Fig. 1

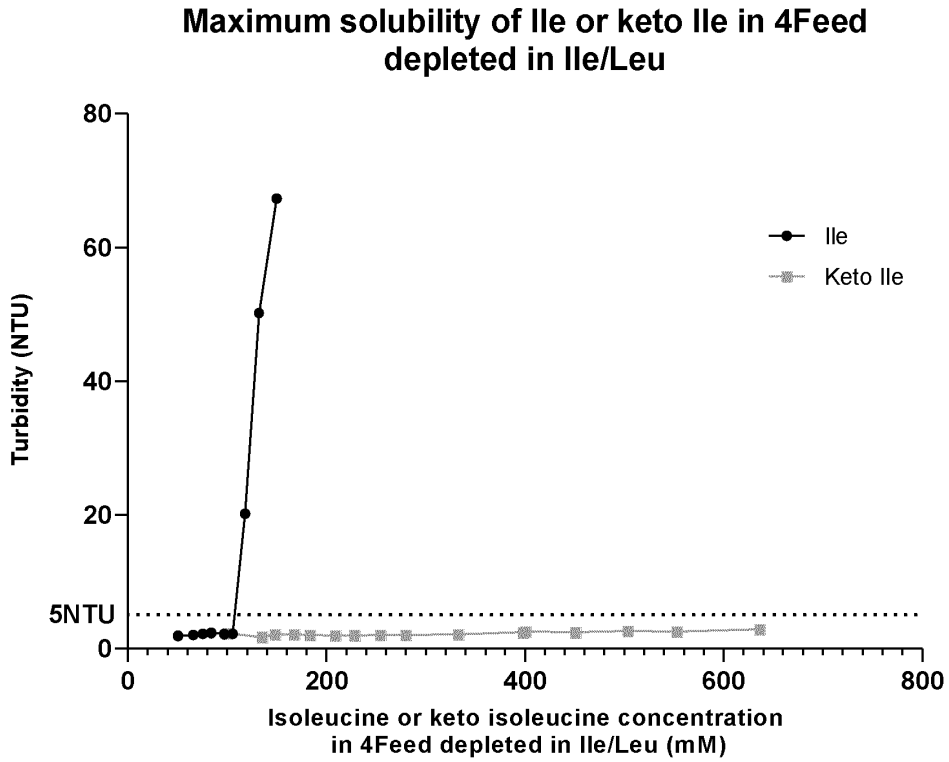

Maximum solubility of Ile or keto Ile in 4Feed depleted in Ile/Leu
Turbidity (NTU)
80
60
40
20
5NTU
0
0
200
400
600
800
Isoleucine or keto isoleucine concentration in 4Feed depleted in Ile/Leu (mM)
Ile
Keto Ile

Fig. 2

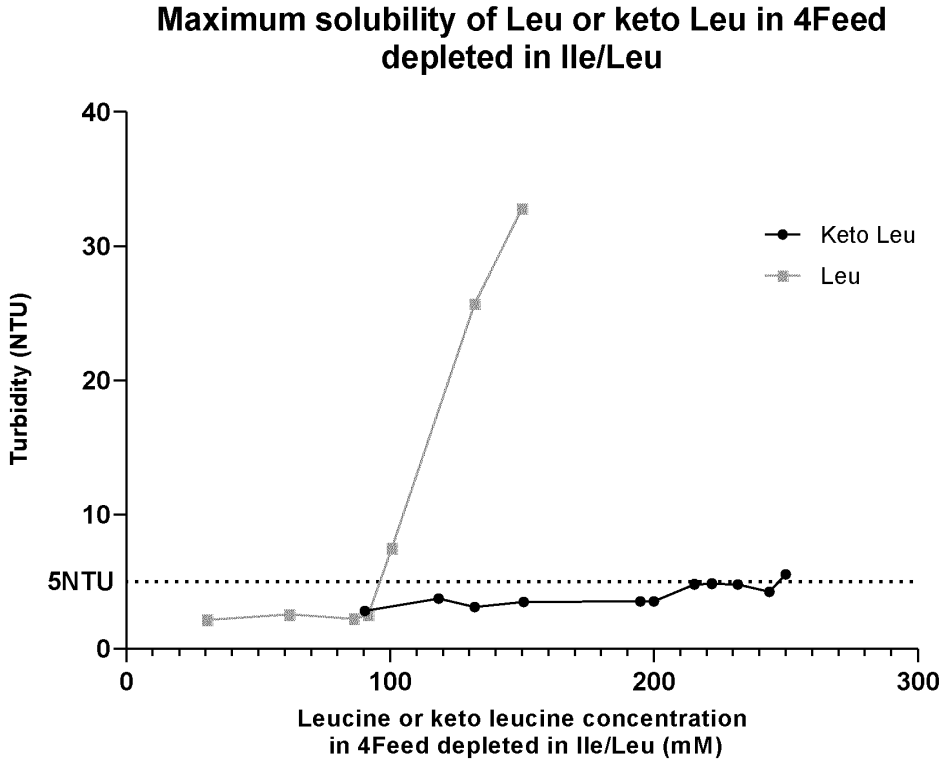

Maximum solubility of Leu or keto Leu in 4Feed depleted in Ile/Leu
Turbidity (NTU)
40
30
20
10
5NTU
0
0
100
200
300
Leucine or keto leucine concentration in 4Feed depleted in Ile/Leu (mM)
Keto Leu
Leu

Fig. 3

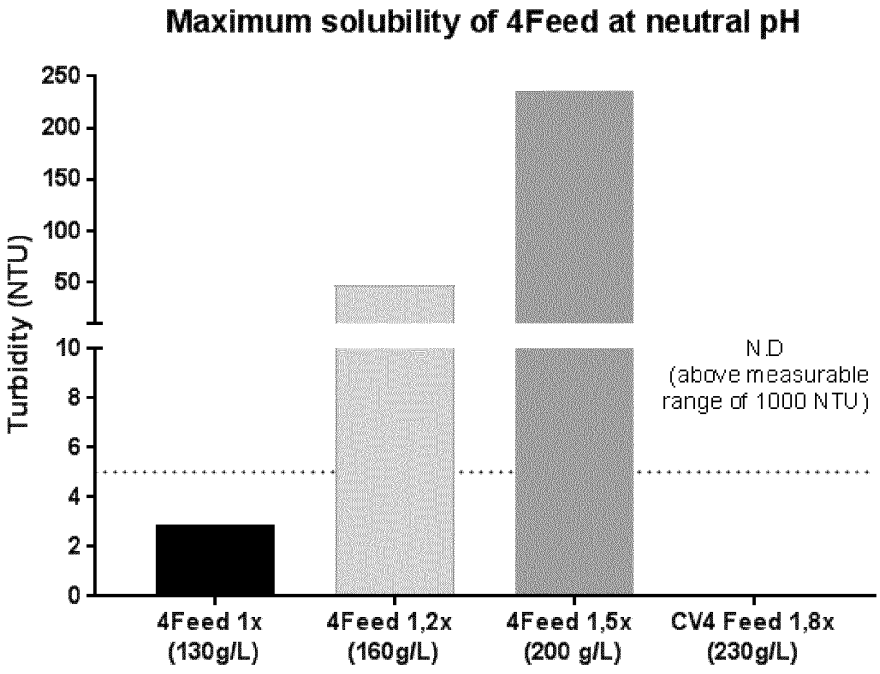

Maximum solubility of 4Feed at neutral pH
Turbidity (NTU)
250
200
150
100
50
10
8
6
4
2
0
N.D
(above measurable
range of 1000 NTU)
4Feed 1x
(130g/L)
4Feed 1,2x
(160g/L)
4Feed 1,5x
(200 g/L)
CV4 Feed 1,8x
(230g/L)

Fig. 4

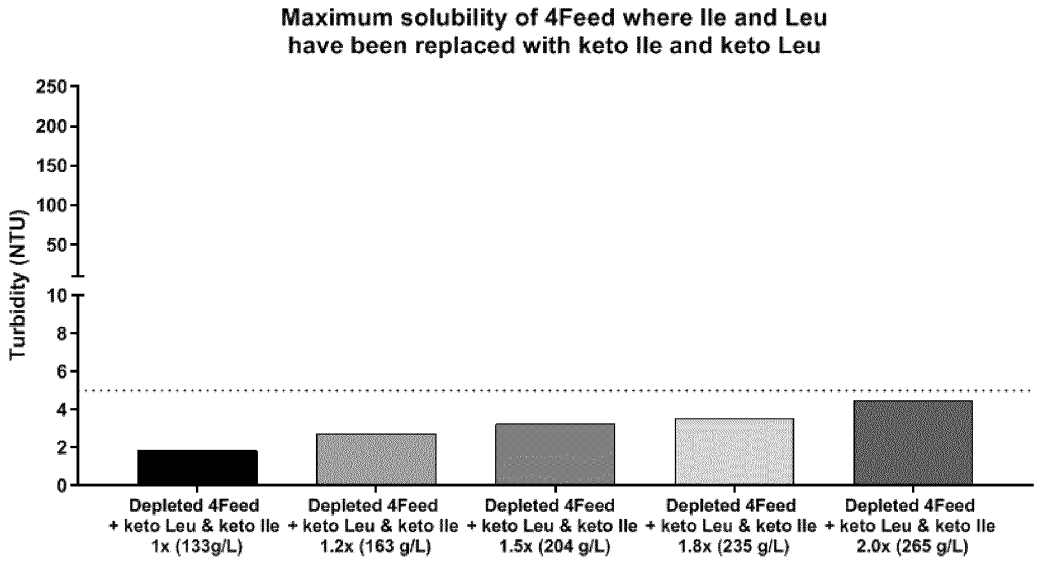
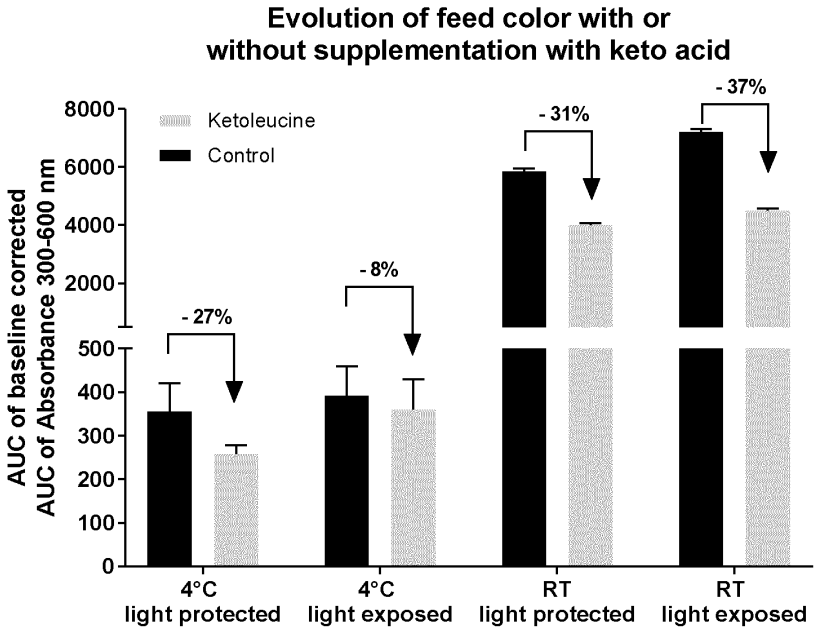

Maximum solubility of 4Feed where Ile and Leu have been replaced with keto Ile and keto Leu
Turbidity (NTU)
250
200
150
100
50
10
8
6
4
2
0
Depleted 4Feed + keto Leu & keto Ile 1x (133g/L)
Depleted 4Feed + keto Leu & keto Ile 1.2x (163 g/L)
Depleted 4Feed + keto Leu & keto Ile 1.5x (204 g/L)
Depleted 4Feed + keto Leu & keto Ile 1.8x (235 g/L)
Depleted 4Feed + keto Leu & keto Ile 2.0x (265 g/L)

Fig. 5

A


Evolution of feed color with or without supplementation with keto acid
AUC of baseline corrected AUC of Absorbance 300-600 nm
Ketoleucine
Control
8000
6000
4000
2000
500
400
300
200
100
0
- 27%
- 8%
- 31%
- 37%
4°C light protected
4°C light exposed
RT light protected
RT light exposed

