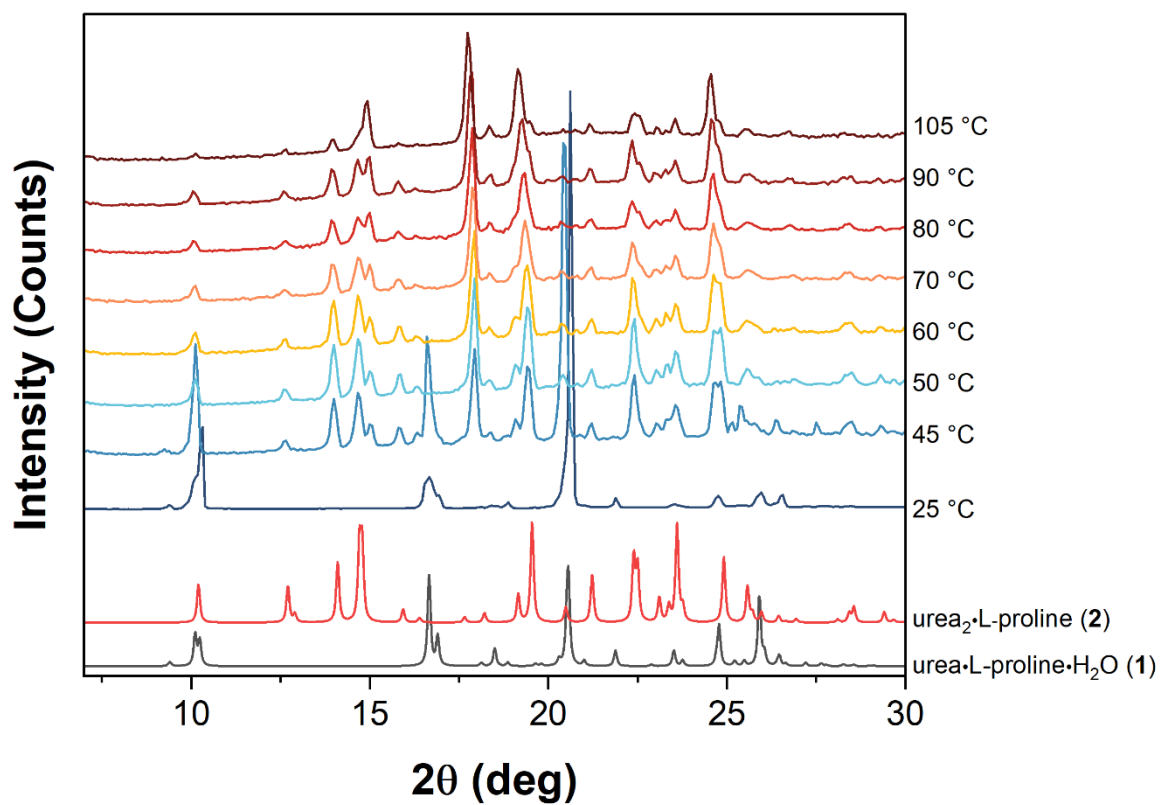


Figure SI-10. VT-PXRD on urea·L-proline·H₂O (1).

