Supporting information

Investigations on the transfer of quinolizidine alkaloids from *Lupinus angustifolius* into the milk of dairy cows

Anna Maria Engel[†], Fenja Klevenhusen[†], Jan-Louis Moenning[†], Jorge Numata[†], Carola Fischer-Tenhagen[‡], Benjamin Sachse[§], Bernd Schäfer[§], Hildburg Fry[†], Oliver Kappenstein[†], Robert Pieper*[†]

[†]German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany

[‡]German Federal Institute for Risk Assessment, Department Experimental Toxicology and ZEBET, Alt-Marienfelde 17-21, 12277 Berlin, Germany

[§]German Federal Institute for Risk Assessment, Department Food Safety, Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany

*Email: Robert.Pieper@bfr.bund.de

Table of contents

1 LC-MS/MS Chromatograms

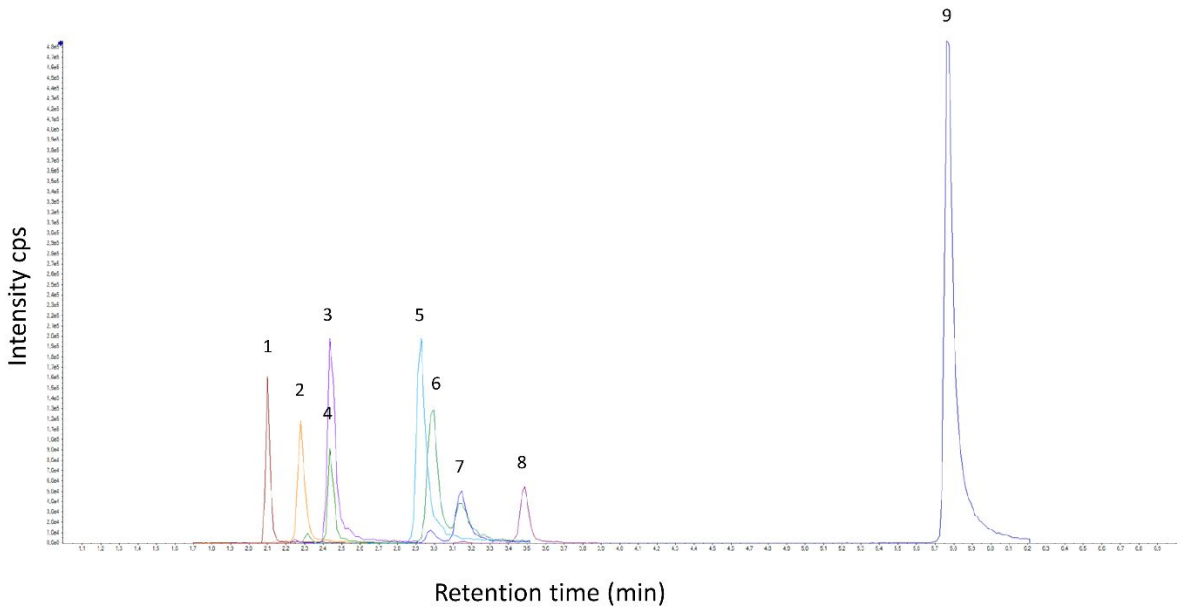

Figure S1. Overlay LC-MS/MS chromatogram of the quantifier MRM transitions of nine QAs in a standard solution with a concentration of 2.5 ng/ml each (1. cytisine (Rt = 2.1 min), 2. lupinine (Rt = 2.3 min), 3. thermopsine (Rt = 2.45 min), 4. 13-hydroxylupanine (Rt = 2.45 min), 5. multiflorine (Rt = 2.95 min), 6. lupanine (Rt = 6.0 min), 7. iso-lupanine (Rt = 3.15 min), 8. angustifoline (Rt = 3.5 min), 9. sparteine (Rt = 5.8 min).

