DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation-Report
proficiency test

DLA 33/2014

Free amino acids

Dienstleistung Lebensmittel Analytik GbR
Waldemar Bonsels Weg 170
22926 Ahrensburg
Deutschland

proficiency-testing@dla-lvu.de
www.dla-lvu.de

Coordinator of this PT:
Dipl.Chem. Udo Kasel

Contents

1 Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009.

2 Evaluation

2.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X. The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

2.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (Sx) was calculated.

2.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (95% significance). Detected outliers were stated for information only, when z-score was < -2 or > 2.

2.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

2.4.1 General model (Horwitz / Thompson)

The relative target standard deviation in % of the assigned value was derived from following equation (Horwitz)

$$\sigma_{(\%)} = 2^{(1-0,5\log X)} .$$

From the result the target standard deviation was calculated

$$\sigma = X * \sigma_{(\%)} / 100.$$

For analytes with a content below 120 µg/kg after the evaluation of a lot of mycotoxin- proficiency testing schemes after 1997 it was suggested for the target standard deviation a steady value of 22 % (Thompson), analogical:

$$\sigma = 0,22\ C / mr;$$

with σ = Target standard deviation for contents < 120 µg/kg
C = assigned content, expressed as a dimensionless mass ratio
mr = dimensionless mass ratio.

2.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated :

$$\sigma = \sqrt{(\sigma_L^2 + (\sigma_r^2 / n))} .$$

If available, the precision data from official methods for each parameter are used to calculate the target standard deviation.

2.5 *z-Score*

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ) the result (x) of the participant is deviating from the assigned value (X).

Participants' z-scores are derived as:

$$z = (x - X) / \sigma \quad ;$$

the requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

2.6 *z'-Score*

The z'-Score can be used to assess the results of the participants in case the standard uncertainty must be considered (s. 2.8).

The calculation is carried out as follows (3)

$$z' = (x - X) / \sqrt{\hat{\sigma}^2 + u_X^2}$$

For the following evaluation $\sqrt{\hat{\sigma}^2 + u_X^2}$ is defined as $\hat{\sigma}'$, the target standard deviation considering the standard uncertainty of the results.

The requirements for the analytical performance are considered as fulfilled then, if

$$-2 \leq z' \leq 2 \ .$$

2.7 *Quotient S^x/σ*

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given.

2.8 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty u_X for this PT is calculated as follows

$$u_X = 1{,}25 * S^x / \sqrt{(p)} \quad .$$

If $u_X \leq 0{,}3 * \sigma$ the standard uncertainty of the assigned value needs not be included in the interpretation of the results of the PT. The quotient u_X / σ is given in the evaluation.

3 Realisation

3.1 Test material

Test material was an italian hard cheese, 18 month old. App. 500g of the material were grated, mixed with app. 0,5% sorbic acid, mixed and put in portions of app. 10 gram. The portions were numbered chronologically.

3.1.1 *Homogeneity*

The calculation of the repeatability standard deviation of the participants for alanine and histidine was used as an indicator of homogeneity. The results are 4,3% and 3,5% and in the same magnitude as the repeatability standard deviation of the German official method. The repeatability standard deviation of the participants is given in the documentation.
In the documentation the portion numbers are graphically assigned to the results of alanine and histidine. There is no trend recognizable in the results which could suggest inhomogeneity.

3.2 Tests

Two test samples were sent to every participating laboratory in the 40th week of 2014. The test method was optional. The tests should be finished at 14.11.2014.

3.3 Results and statistic evaluation

The participants submitted their results in standard forms, which have been handed out with the samples.

The statistical evaluation was carried out according to 2.4.2 of this report if at least 7 results were submitted, which were:
The target standard deviation,σ, for each amino acid was calculated from ASU L 07.00-64.
For information the target standard deviation according to 2.4.1, „Horwitz", is stated additionally.
Tyrosine was evaluated with the precision data from ASU L 49.07-2, extended according to 2.6 of this report.

Two participants submitted results, differing about a factor of app. 10000 from the others. Because these two sets of results distorted the whole evaluation, they were eliminated as extreme outliers.

Single results for Asparagine, Citrulline and Glutamine are documented.
Queried and documented were single results and the testing method applied.
12 out of 12 participants submitted their results in time.

4 Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

In the upper table the characteristics **without participants 1 and 4** are listed (see 3.3):