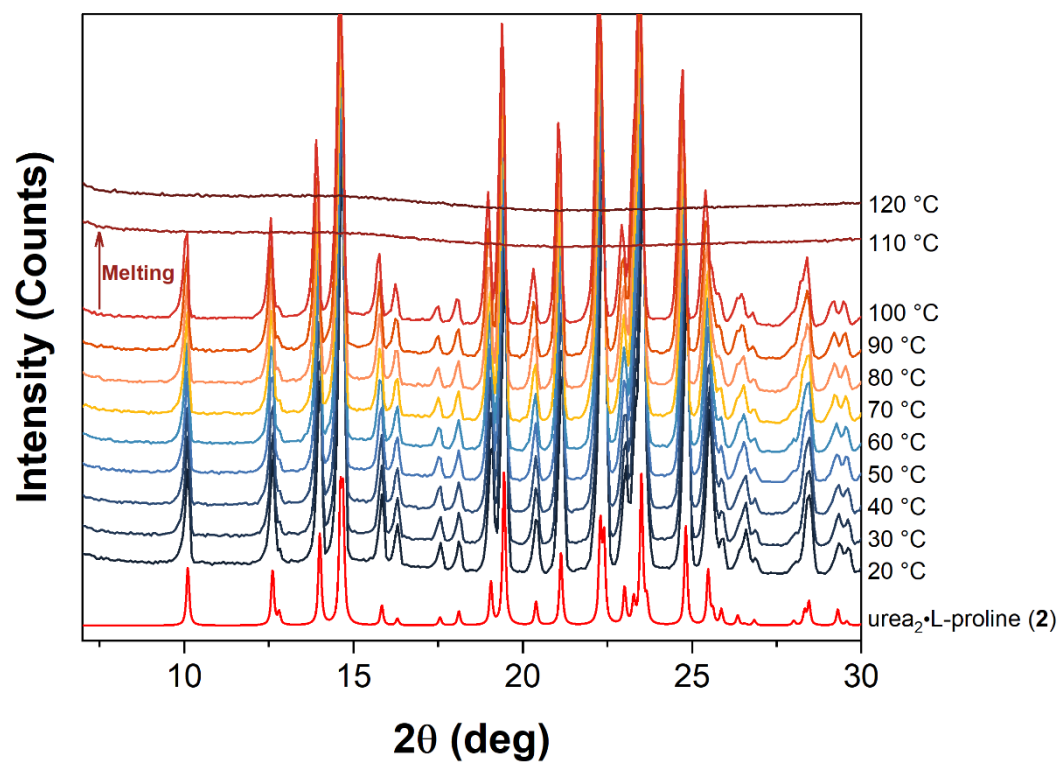


Figure SI-11. VT-PXRD on urea₂·L-proline (2).