B

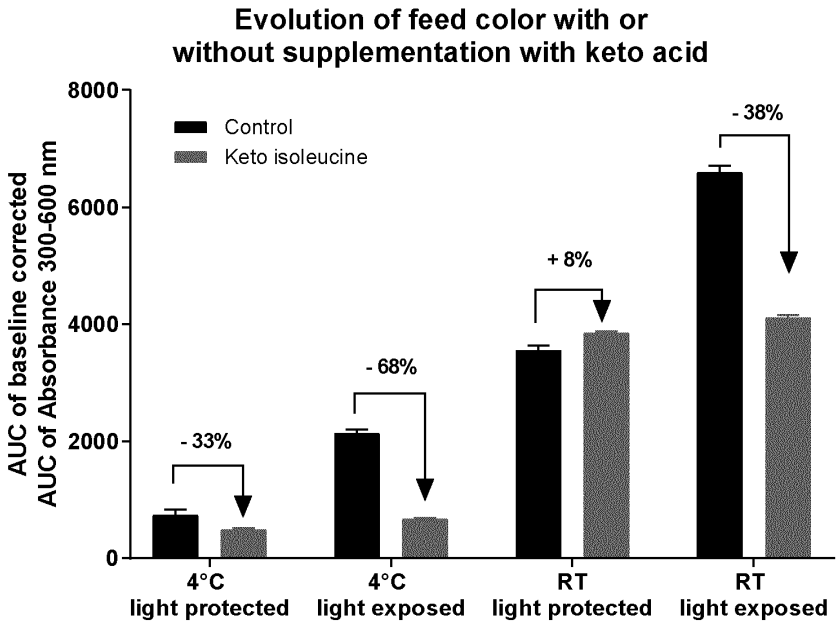
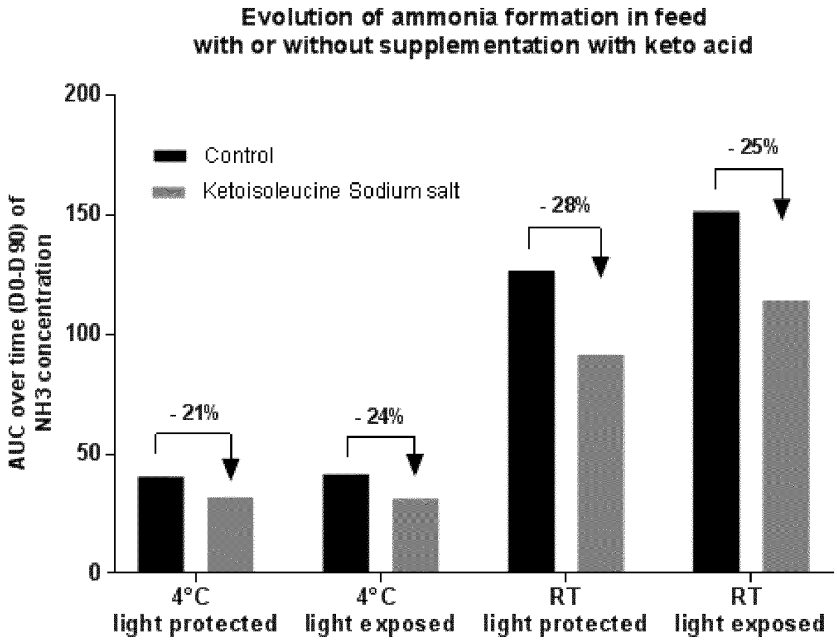

Evolution of feed color with or without supplementation with keto acid
AUC of baseline corrected AUC of Absorbance 300-600 nm
Control
Keto isoleucine
8000
6000
4000
2000
0
- 33%
- 68%
+ 8%
- 38%
4°C light protected
4°C light exposed
RT light protected
RT light exposed

Fig. 6

A


Evolution of ammonia formation in feed with or without supplementation with keto acid
AUC over time (D0-D90) of NH3 concentration
200
150
100
50
0
Control
Ketoisoleucine Sodium salt
- 21%
- 24%
- 28%
- 25%
4°C light protected
4°C light exposed
RT light protected
RT light exposed

B

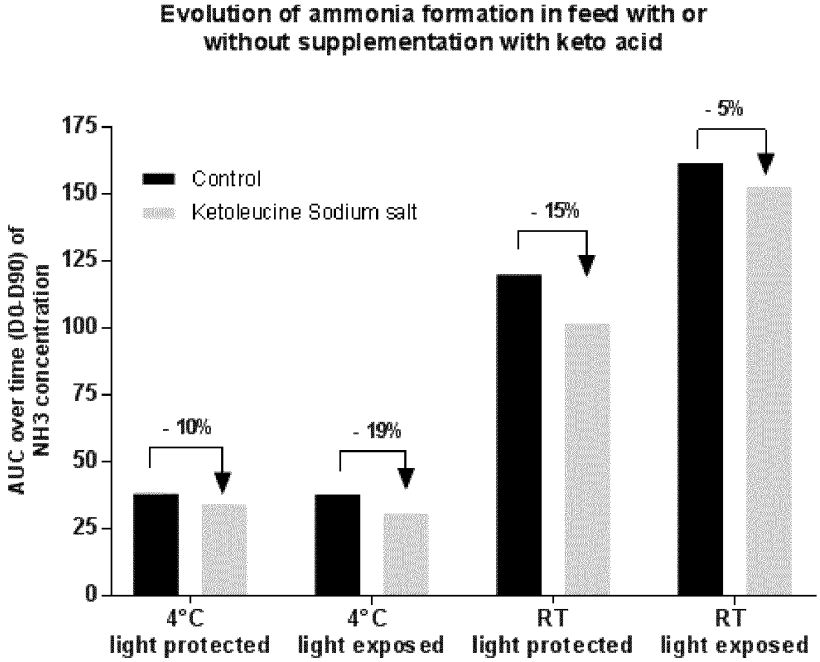
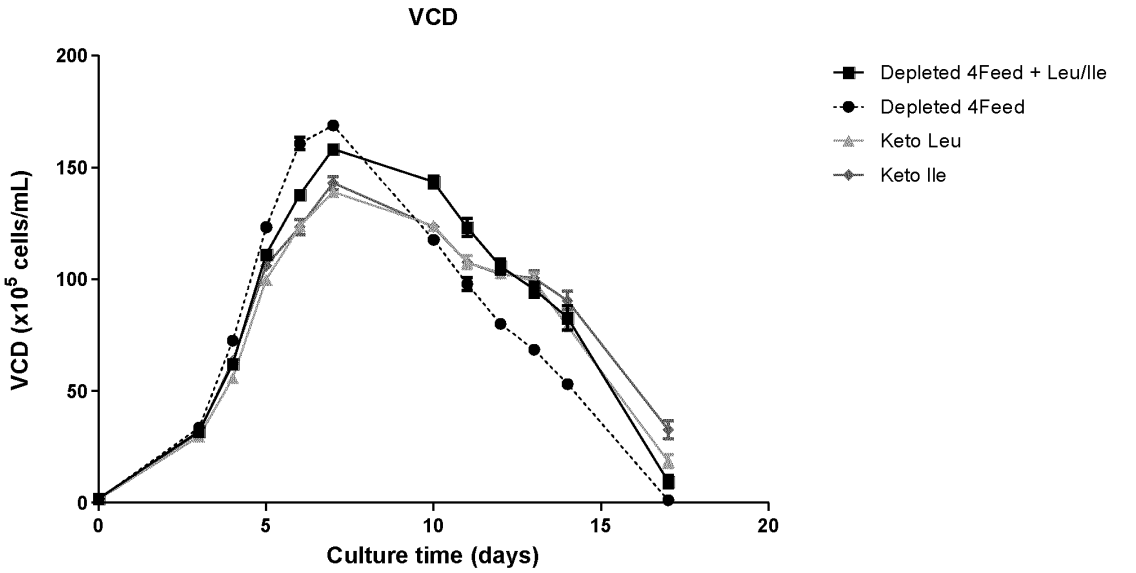

Evolution of ammonia formation in feed with or without supplementation with keto acid
AUC over time (D0-D90) of NH3 concentration
175
150
125
100
75
50
25
0
Control
Ketoleucine Sodium salt
- 10%
- 19%
- 15%
- 5%
4°C light protected
4°C light exposed
RT light protected
RT light exposed

Fig. 7

A


VCD
VCD (x10^5 cells/mL)
200
150
100
50
0
0
5
10
15
20
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile

B

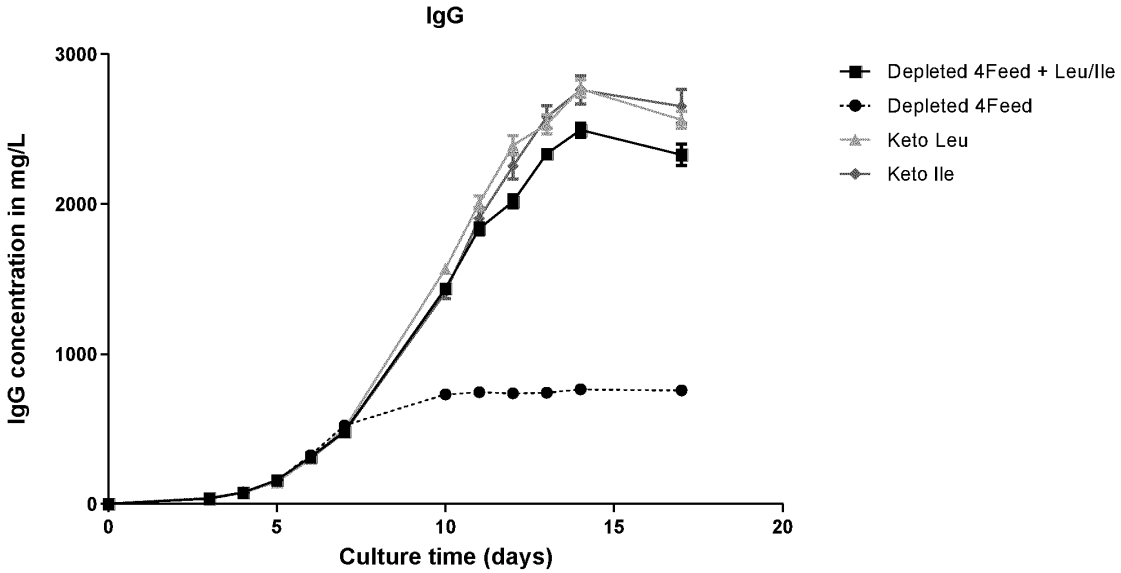

IgG
IgG concentration in mg/L
3000
2000
1000
0
0
5
10
15
20
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile

Fig. 8

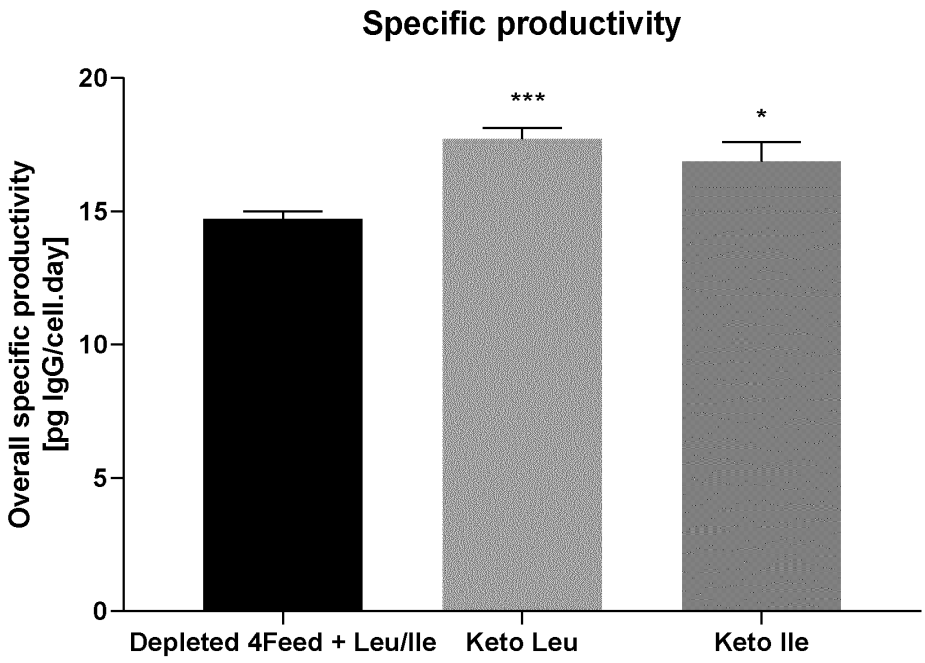
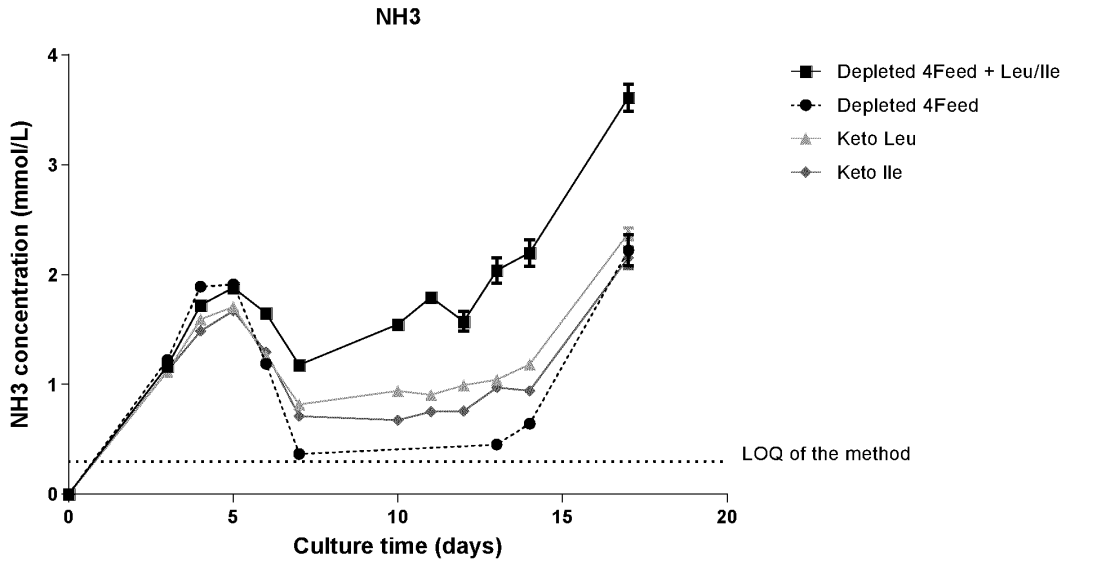