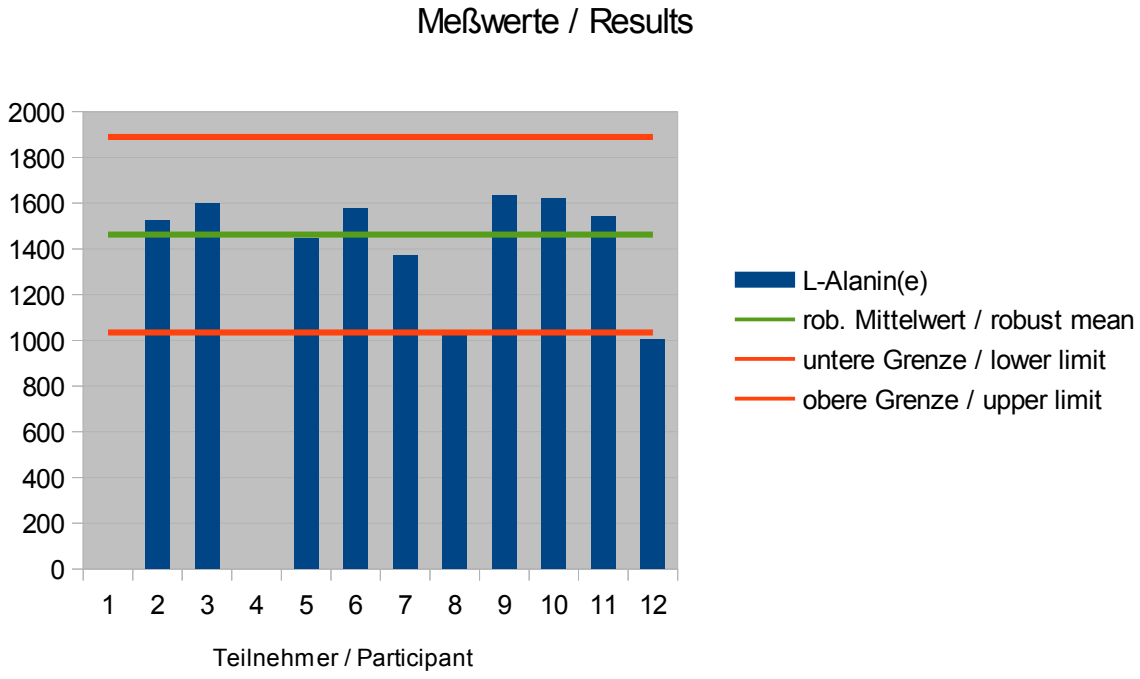
Number of the results
Number of outliers
Mean
Median
Robust mean (X)
Robust standard deviation(S^x)
Target standard deviation (σ´)
Target standard deviation Horwitz (for information)
Lower limit of target range (X – 2σ´)
Upper limit of target range (X + 2σ´)
Quotient S^x/σ
Standard uncertainty u_x
Quotient u_X/σ
Results in target range.

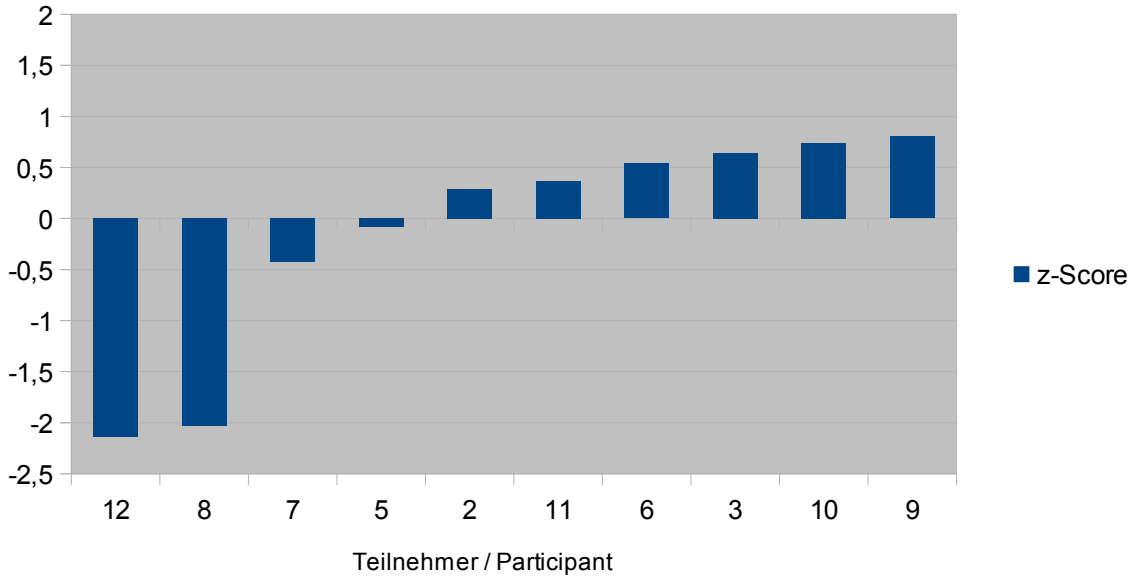
In the lower table -laboratories- the individual results of the participating laboratory are listed:

Evaluation number	test result	deviation from assigned value	Z-Score (σ)	Z-Score (Horwitz) for information	remarks

4.1 Alanin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	1435
Median	1533
Robust mean (X)	1462
Robust standard deviation (S^x)	205
Target standard deviation (sigma)	214
Lower limit of target range	1035
Upper limit of target range	1889
Quotient S^x/σ	1,0
Standard uncertainty U^x	81
Quotient U^x/σ	0,4
Results in target range	9
Percent in target range	90