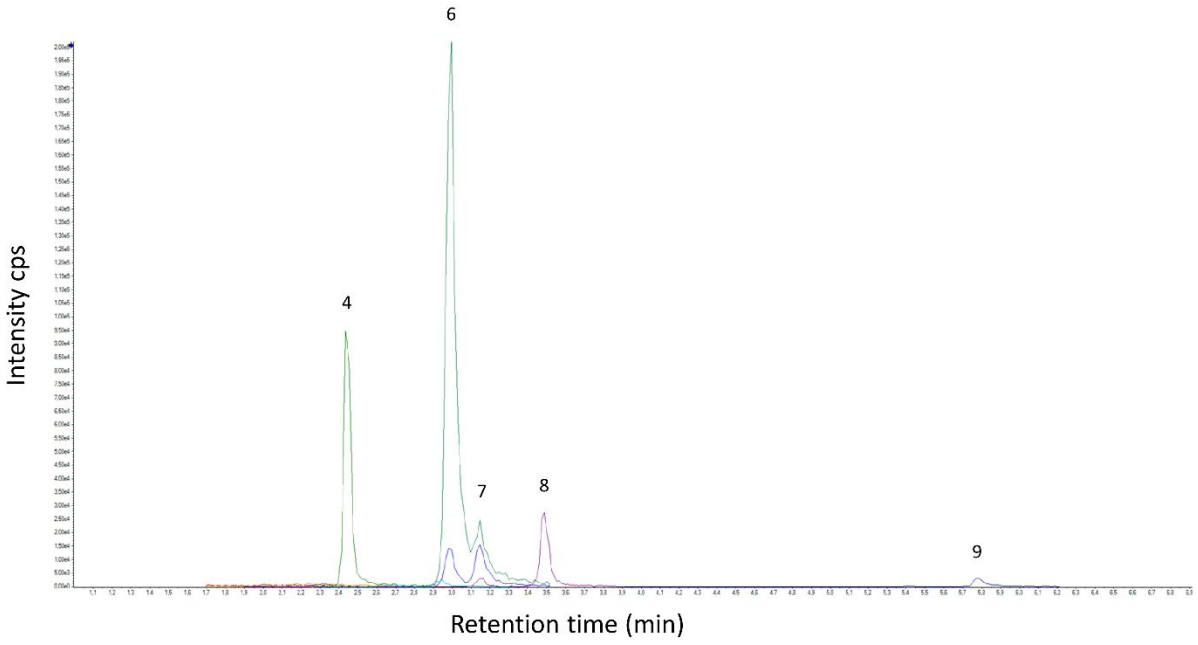

Figure S2. Overlay LC-MS/MS chromatogram of the quantifier MRM transitions of five QAs analysed in lupin seeds (whole grain, untoasted) used for feeding (4. 13-hydroxylupanine 3.6 ng/ml (715 mg/kg), 6. lupanine 3.8 ng/ml (765 mg/kg), 7. iso-lupanine 0.7 ng/ml (140 mg/kg), 8. angustifoline 156 ng/ml (156 mg/kg), 9. sparteine < LOD; Dilution 1:8000).