Specific productivity
Overall specific productivity [pg IgG/cell.day]
20
15
10
5
0
***
*
Depleted 4Feed + Leu/Ile
Keto Leu
Keto Ile

Fig. 9

A


NH3
NH3 concentration (mmol/L)
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile
LOQ of the method

B

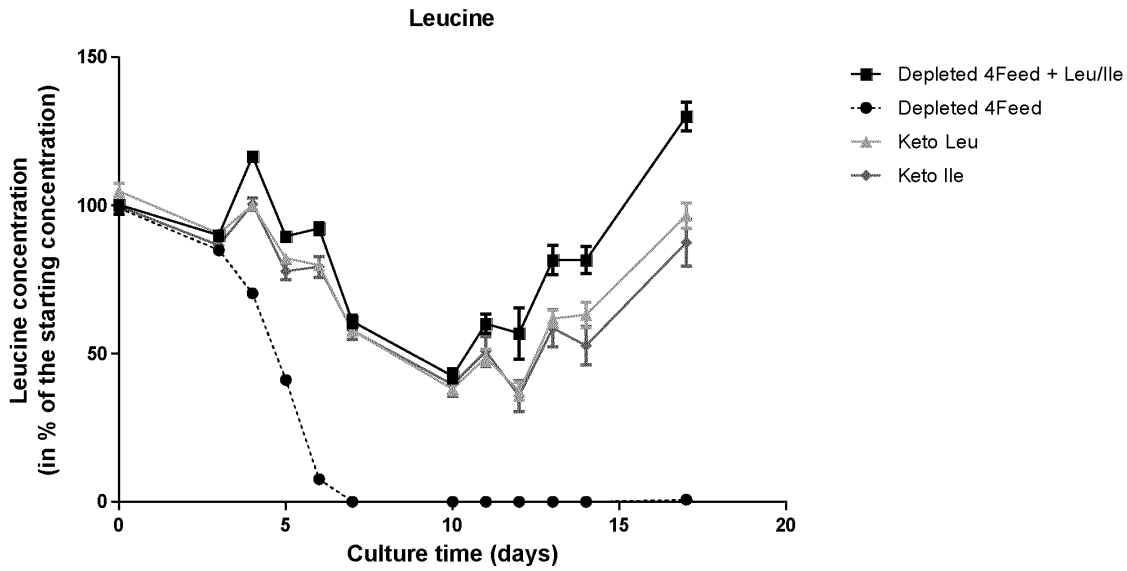
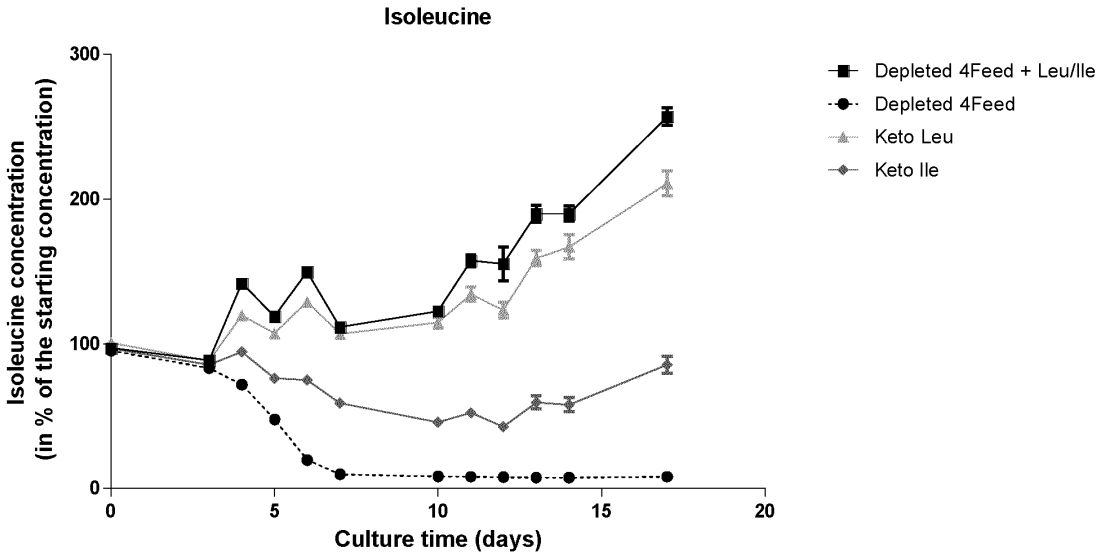

Leucine
Leucine concentration
(in % of the starting concentration)
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile

Fig. 10

A


Isoleucine
Isoleucine concentration (in % of the starting concentration)
300
200
100
0
0
5
10
15
20
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile

B

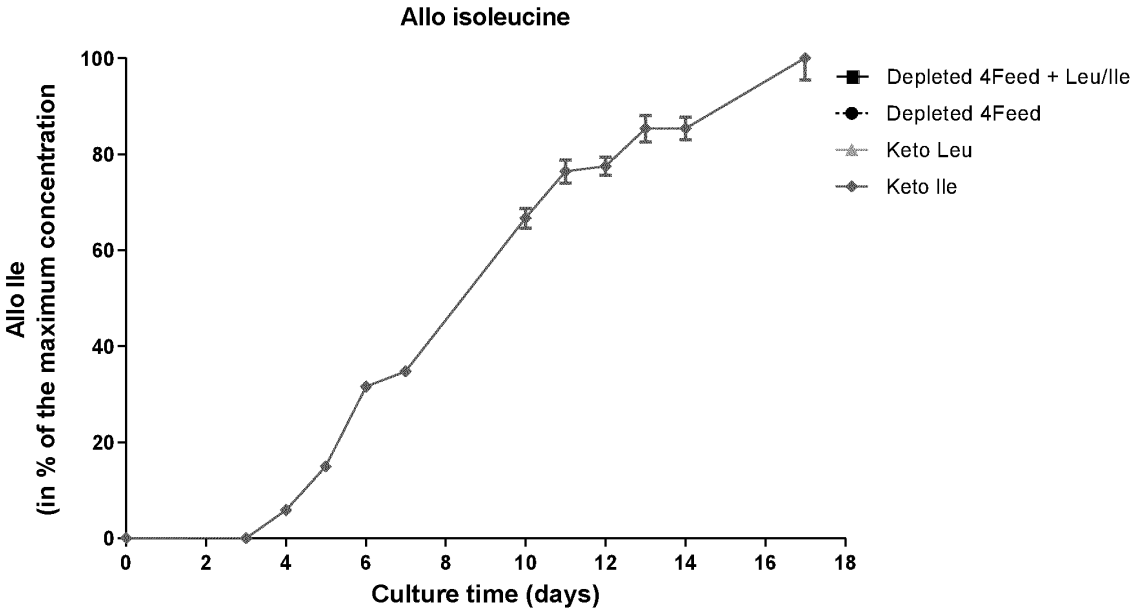

Allo isoleucine
Allo Ile (in % of the maximum concentration
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
Culture time (days)
Depleted 4Feed + Leu/Ile
Depleted 4Feed
Keto Leu
Keto Ile

Fig. 11

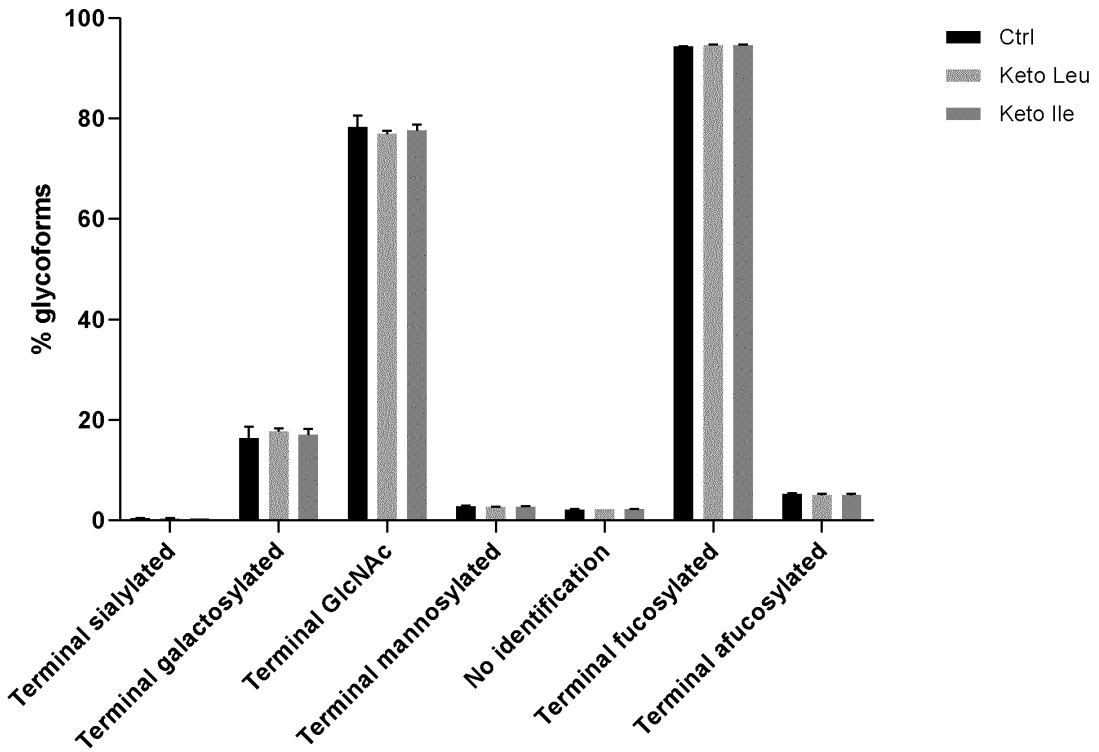

100
80
60
40
20
0
% glycoforms
Ctrl
Keto Leu
Keto Ile
Terminal sialylated
Terminal galactosylated
Terminal GlcNAc
Terminal mannosylated
No identification
Terminal fucosylated
Terminal afucosylated