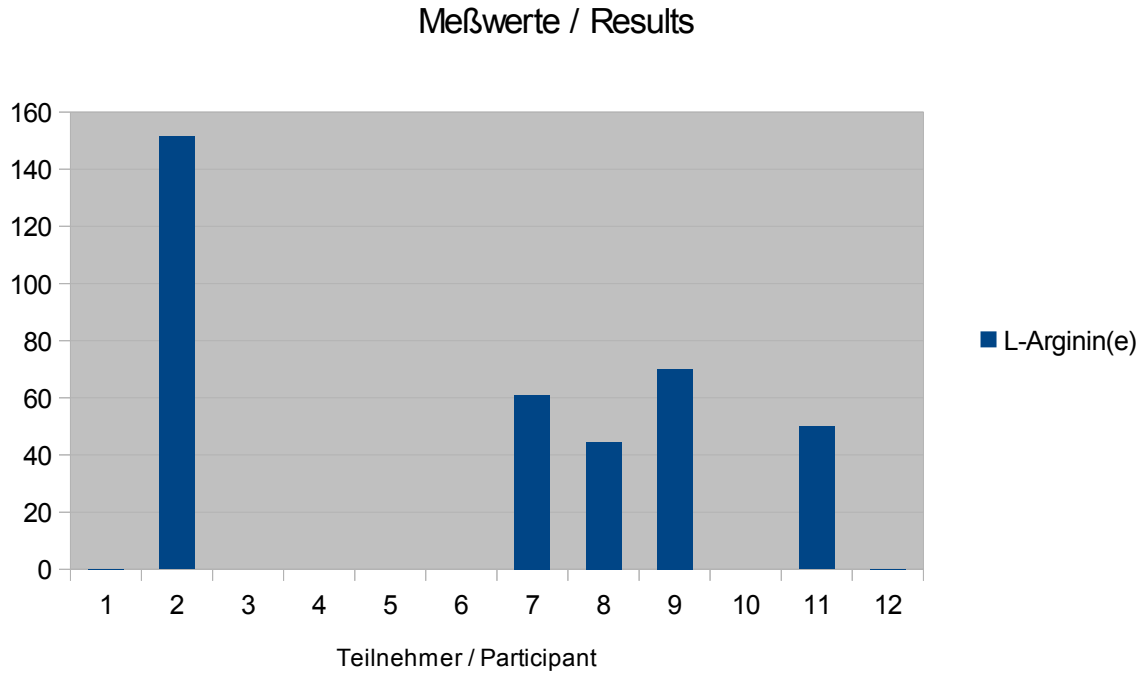




Auswerte nummer / Evaluation number	L-Alanin(e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,314				
2	1525	62,69	0,3	0,8	
3	1600	137,69	0,6	1,8	
4	0,16				
5	1444,25	-18,06	-0,1	-0,2	
6	1577,5	115,19	0,5	1,5	
7	1371	-91,31	-0,4	-1,2	
8	1029	-433,31	-2,0	-5,5	
9	1634	171,69	0,8	2,2	
10	1620	157,69	0,7	2,0	
11	1541	78,69	0,4	1,0	
12	1005	-457,31	-2,1	-5,9	Ausreisser / Outlier

4.2 Arginin(e) in mg/kg

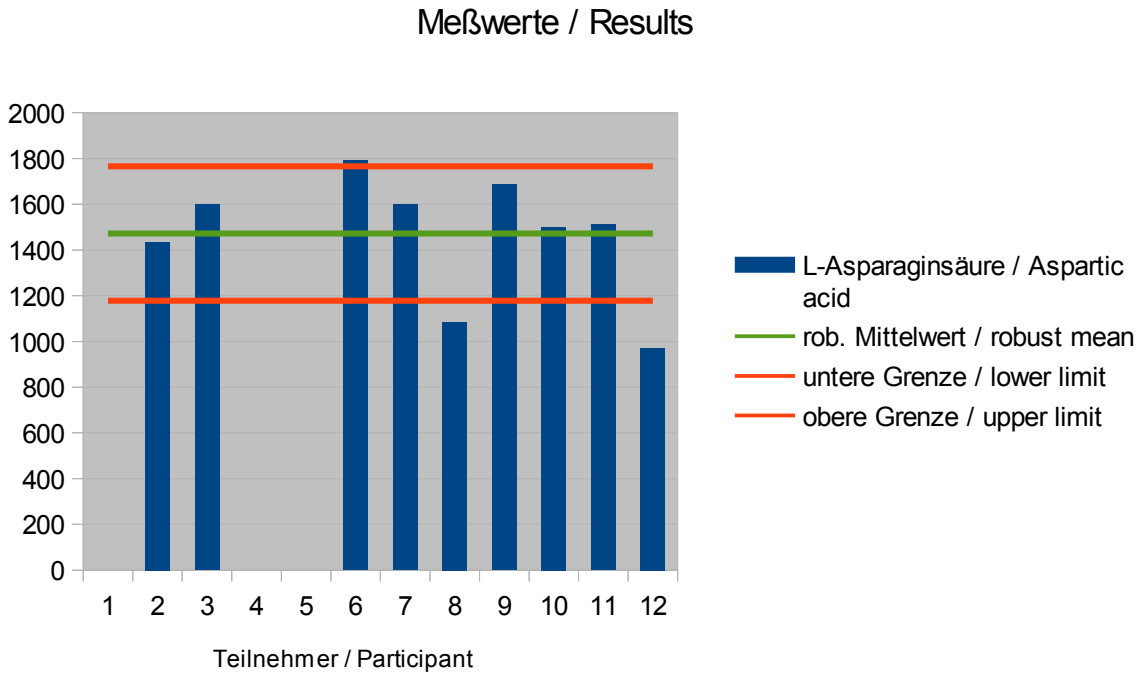
Number of the results	5
Number of outliers	1
Mean	not evaluated
Median	not evaluated
Robust mean (X)	not evaluated
Robust standard deviation (S^x)	not evaluated
Target standard deviation (sigma)	not evaluated
Lower limit of target range	not evaluated
Upper limit of target range	not evaluated
Quotient S^x/σ	not evaluated
Standard uncertainty U^x	not evaluated
Quotient U^x/σ	not evaluated
Results in target range	not evaluated
Percent in target range	not evaluated