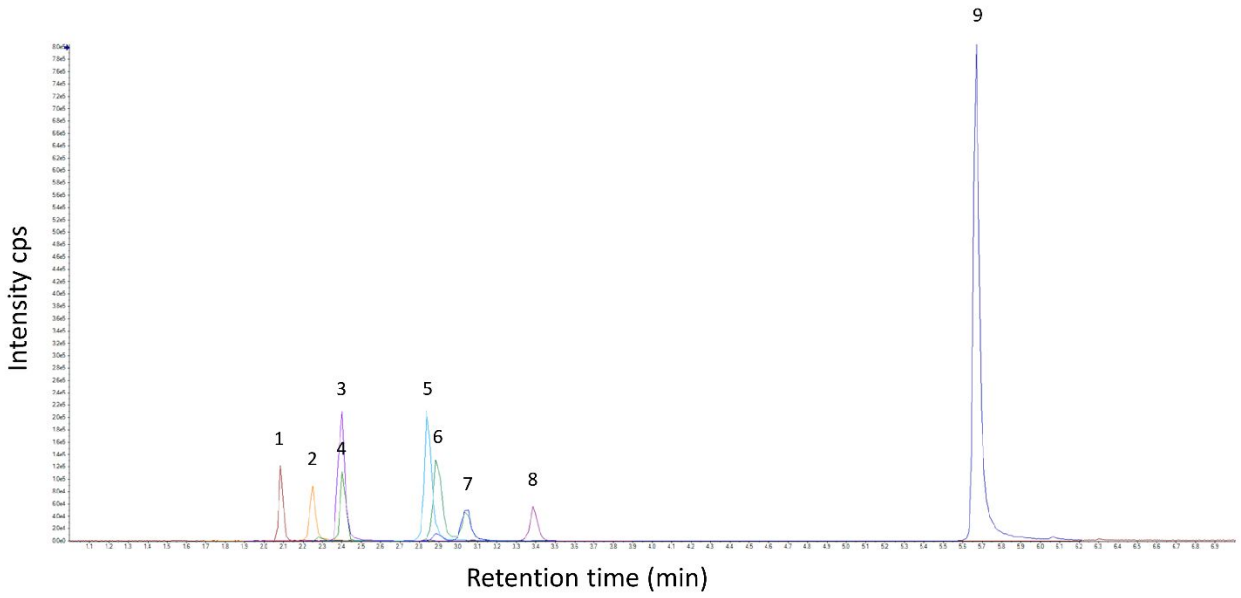

Figure S3. Overlay LC-MS/MS chromatogram of the quantifier MRM transitions of nine QAs in a matrix matched calibration by utilizing cow milk (dilution 1:20) fortified at a level of 2.5 ng/ml (substances and Rt see figure 1).

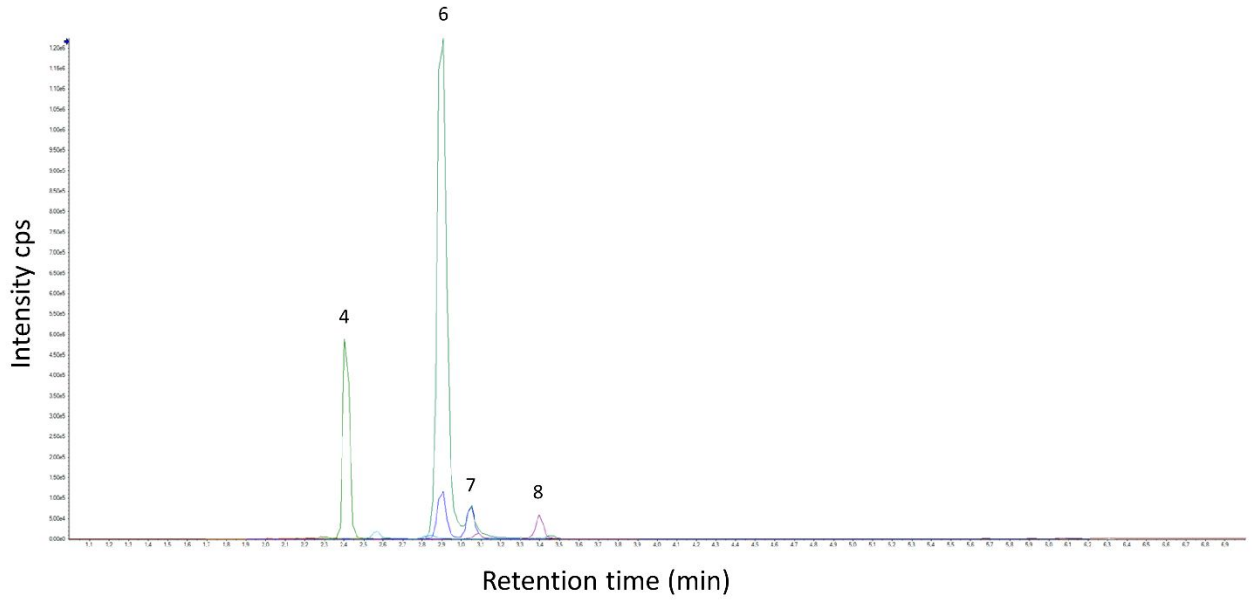

Figure S4. Overlay LC-MS/MS chromatogram of the quantifier MRM transitions of four QAs analysed in a cow milk sample (dilution 1:20) 4. 13-hydroxylupanine and 6. lupanine (both shown QAs are outside of the linear range), 7. iso-lupanine 3.5 ng/ml (117 µg/kg), 8. angustifoline 2.9 ng/ml (97 µg/kg).