Fig. 12

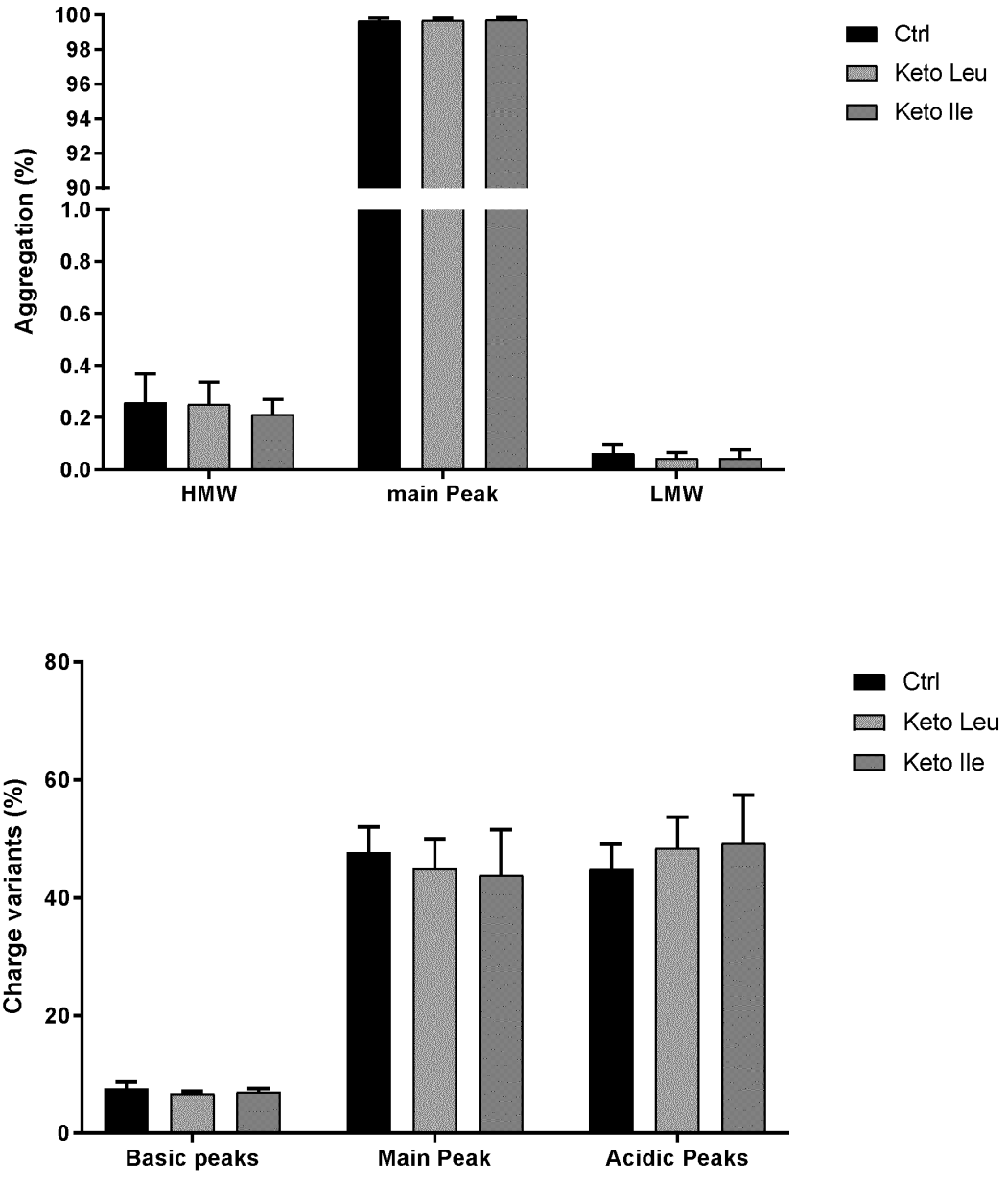
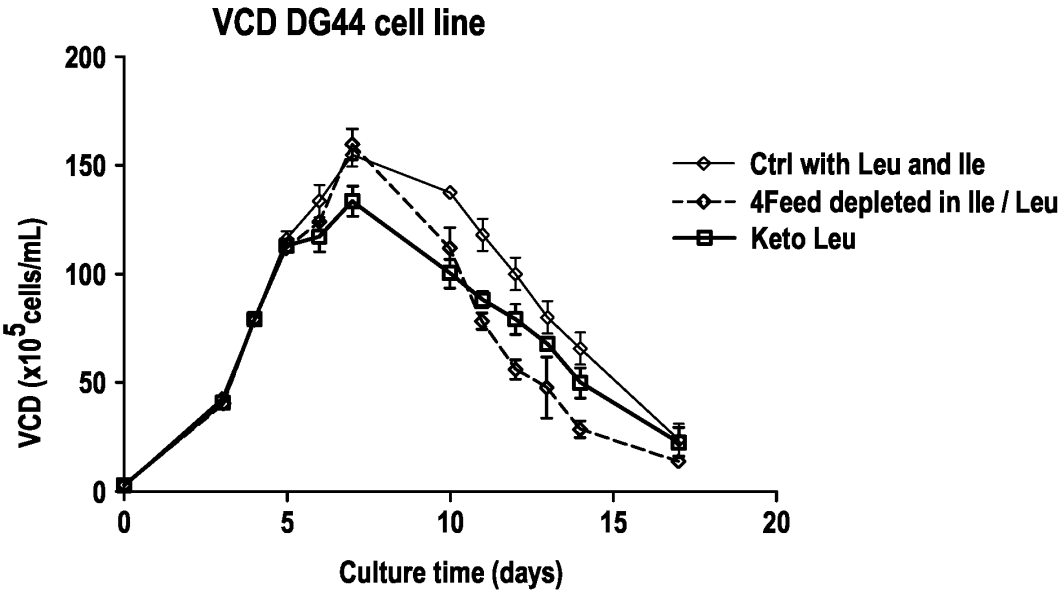

Aggregation (%)
100
98
96
94
92
90
1.0
0.8
0.6
0.4
0.2
0.0
HMW
main Peak
LMW
Ctrl
Keto Leu
Keto Ile
A
B
Charge variants (%)
80
60
40
20
0
Basic peaks
Main Peak
Acidic Peaks
Ctrl
Keto Leu
Keto Ile

Fig. 13 part 1


VCD DG44 cell line
VCD (x10^5cells/mL)
200
150
100
50
0
0
5
10
15
20
Culture time (days)
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu

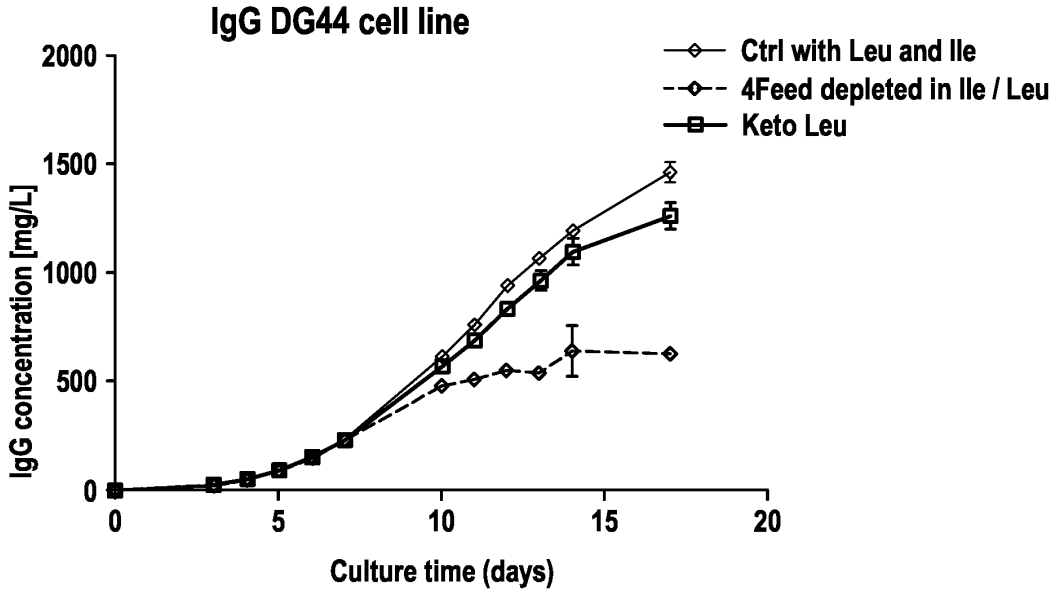
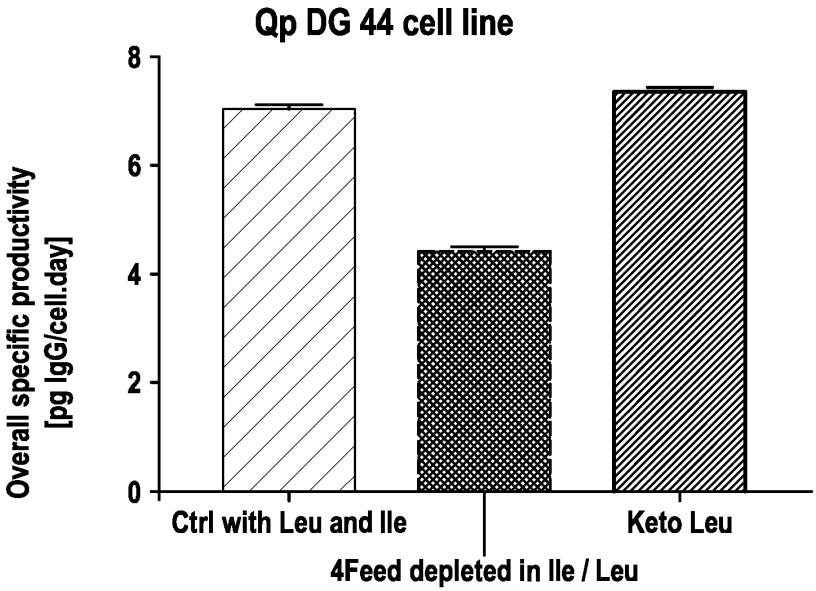

IgG DG44 cell line
IgG concentration [mg/L]
2000
1500
1000
500
0
0
5
10
15
20
Culture time (days)
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu

Fig. 13 part 2


Qp DG 44 cell line
Overall specific productivity [pg IgG/cell.day]
8
6
4
2
0
Ctrl with Leu and Ile
Keto Leu
4Feed depleted in Ile / Leu

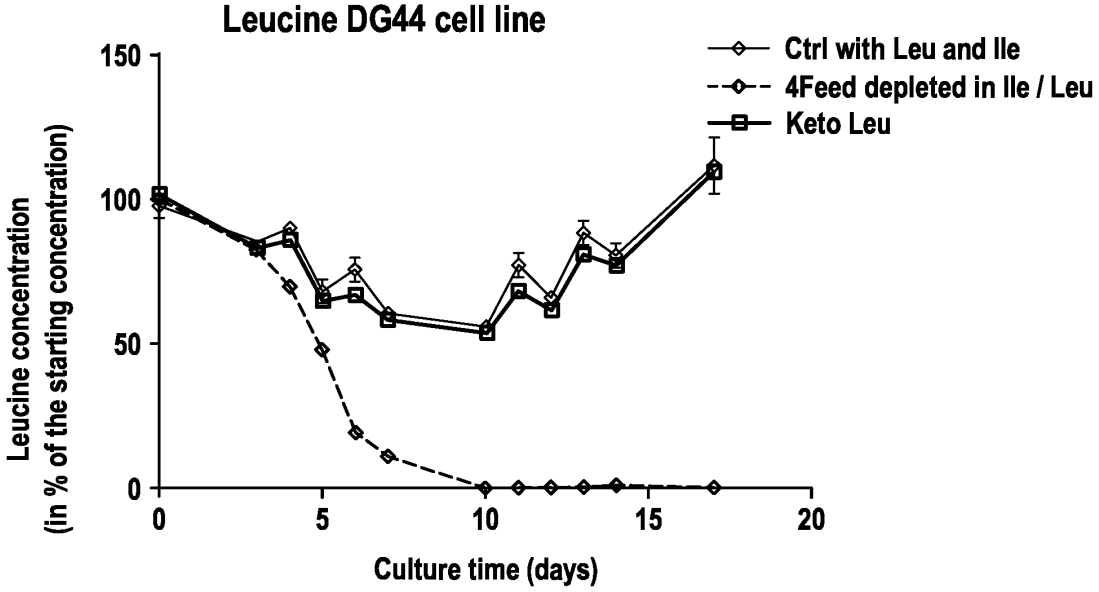
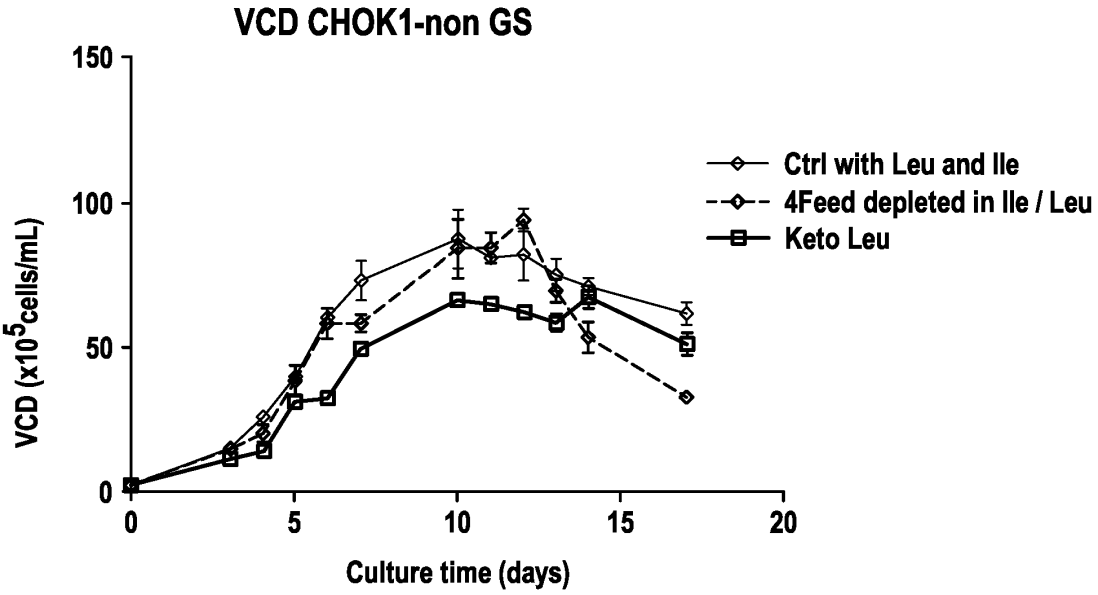