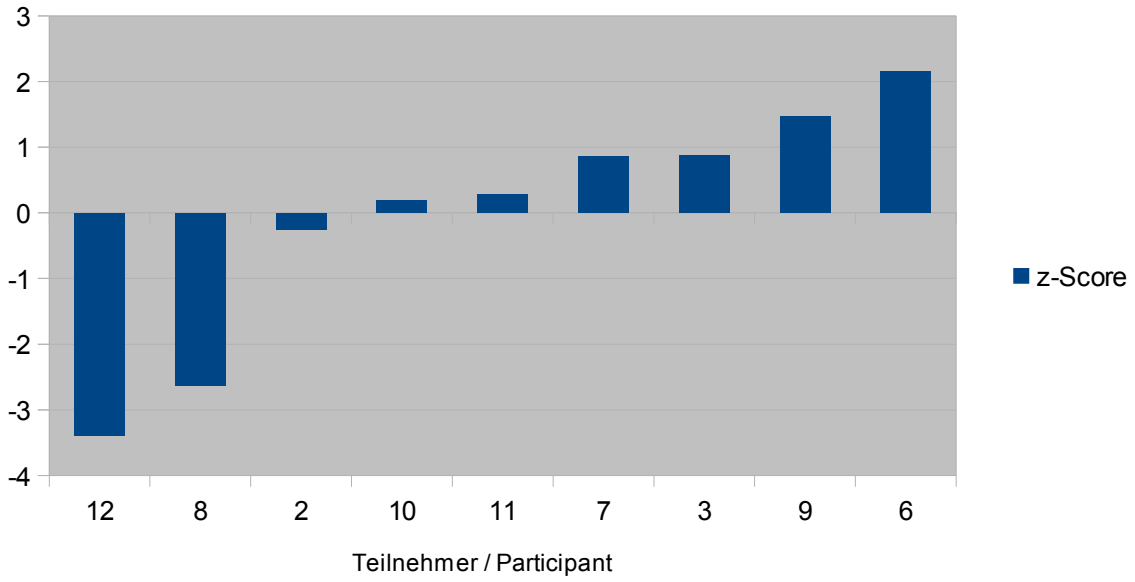


Auswerte nummer / Evaluation number	L-Arginin(e)
1	0,022
2	151,45
3	
4	
5	<100
6	
7	61
8	44,6
9	70
10	<200
11	50
12	

4.3 Asparaginsäure (Aspartic acid) in mg/kg

Statistic Data	
Number of the results	9
Number of outliers	1
Mean	1465
Median	1513
Robust mean (X)	1472
Robust standard deviation (S^x)	290
Target standard deviation (sigma)	147
Lower limit of target range	1178
Upper limit of target range	1766
Quotient S^x/σ	2,0
Standard uncertainty U^x	121
Quotient U^x/σ	0,8
Results in target range	6
Percent in target range	67