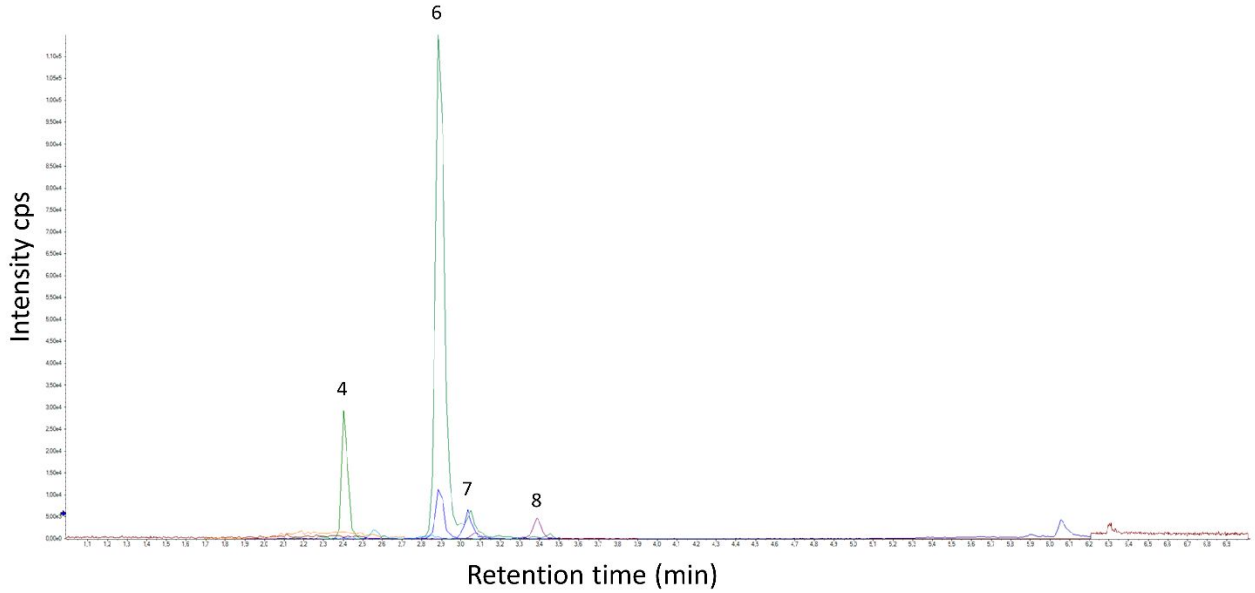

Figure S5. Overlay LC-MS/MS chromatogram of the quantifier MRM transitions of four QAs analysed in a cow milk sample (dilution 1:200) 4. 13-hydroxylupanine 1.2 ng/ml (404 µg/kg), 6. lupanine 1.9 ng/ml (642 µg/kg).

2 Calculations of transfer parameters

2.1 Transfer rates (TR)

The steady state transfer rates were approximated by assuming that a constant feeding period with the same daily intake D[ng/d] (for simplicity D=1 ng/d). Then, the total output via milk at the 100th day M_{100}[ng] was derived via simulating the system until the 100th day, whereupon the transfer rate is given by

$$TR = \frac{M_{100}}{D} 100\% \quad \text{(S1)}$$

2.2 Half-lives

The half-lives of the model are derived analytically. The amount of QA excreted at the n'th morning milk can be described by the following equation

$$X_{n,\,mor} = (\underbrace{e^{M14/24} I_l e^{M10/24} \mathrm{I_l}}_{=:\mathrm{A}})^n X_{0;mor} \tag{S2}$$

for a given starting vector $X_{0;\,\mathrm{mor}}$ at the 0'th morning milk. Here, M is the transition matrix of the PBTK model and I_l is the matrix describing the milking process, i.e.