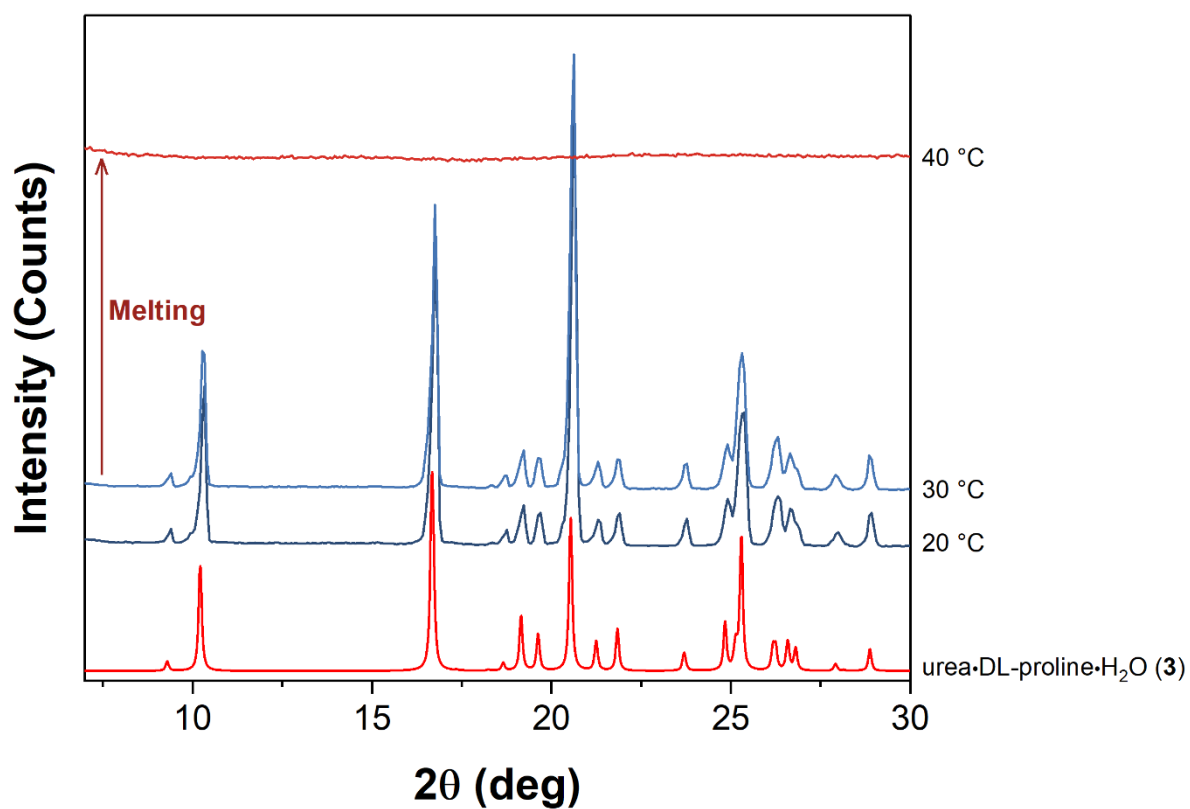


Figure SI-12. VT-PXRD on urea·DL-proline·H₂O (3).