Leucine DG44 cell line
Leucine concentration (in % of the starting concentration)
150
100
50
0
0
5
10
15
20
Culture time (days)
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu

Fig. 14 part 1


VCD CHOK1-non GS
VCD (x10^5cells/mL)
150
100
50
0
0
5
10
15
20
Culture time (days)
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu

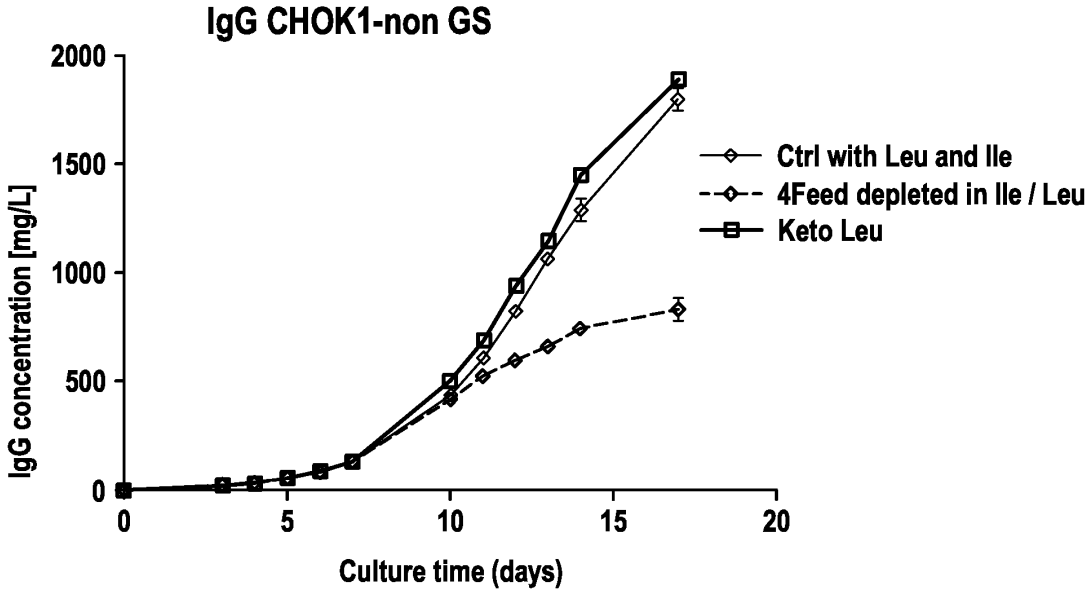
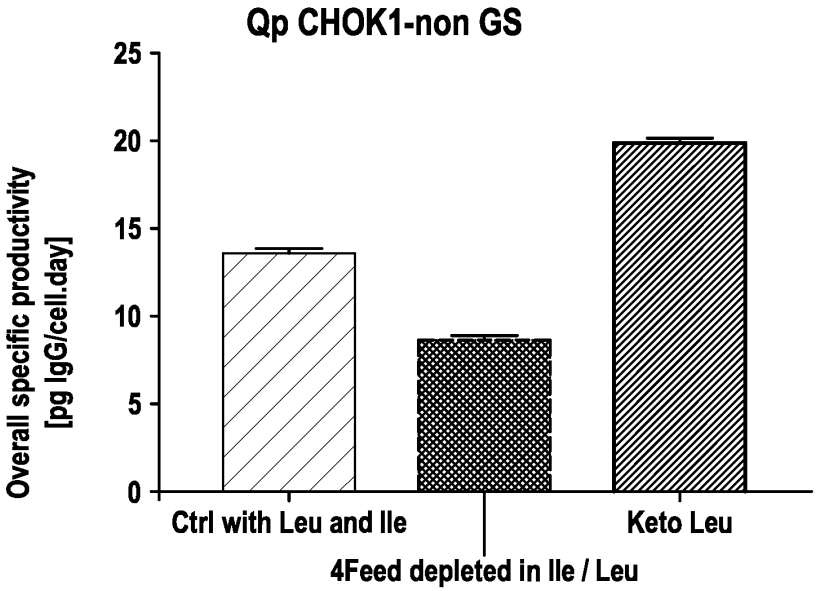

IgG CHOK1-non GS
IgG concentration [mg/L]
2000
1500
1000
500
0
0
5
10
15
20
Culture time (days)
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu

Fig. 14 part 2


Qp CHOK1-non GS
Overall specific productivity [pg IgG/cell.day]
25
20
15
10
5
0
Ctrl with Leu and Ile
Keto Leu
4Feed depleted in Ile / Leu

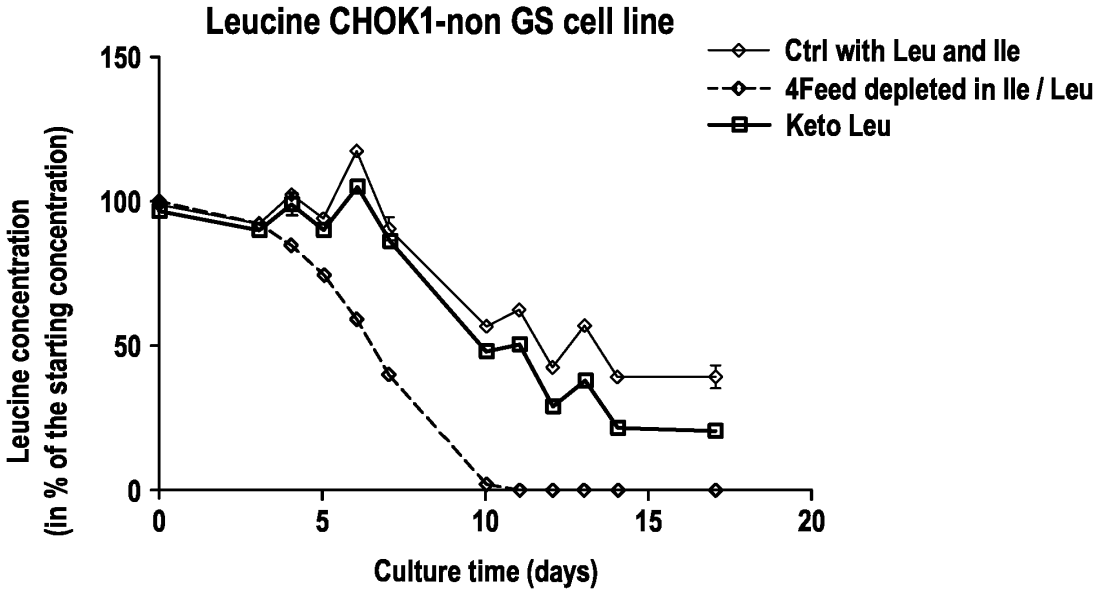
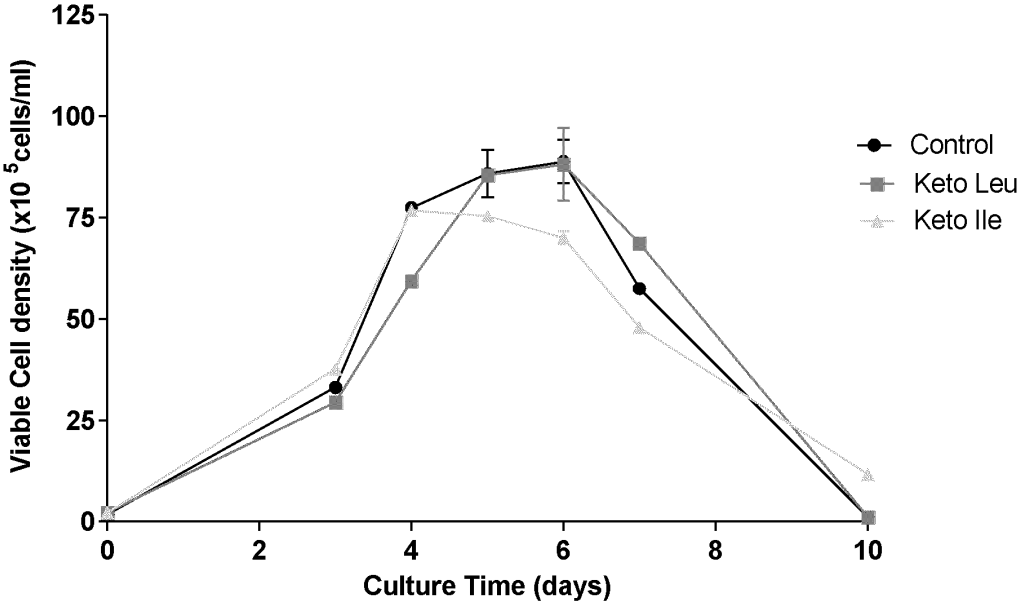
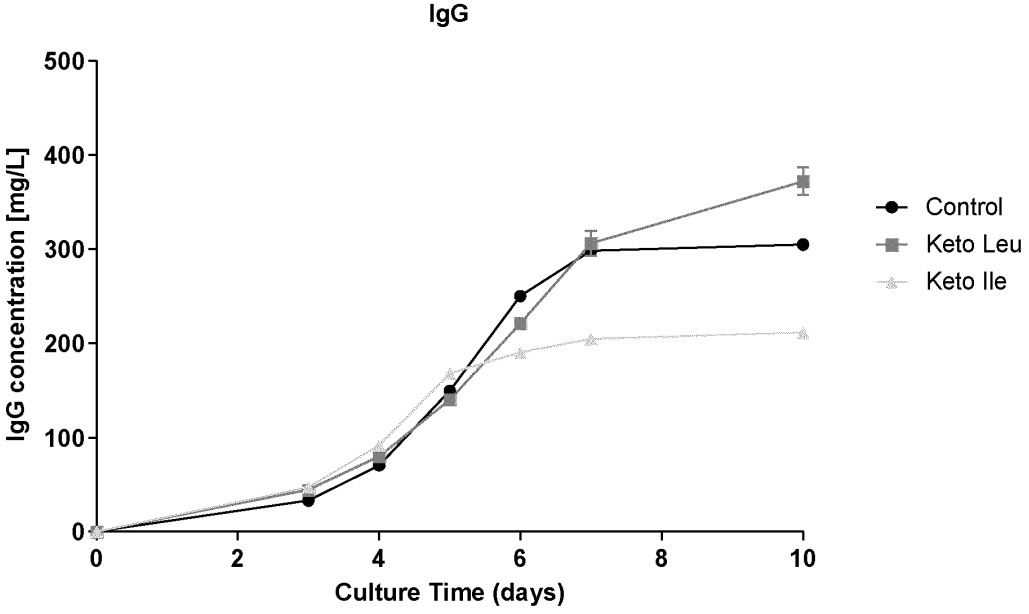

Leucine CHOK1-non GS cell line
Ctrl with Leu and Ile
4Feed depleted in Ile / Leu
Keto Leu
Leucine concentration (in % of the starting concentration)
150
100
50
0
0
5
10
15
20
Culture time (days)

Fig. 15

A


Viable Cell density (x10 5cells/ml)
125
100
75
50
25
0
0
2
4
6
8
10
Culture Time (days)
Control
Keto Leu
Keto Ile


IgG
IgG concentration [mg/L]
500
400
300
200
100
0
0
2
4
6
8
10
Culture Time (days)
Control
Keto Leu
Keto Ile