$$I_l = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix} \tag{S3}$$

Note that I_l only induces two non-zero eigenvalues (1 with multiplicity 2) and $e^{M14/24}$; $e^{M10/24}$ are both invertible, which is why A induces two non-zero eigenvalues λ_1, λ_2. Assuming $\lambda_1 \neq \lambda_2$, then A can be expressed as

$$A = D \begin{pmatrix} \lambda_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & \lambda_2 \end{pmatrix} D^{-1} \tag{S4}$$

for some invertible matrix D. Furthermore, it follows

$$A^{\mathrm{n}} = D \begin{pmatrix} \lambda_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & \lambda_2 \end{pmatrix}^n D^{-1} \tag{S5}$$

$$= D \begin{pmatrix} e^{ln(\lambda_1)} & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & e^{ln(\lambda_2)} \end{pmatrix}^n D^{-1} \tag{S6}$$

Therefore, the half-lives induced by *A* are given by

$$\tau_\alpha = \frac{\ln(2)}{-\ln(\lambda_1)} \quad \text{(S7)}$$

$$\tau_\beta = \frac{\ln(2)}{-\ln(\lambda_2)} \quad \text{(S8)}$$

Finally, note that the amount in each compartment at the evening milking time of the n'th day can be expressed as follows

$$X_{n,\,eve} = e^{M10/24} I_n X_{n,mor} \quad \text{(S9)}$$

Therefore, morning and evening milk have the same half-lives and

so does the whole milk of the day as

$$X_{n,\,tot} = X_{n,\,mor} + X_{n.\,eve} \quad \text{(S10)}$$

2.3 Relative transition amount (RTA)

The relative transition amount describes the amount relative to a steady state at which the decay (starting from steady state) of the amount of QA excreted with milk is better described by the τ_β rather than the τ_α. The decay is described by a biexponential function (except for the first day), i.e.,

$$A(t) = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_2 t} \quad \text{(S11)}$$

Note that A(t) is the continuous expansion of the QA excretion function, as this only makes sense in a discrete setting, i.e., $A|_{\mathbb{N}}(t) = \pi_{Udder}(X_{t;tot})$ with π_{Udder} being the projection onto the udder compartment. The time point at which this happens can be expressed by $\tilde{t}$

$$\frac{d}{dt} C_1 e^{\lambda_1 t}\big|_{t\,=\,\tilde{t}} = \frac{d}{dt} C_2 e^{\lambda_2 t}\big|_{t\,=\,\tilde{t}} \quad \text{(S12)}$$

$$\Leftrightarrow \lambda_1 C_1 e^{\lambda_1 \tilde{t}} = \lambda_2 C_2 e^{\lambda_2 \tilde{t}} \quad \text{(S13)}$$

$$\Leftrightarrow \tilde{t} = \frac{ln\left(\frac{\lambda_1 C_1}{\lambda_2 C_2}\right)}{\lambda_2 - \lambda_1} \quad \text{(S14)}$$

Thus, knowing the half-lives (section 1.2), only C_1; C_2 are unknown. To derive these, the function A(t) is solved for two different time points, i.e. for simplicity t0 = 0 and t10=10. This can be done by simulating the 101th and the 111th day assuming a 100 day feeding period. Note that the 101st day is chosen as the start of A instead of the 100th, due to the partial influence of the feeding on the decay of the first day of the depuration phase. Then C_1 and C_2 can be calculated as follows

$$C_1 = A(0) - C_2 \tag{S15}$$

$$C_2 = \frac{A(10) - A(0)e^{\lambda_1 10}}{e^{\lambda_2 10} - e^{\lambda_1 10}} \tag{S16}$$

Together with equations (S11) and (S14), the amounts at the transition time can now be calculated. Then the relative transition amount (RTA) is given by

$$RTA = \frac{A(\tilde{t})}{A_{ss}} 100\% \tag{S17}$$

where A_{ss} are the amounts excreted during steady state.