5. Hot-Stage Microscopy

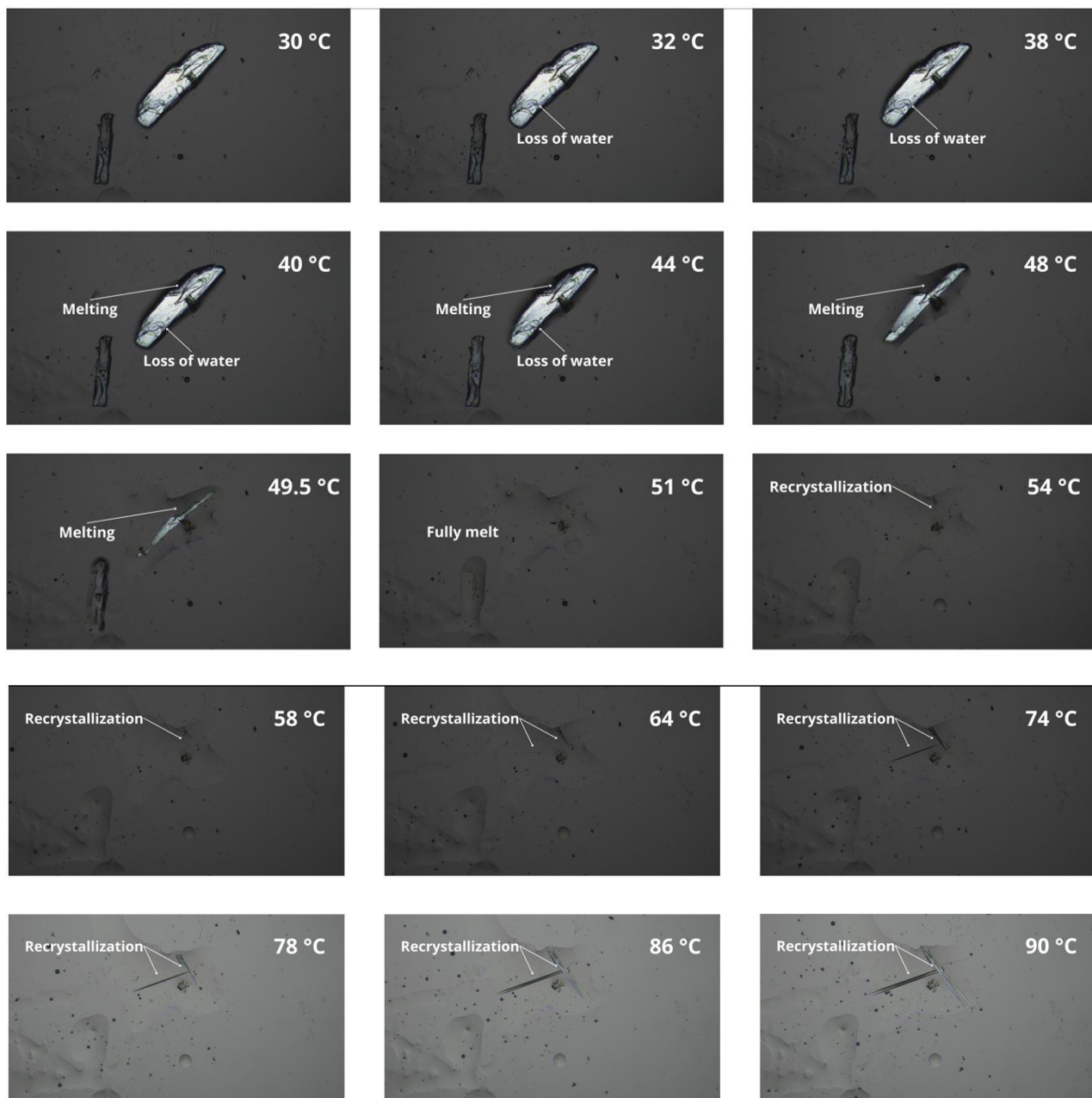


Figure SI-13. Hot-stage microscopy analysis on a single crystal of urea·L-proline·H₂O (1). Water loss, evidenced by bubbles trapped in the oil covering the crystal, is observed starting at 32 °C, followed by melting at around 40 °C; recrystallization to urea₂·L-proline (2) can be detected at ca. 54 °C, and it is complete around 85-90 °C.