B

Fig. 16 part 1

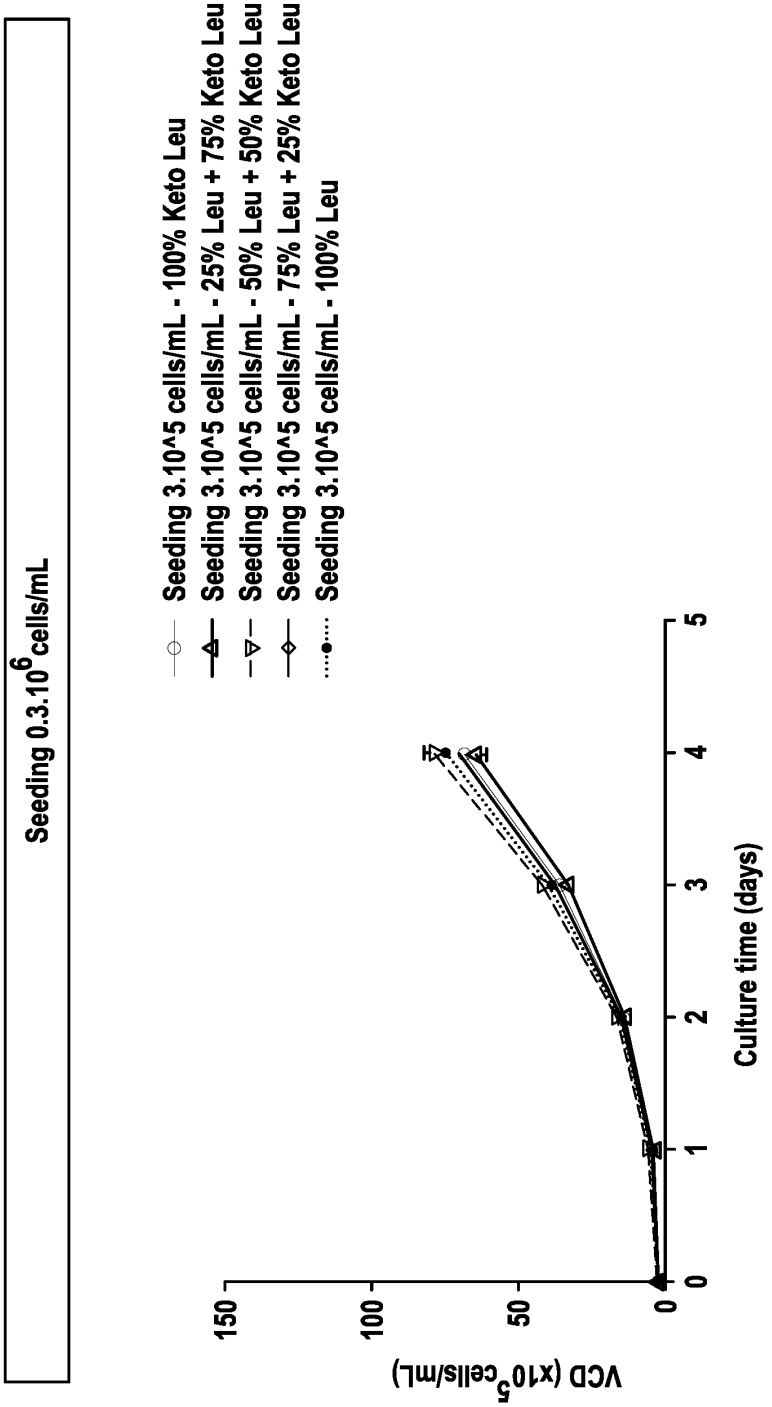

Seeding 0.3.10^6 cells/mL
Seeding 3.10^5 cells/mL - 100% Keto Leu
Seeding 3.10^5 cells/mL - 25% Leu + 75% Keto Leu
Seeding 3.10^5 cells/mL - 50% Leu + 50% Keto Leu
Seeding 3.10^5 cells/mL - 75% Leu + 25% Keto Leu
Seeding 3.10^5 cells/mL - 100% Leu
VCD (x10^5 cells/mL)
150
100
50
0
0
1
2
3
4
5
Culture time (days)

Fig. 16 part 2

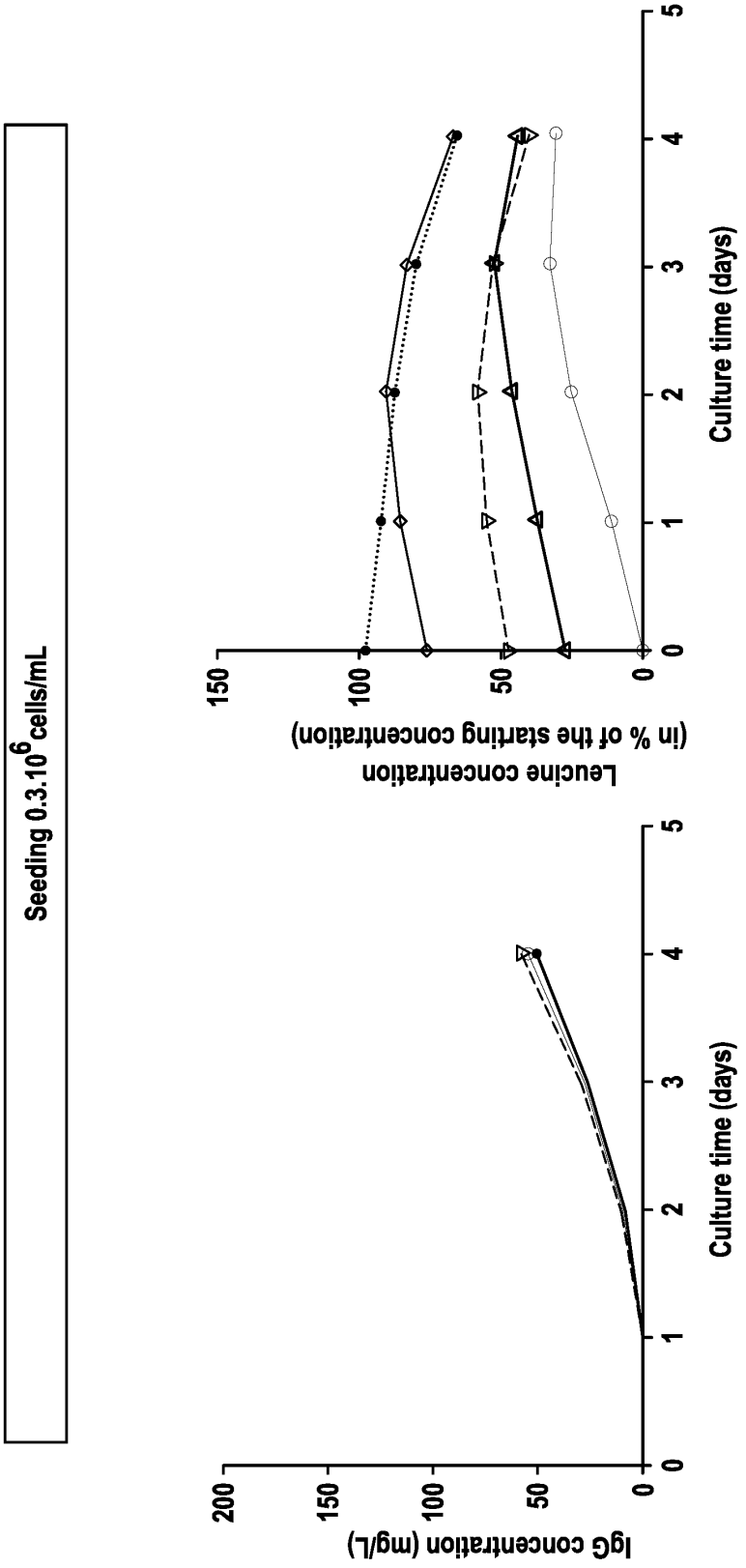

Seeding 0.3.10^{6}cells/mL
IgG concentration (mg/L)
Culture time (days)
Leucine concentration
(in % of the starting concentration)
Culture time (days)

Fig. 16 part 3

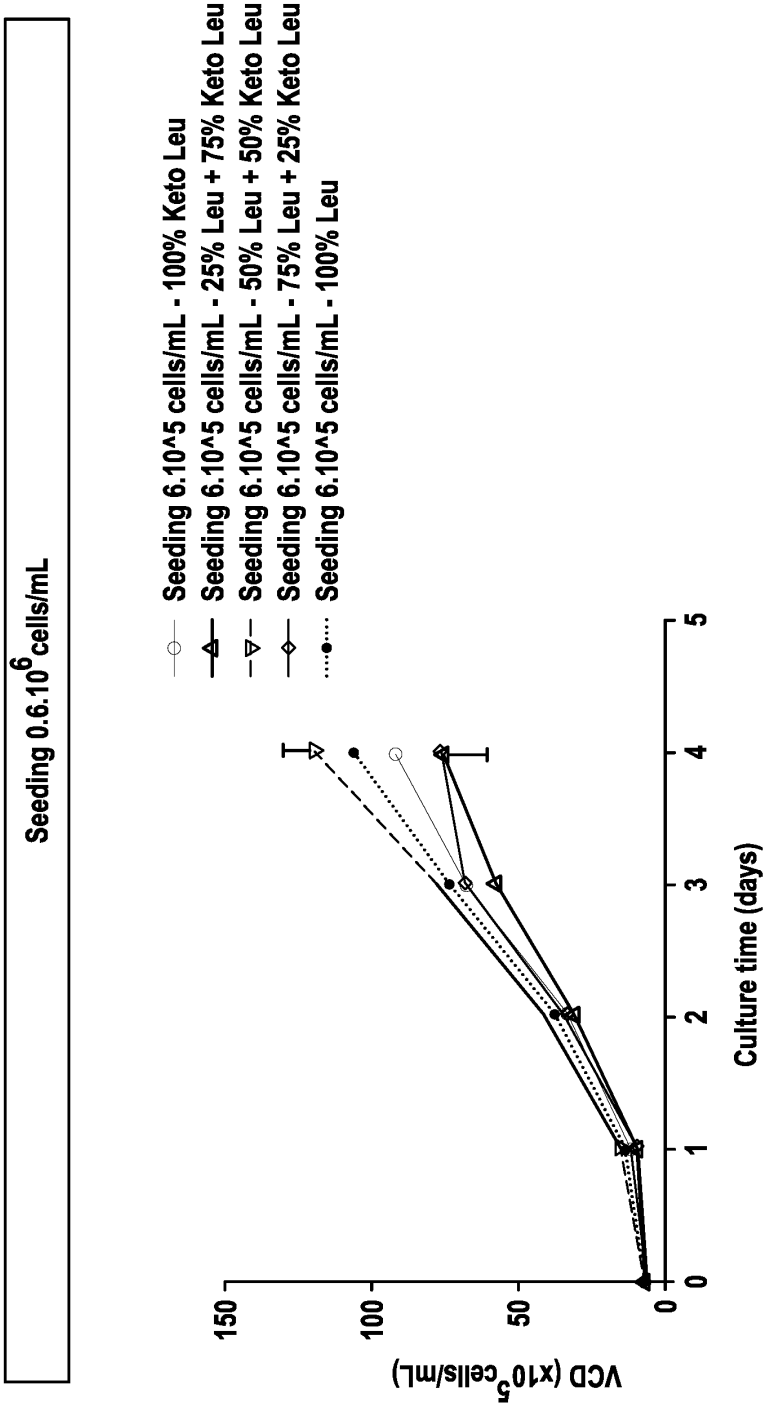

Seeding 0.6.10^6 cells/mL
Seeding 6.10^5 cells/mL - 100% Keto Leu
Seeding 6.10^5 cells/mL - 25% Leu + 75% Keto Leu
Seeding 6.10^5 cells/mL - 50% Leu + 50% Keto Leu
Seeding 6.10^5 cells/mL - 75% Leu + 25% Keto Leu
Seeding 6.10^5 cells/mL - 100% Leu
VCD (x10^5 cells/mL)
150
100
50
0
0
1
2
3
4
5
Culture time (days)