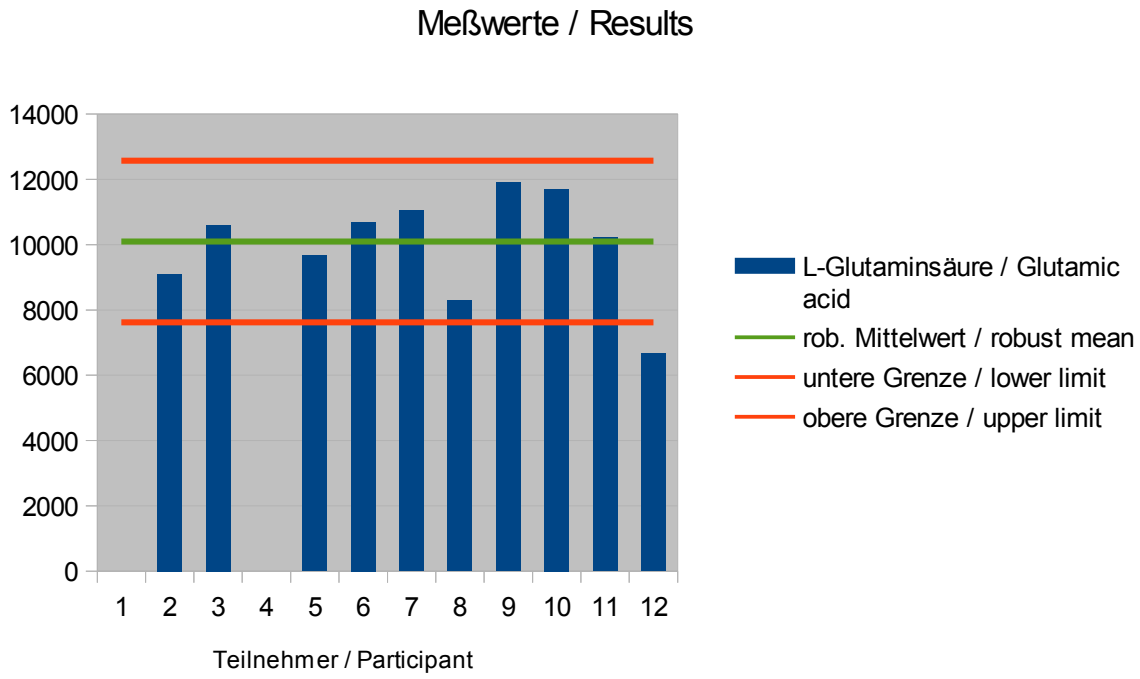


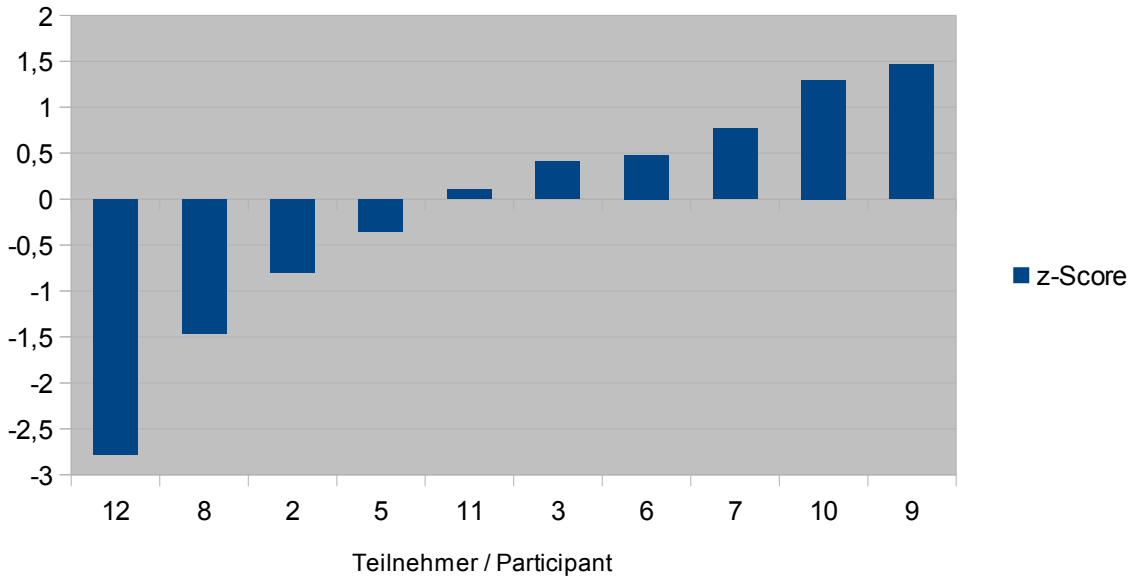


Auswerte nummer / Evaluation number	L-Asparaginsäure / Aspartic acid	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,304				
2	1435	-36,77	-0,3	-0,5	
3	1600	128,23	0,9	1,6	
4	0,15				
5	nicht auswertbar / not evaluable				
6	1790	318,23	2,2	4,1	
7	1598	126,23	0,9	1,6	
8	1085	-386,77	-2,6	-4,9	
9	1688	216,23	1,5	2,8	
10	1500	28,23	0,2	0,4	
11	1513	41,23	0,3	0,5	
12	972	-499,77	-3,4	-6,4	Ausreisser / Outlier

4.4 Glutaminsäure (Glutamic acid) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	9988
Median	10413
Robust mean (X)	10095
Robust standard deviation (S^x)	1578
Target standard deviation (sigma)	1238
Lower limit of target range	7619
Upper limit of target range	12570
Quotient S^x/σ	1,3
Standard uncertainty U^x	624
Quotient U^x/σ	0,5
Results in target range	9
Percent in target range	90