3 Transfer parameters

Table S1. α-half-lives τ_α of the simulated QA. The mean value was derived via fitting the model to the four experimental cows and the confidence interval (α=0.05) was derived using the delete-one jackknife method.

	Mean (d)	95% confidence interval (d)
Hydroxylupanine	0.28	0.26 - 0.31
Lupanine	0.26	0.25 - 0.28
Isolupanine	0.26	0.23 - 0.29
Angustifoline	0.27	0.24 - 0.29

Table S2. *β*-half-lives τ_β of the simulated QAs. The mean value was derived via fitting the model to all four cows and the confidence interval (α=0.05) was derived using the delete-one jackknife method.

	Mean (d)	95% confidence interval (d)
Hydroxylupanine	3.51	2.66 – 5.41
Lupanine	3.04	2.00 – 5.93
Isolupanine	2.48	2.17 – 2.95
Angustifoline	5.18	2.85 – 25.79

Table S3. The relative transition amount from alpha into beta phase of the simulated QA. The mean value was derived via fitting the model to all four cows and the confidence interval (α=0.05) was derived using the delete-one jackknife method.

	Mean (%)	95% confidence Interval (%)
Hydroxylupanine	0.14	0.11 - 0.17
Lupanine	0.11	0.01 - 0.17
Isolupanine	0.34	0.19 - 048
Angustifoline	0.14	0.10 – 0.18

4 Complete toxicokinetic model

The PBTK model (Fig 2) between milking events can be described by a linear equation system of the form

$$\dot{\boldsymbol{A}}(t) = \boldsymbol{MA}(t) + \boldsymbol{I}(t) \tag{S18}$$

where M is the transition Matrix given by

$$\mathbf{M} = \begin{pmatrix} -(k_{CP} + k_{CU} + k_{CE}) & k_{UC} & k_{PC} \\ k_{CU} & -k_{UC} & 0 \\ k_{CP} & 0 & -k_{PC} \end{pmatrix}. \tag{S19}$$

Here the model parameters k_{ij} represent the transition rates from compartment i to compartment j for the following compartments: i,j=C, Central; i,j=P, Peripheral; i,j=U , Milk

and i,j=E, Eliminated (conceptually lumping any metabolization and excretion). Alternatively, the same model can be written as the system of differential equations

$$\dot{A}_C(t) = -(k_{CP} + k_{CU} + k_{CE})A_C(t) + k_{UC}A_U(t) + k_{PC}A_P(t) \quad \text{(S20)}$$

$$\dot{A}_U(t) = k_{CU}A_C(t) - k_{UC}A_U(t) \quad \text{(S21)}$$

$$\dot{A}_P(t) = k_{CP}A_C(t) - k_{PC}A_P(t) \quad \text{(S22)}$$

4.1 Periodic milking

The last piece of the model is the implementation of the periodic milking or emptying of the udder at each milking time, which is calculated algorithmically as follows:

$X = (0,0,0)^T$ #Initialization

MilkList=[] #Intialzing the array containing the milk data

for i=0:numberOfExperimentHours-1:

    if [i,i+1] is feeding time:

        I=$(\frac{daily\ dose}{10},0,0)^T$

    else:

        I=$(0,0,0)^T$

    $x^* = -M^{-1}I$

    $X = x^* + e^M(X - x^*)$

    if i+1 is milking time:

        MilkList.append(X["Udder"])

        $X = I_l X$

Here MilkList contains the QA amount excreted at each milking time, thereby alternating between morning and evening milk. The feeding times and milking times follow the experimental schedule for fitting the data, and can be fixed for a predictive model for the

general case. The total QA amount excreted per day can be calculated by adding the amounts for morning and evening milking, as is done for the predictive model included as code. The best-fit values for the model parameters in eq S19 are reproduced in Table S4.