Fig. 16 part 4

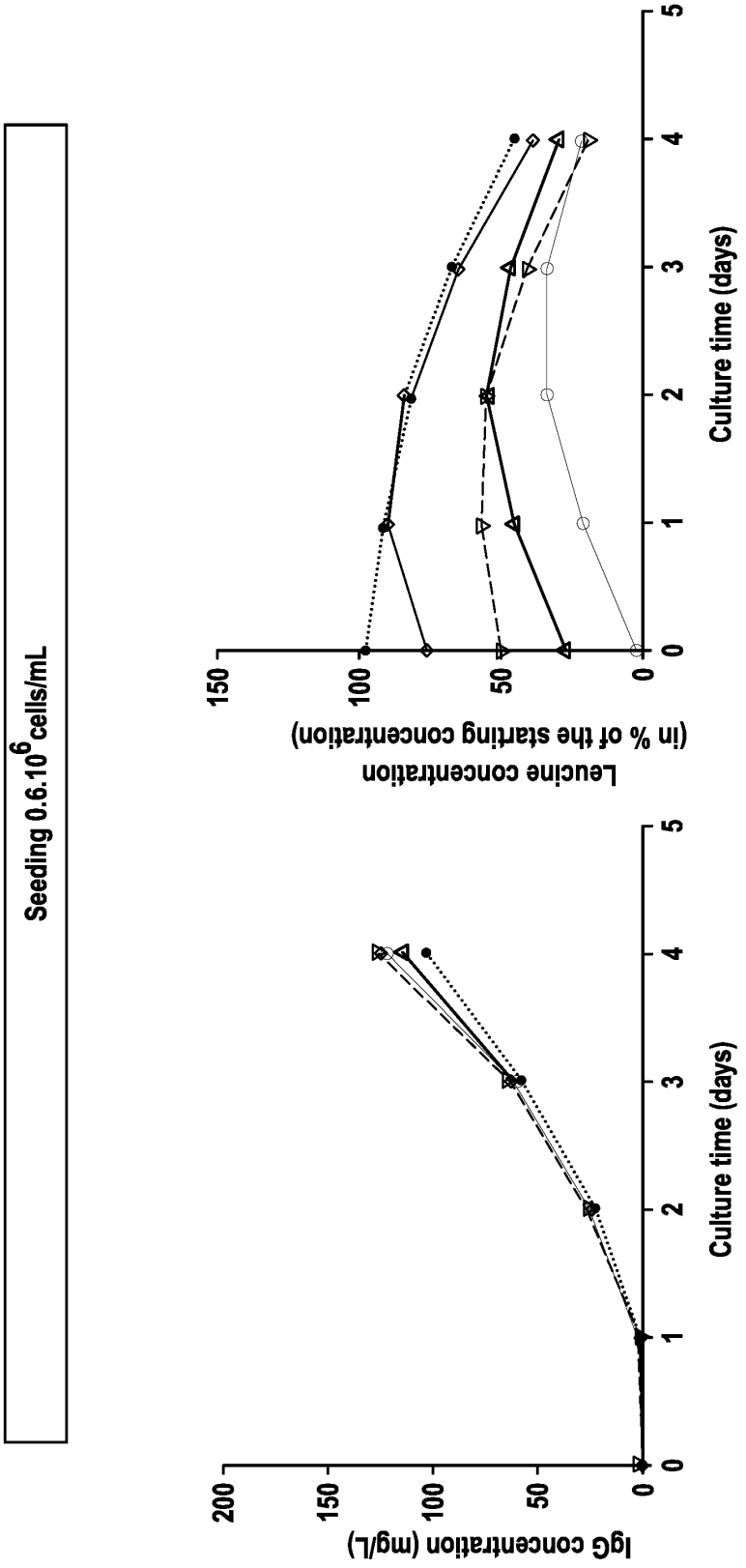

Seeding 0.6.10^6 cells/mL
IgG concentration (mg/L)
Culture time (days)
Leucine concentration
(in % of the starting concentration)
Culture time (days)

Fig. 16 part 5

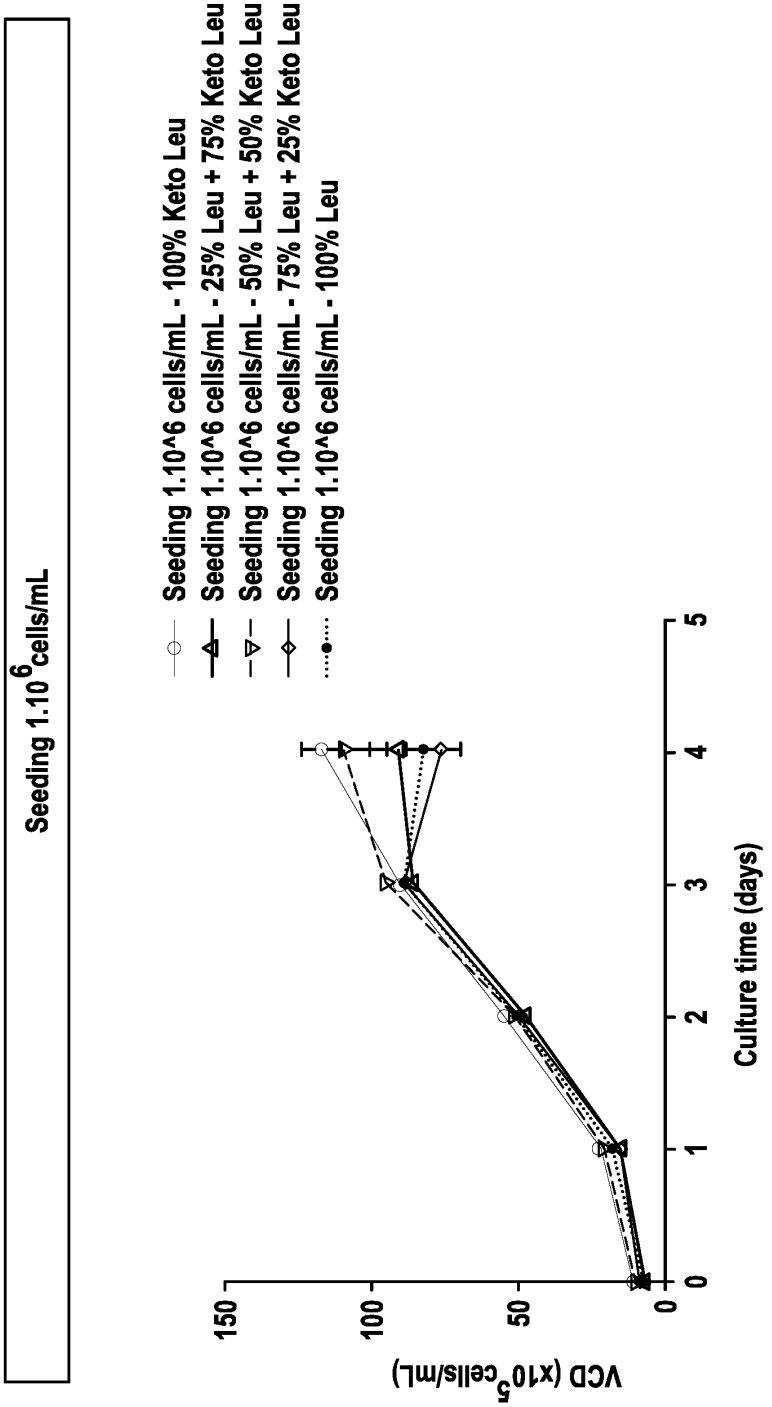

Seeding 1.10^6 cells/mL
Seeding 1.10^6 cells/mL - 100% Keto Leu
Seeding 1.10^6 cells/mL - 25% Leu + 75% Keto Leu
Seeding 1.10^6 cells/mL - 50% Leu + 50% Keto Leu
Seeding 1.10^6 cells/mL - 75% Leu + 25% Keto Leu
Seeding 1.10^6 cells/mL - 100% Leu
VCD (x10^5 cells/mL)
150
100
50
0
0
1
2
3
4
5
Culture time (days)

Fig. 16 part 6

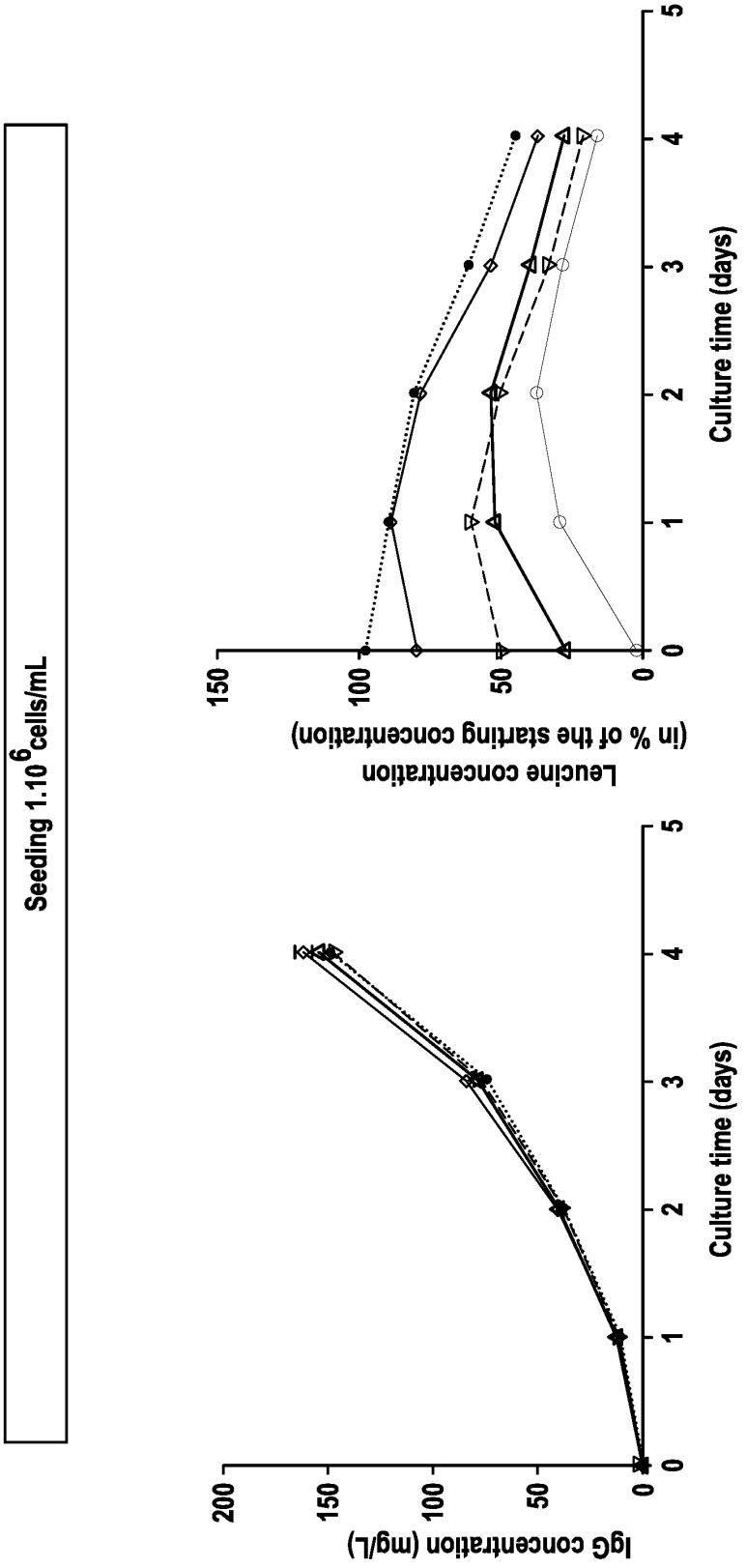

Seeding 1.10^6 cells/mL
IgG concentration (mg/L)
Culture time (days)
Leucine concentration
(in % of the starting concentration)
Culture time (days)