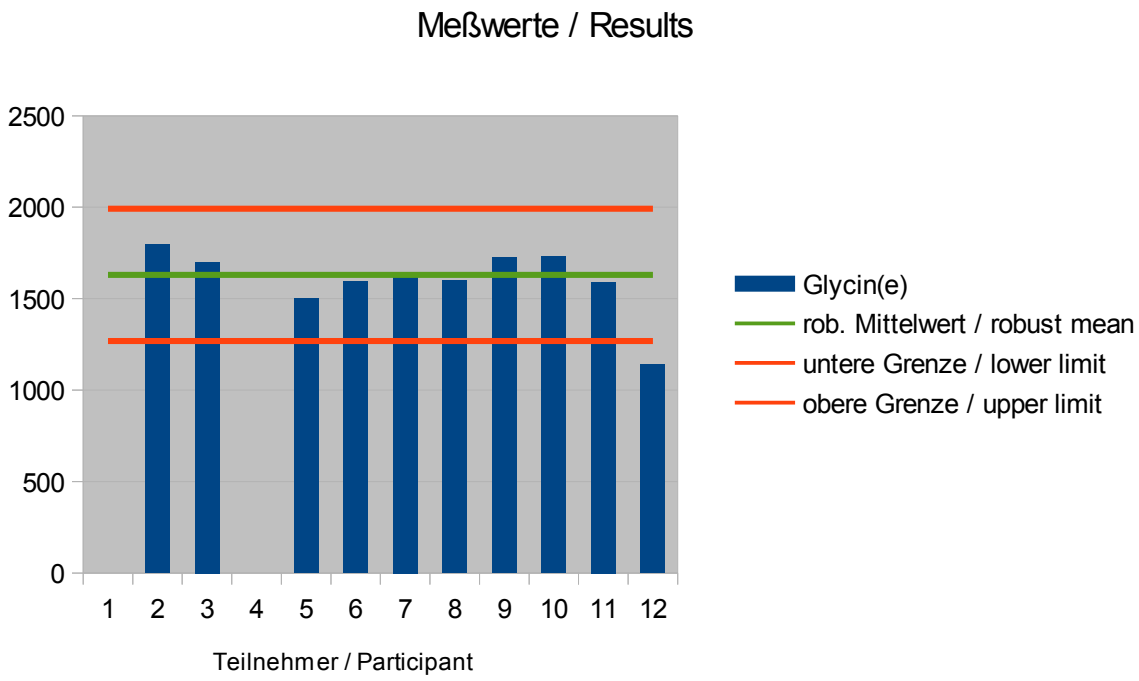


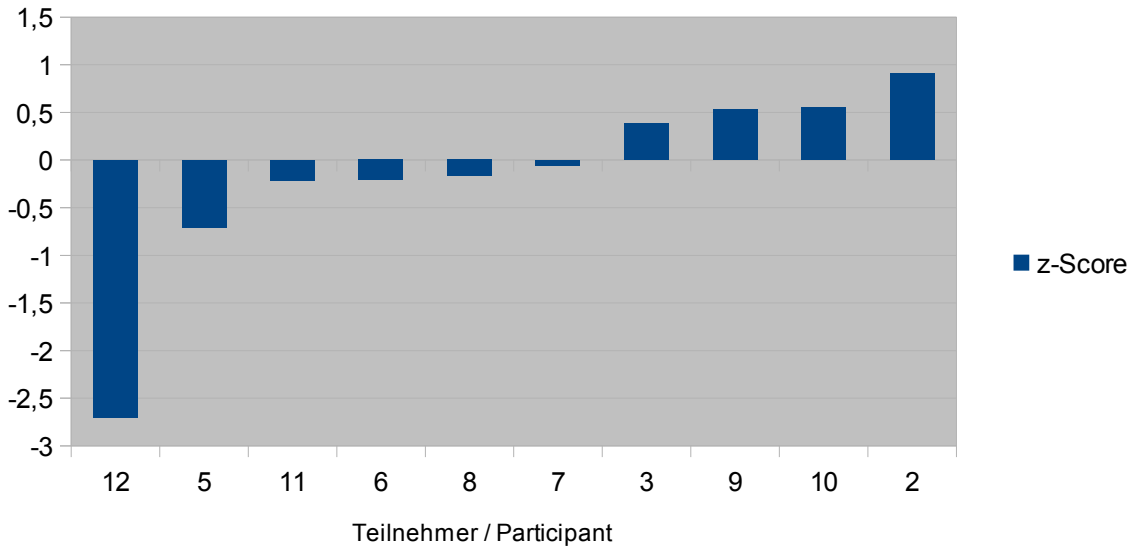


Auswerte nummer / Evaluation number	L-Glutaminsäure / Glutamic acid	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	1,95				
2	9105	-989,75	-0,8	-2,5	
3	10600	505,25	0,4	1,3	
4	1,05				
5	9664	-430,75	-0,3	-1,1	
6	10687,5	592,75	0,5	1,5	
7	11045	950,25	0,8	2,4	
8	8285	-1809,75	-1,5	-4,5	
9	11908	1813,25	1,5	4,5	
10	11700	1605,25	1,3	4,0	
11	10226	131,25	0,1	0,3	
12	6660	-3434,75	-2,8	-8,5	Ausreisser / Outlier

4.5 Glycin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	1600
Median	1610
Robust mean (X)	1630
Robust standard deviation (S^x)	123
Target standard deviation (sigma)	181
Lower limit of target range	1268
Upper limit of target range	1992
Quotient S^x/σ	0,7
Standard uncertainty U^x	48
Quotient U^x/σ	0,3
Results in target range	9
Percent in target range	90