Table S4. Optimized model parameters k_{ij} for each of the modeled QAs.

	k_{CP} (1/d)	k_{PC} (1/d)	k_{CU} (1/d)	k_{UC} (1/d)	k_{CE} (1/d)
Hydroxylupanine	$5.40*10^{-3}$	$2.00*10^{-1}$	$6.57*10^{-2}$	1.69	2.41
Lupanine	$4.87*10^{-3}$	$2.28*10^{-1}$	$2.24*10^{-1}$	6.25	2.61
Isolupanine	$1.44*10^{-2}$	$2.81*10^{-1}$	$2.87*10^{-1}$	6.12	2.67
Angustifoline	$4.65*10^{-3}$	$1.34*10^{-1}$	$1.05*10^{-1}$	6.59	2.59

5 Semilogarithmic plot to show biphasic behavior during depuration

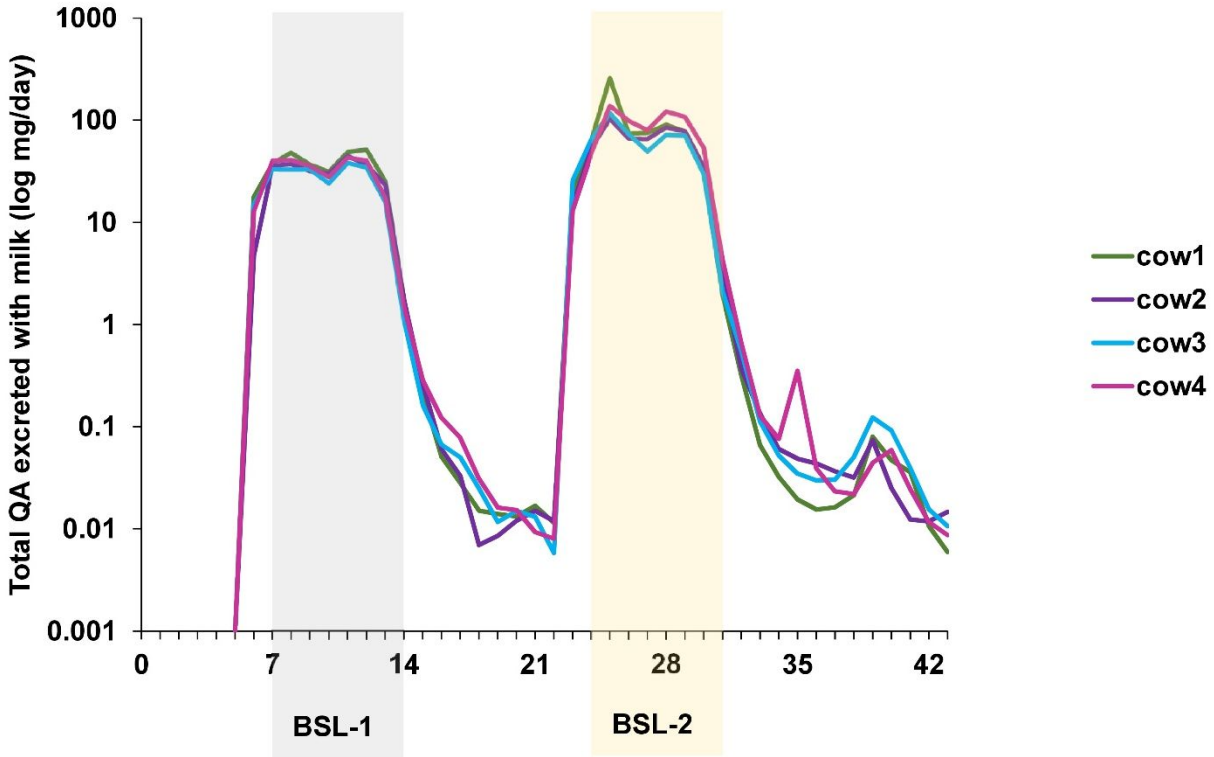

Figure S6. Logarithmic plot of total QA excreted with milk daily in mg/d. The depuration periods following BSL–1 (blue sweet lupine 1 kg/d) and BSL–2 (blue sweet lupine 2 kg/d) show a biphasic behavior: an initial fast α-phase and a later slow β-phase.