Fig. 17 part 1

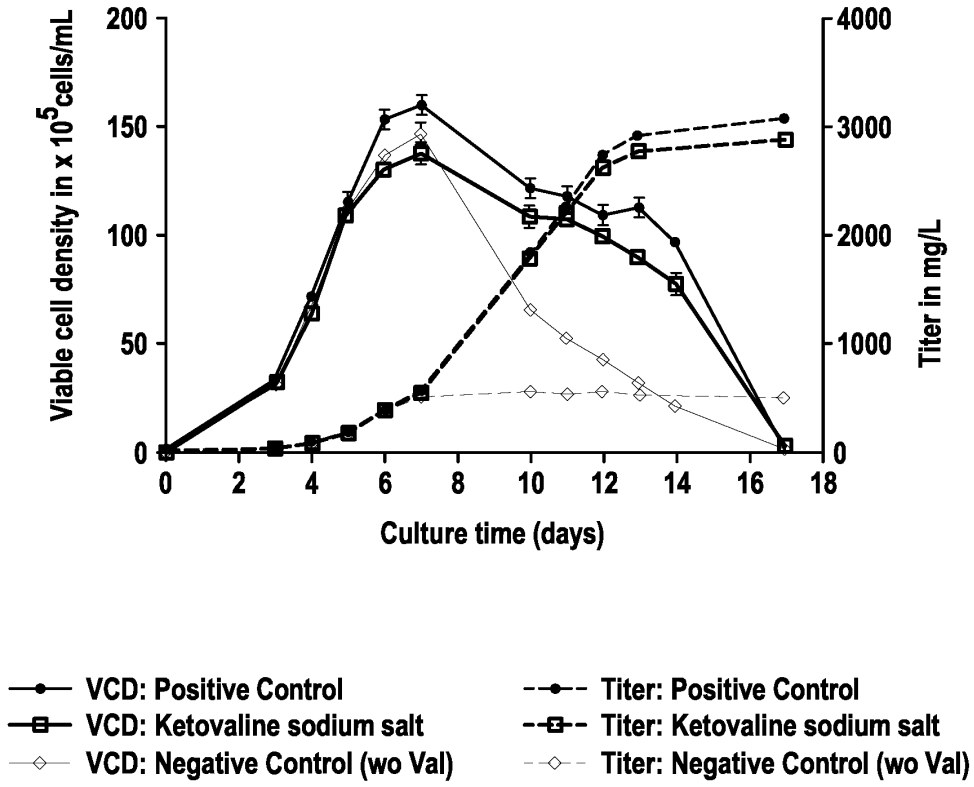
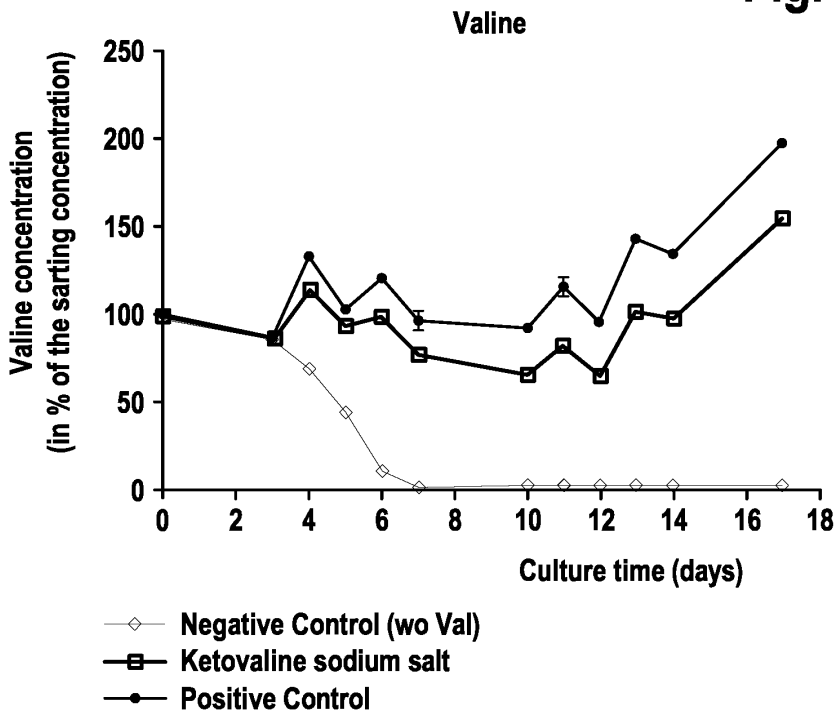

Viable cell density in x 10^5 cells/mL
Titer in mg/L
Culture time (days)
0
50
100
150
200
1000
2000
3000
4000
0
2
4
6
8
10
12
14
16
18
VCD: Positive Control
VCD: Ketovaline sodium salt
VCD: Negative Control (wo Val)
Titer: Positive Control
Titer: Ketovaline sodium salt
Titer: Negative Control (wo Val)

Fig. 17 part 2


Valine
Valine concentration (in % of the sarting concentration)
250
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18
Culture time (days)
Negative Control (wo Val)
Ketovaline sodium salt
Positive Control

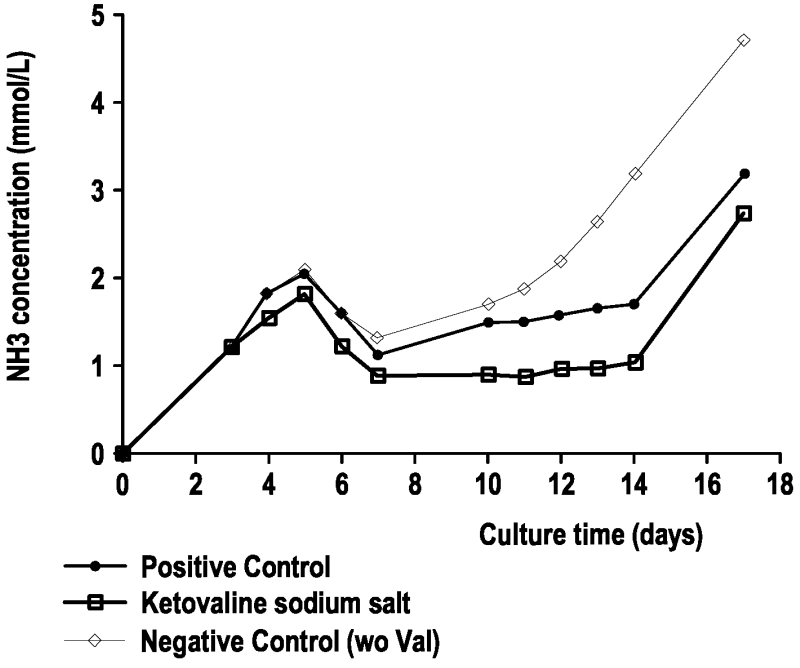
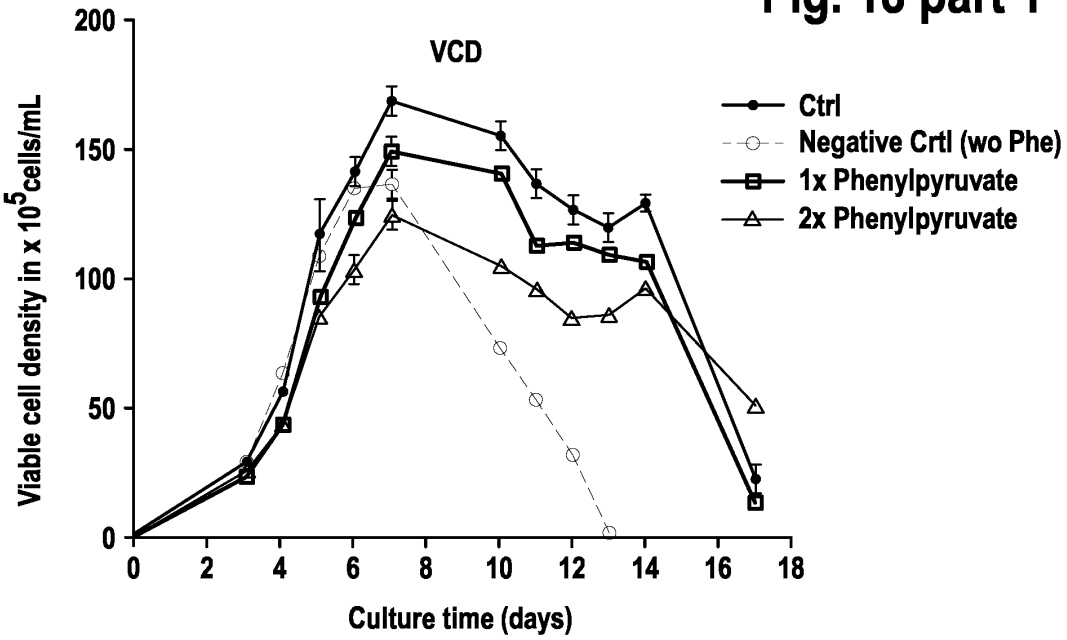

NH3 concentration (mmol/L)
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18
Culture time (days)
Positive Control
Ketovaline sodium salt
Negative Control (wo Val)

Fig. 18 part 1


VCD
Viable cell density in x 10^5cells/mL
Culture time (days)
Ctrl
Negative Crtl (wo Phe)
1x Phenylpyruvate
2x Phenylpyruvate

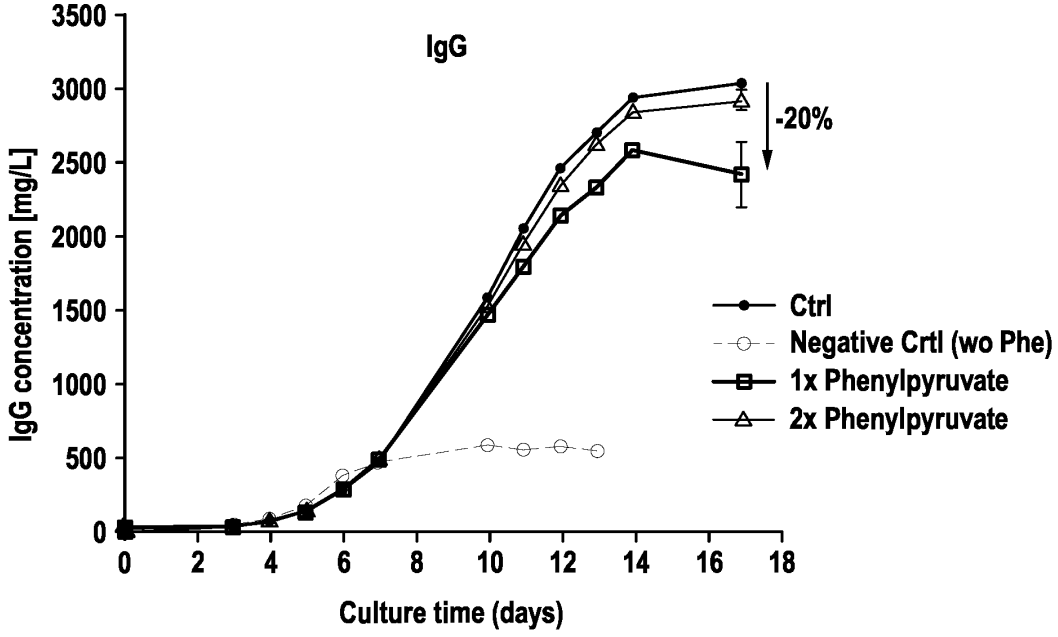
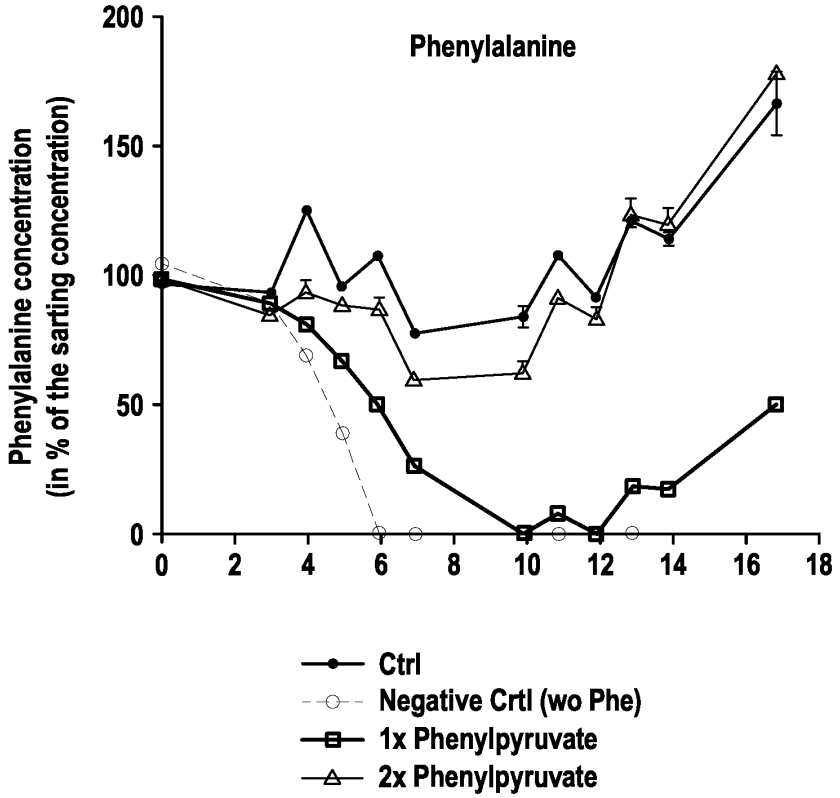

IgG
IgG concentration [mg/L]
Culture time (days)
-20%
Ctrl
Negative Crtl (wo Phe)
1x Phenylpyruvate
2x Phenylpyruvate

Fig. 18 part 2


Phenylalanine
Phenylalanine concentration
(in % of the sarting concentration)
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
Ctrl
Negative Crtl (wo Phe)
1x Phenylpyruvate
2x Phenylpyruvate