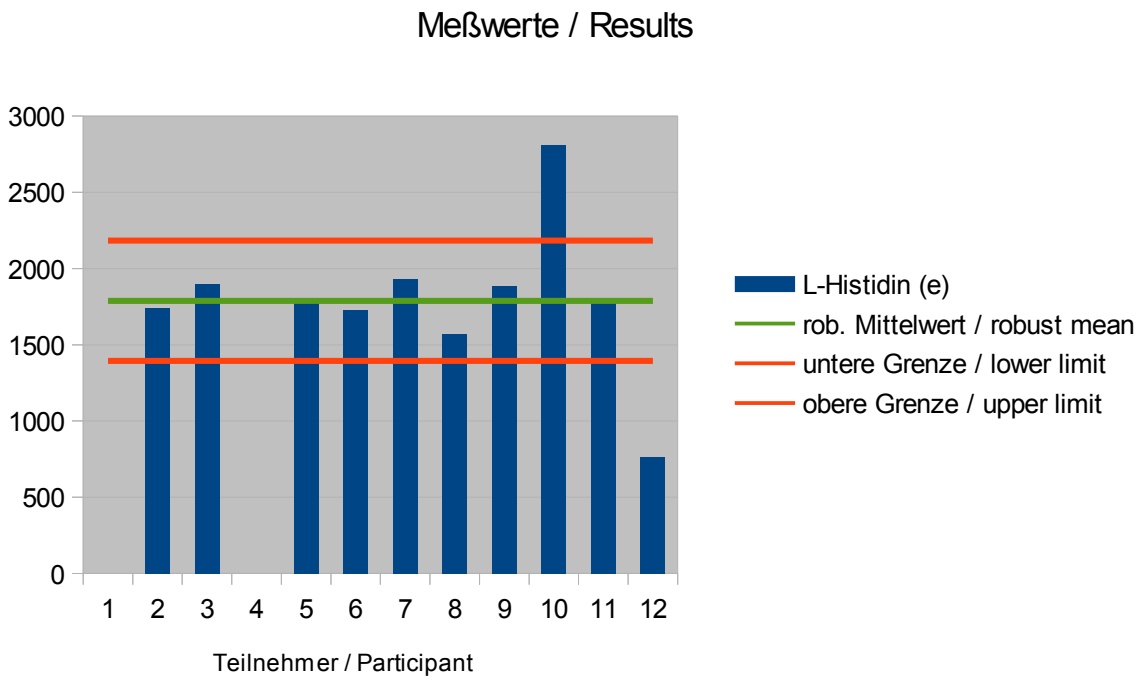


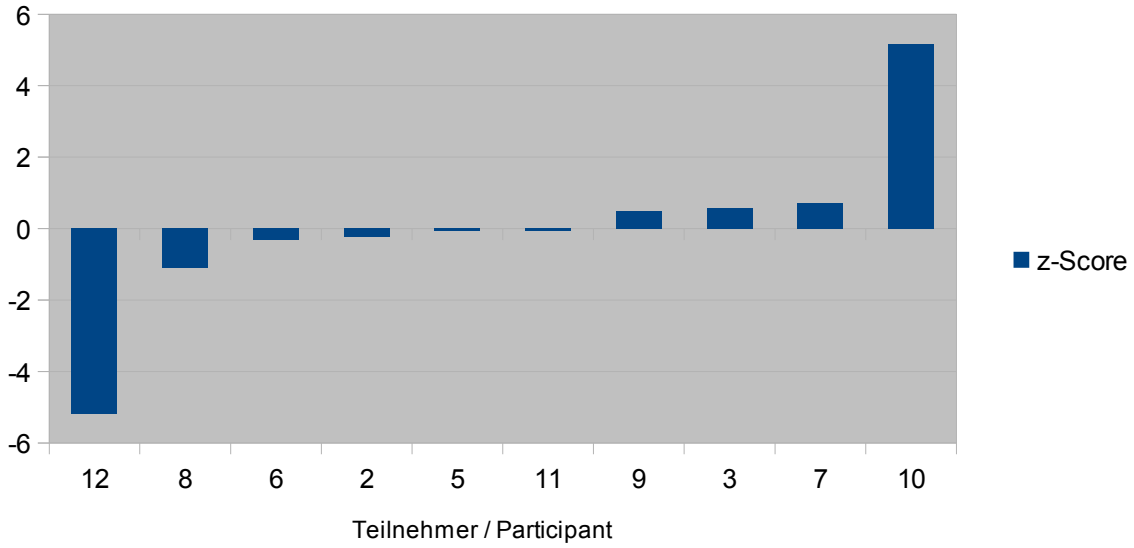


Auswerte nummer / Evaluation number	Glycin(e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,323				
2	1795	164,78	0,9	1,9	
3	1700	69,78	0,4	0,8	
4	0,17				
5	1502,5	-127,72	-0,7	-1,5	
6	1592,5	-37,72	-0,2	-0,4	
7	1619	-11,22	-0,1	-0,1	
8	1600	-30,22	-0,2	-0,4	
9	1726	95,78	0,5	1,1	
10	1730	99,78	0,6	1,2	
11	1591	-39,22	-0,2	-0,5	
12	1142	-488,22	-2,7	-5,7	Ausreisser / Outlier

4.6 Histidin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	2
Mean	1789
Median	1775
Robust mean (X)	1789
Robust standard deviation (S^x)	194
Target standard deviation (sigma)	197
Lower limit of target range	1394
Upper limit of target range	2183
Quotient S^x/σ	1,0
Standard uncertainty U^x	77
Quotient U^x/σ	0,4
Results in target range	8
Percent in target range	80