6. Raw digestibility data of urea·L-proline·H₂O (1)

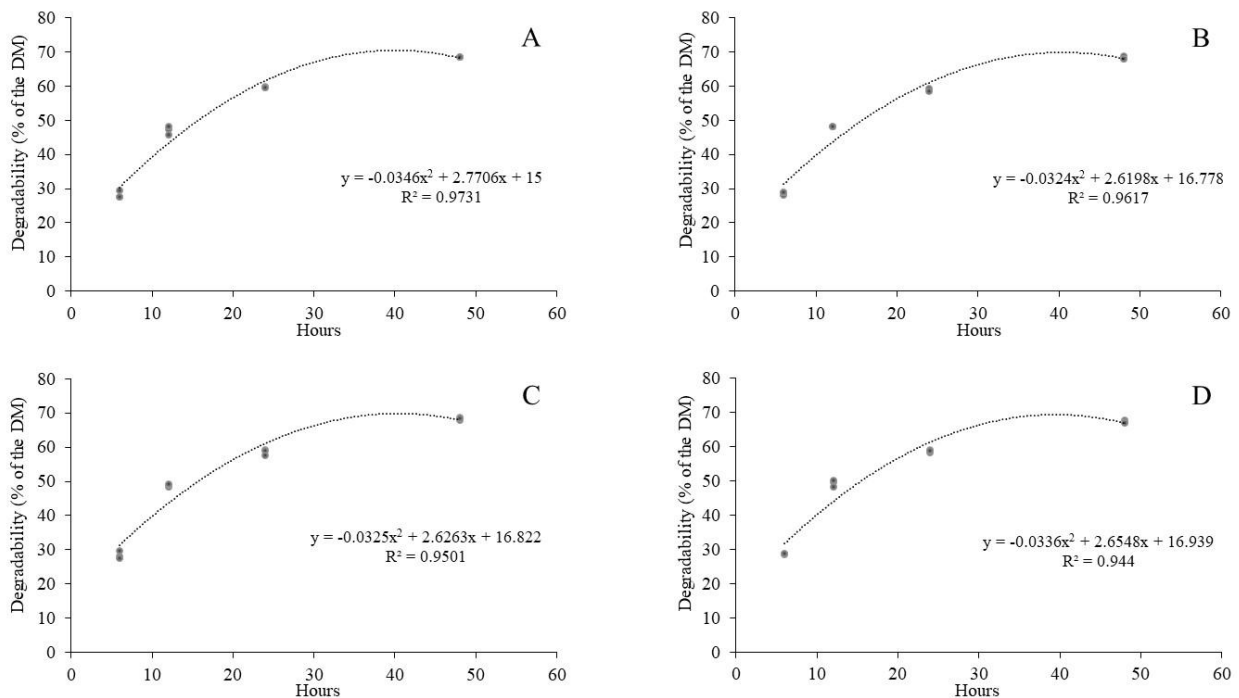


Figure SI-14. *In vitro* degradability curve (6 to 48 hours) of dry matter (DM). A – Corn Silage; B - Corn Silage + Urea; C- Corn Silage + L-Proline; D - Corn Silage + 1As as shown in Figure 4, the ruminal degradability of dry matter tended to stabilize across all treatments after 24 hours of incubation. The degradation curves revealed a consistent pattern among treatments, with no significant differences in the percentage of degraded material.

7. Solubility experiments.

Solubility in water at room temperature is in the range 1-1.2 g mL⁻¹ for urea, and ranges between 0.1 and 0.2 g mL⁻¹ for DL-proline.¹⁻³ Solubility in water of L-proline at 25 °C is apparently controversial, as it spans wide ranges in the literature, with possibly special conditions used for its determination. The Sigma-Aldrich safety data sheet⁴ reports a value of 162.3 g L⁻¹, PubChem⁴ reports a value of 0.162 g mL⁻¹ and one of 1.62 g mL⁻¹, but a value of 1.725 g mL⁻¹ was recently measured,⁵ value that we have chosen to consider as reliable.

Urea·L-proline·H₂O (1)

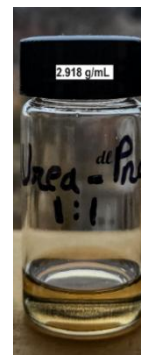
A qualitative analysis was performed for urea·L-proline·H₂O (1) as described in the following. Urea·L-proline·H₂O (1) was progressively added to a vial containing 1 mL of bidistilled water, until saturation was achieved with a total mass of cocrystal of 3.843 g, corresponding to 1.193 g of urea and 2.289 g of L-proline. Cocrystallization of urea with L-proline, therefore, leaves unchanged the solubility of urea, and slightly increases the solubility of L-proline.

Urea·DL-proline·H₂O (3)

Similarly, a qualitative analysis was performed for urea·DL-proline·H₂O (3).

Urea·DL-proline·H₂O (3) was progressively added to a vial containing 1 mL of bidistilled water, until saturation was achieved with a total mass of cocrystal of 2.918 g, corresponding to 0.906 g of urea and 1.738 g of DL-proline.

Cocrystallization of urea with DL-proline therefore results in a slightly lower solubility of urea, while the solubility of DL-proline is increased of ca. one order of magnitude with respect to pure DL-proline.



8. Bibliography

- (1) *DL-Proline (CAS 609-36-9)* | Glenthams Life Sciences.
<https://www.glenthams.com/en/products/product/GM7043/> (accessed 2025-02-17).
- (2) *DL-Proline (CAS 609-36-9)*. <https://go.drugbank.com/drugs/DB00172> (accessed 2025-02-17).
- (3) *DL-Proline (CAS 609-36-9)* | Sigma-Aldrich: St. Louis, MO.
<https://www.sigmaaldrich.com/IT/it/product/aldrich/171824?srsltid=AfmBOoq99D8GKtNzMKLQkeNmCoube-ww3yvTBWBcFJUZENVYY2G9xW6g> (accessed 2025-02-17).
- (4) <https://www.sigmaaldrich.com>
- (5) <https://pubchem.ncbi.nlm.nih.gov/compound/Proline>
- (6) Saha, A.; Mahali, K.; Ganai, S.; Mukherjee, P.; Shrestha, N. K.; Henaish, A.M.A.; Ahmed, J.; Kundu, S.; Roy, S. Solubility and the solution thermodynamics of l-proline in the aqueous binary mixture of NaCl and KCl solution *Journal of Molecular Liquids* **2023**, *391*, 123352.
<https://doi.org/10.1016/j.molliq.2023.123352>