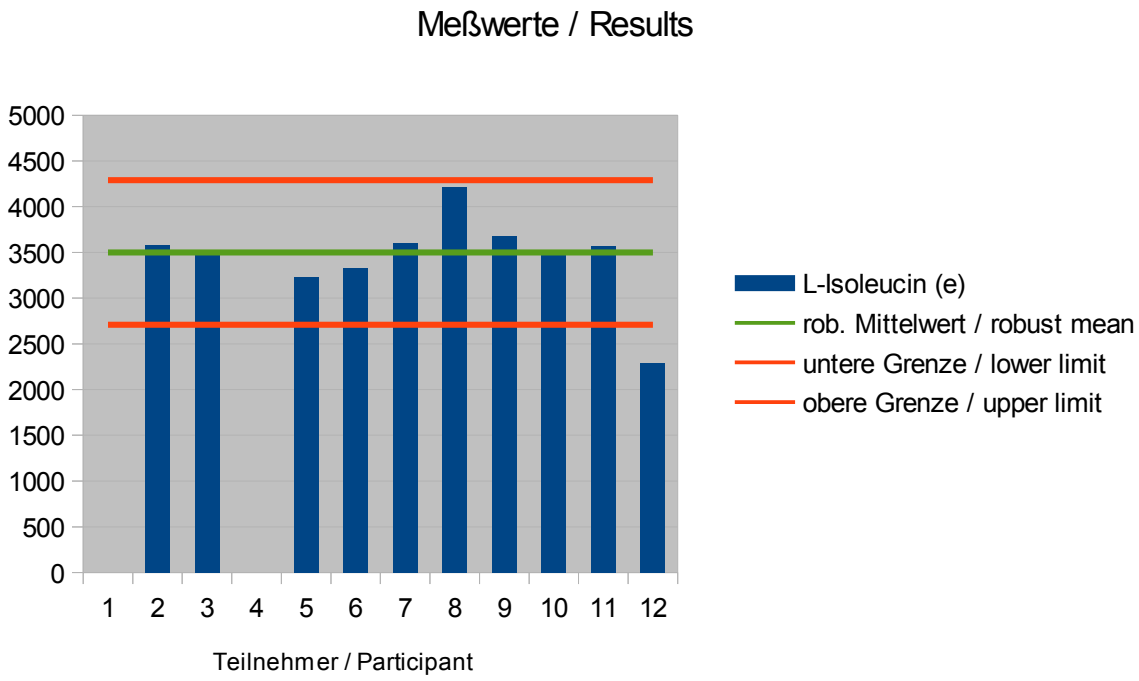


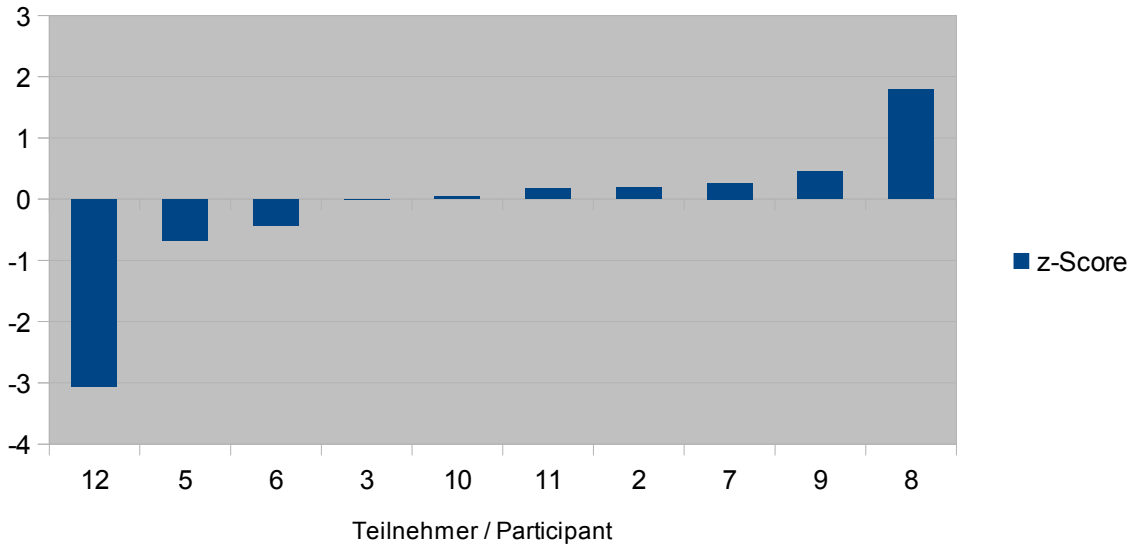


Auswerte nummer / Evaluation number	L-Histidin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,531				
2	1744	-44,66	-0,2	-0,5	
3	1900	111,34	0,6	1,2	
4	0,17				
5	1773,75	-14,91	-0,1	-0,2	
6	1727,5	-61,16	-0,3	-0,7	
7	1931	142,34	0,7	1,5	
8	1573	-215,66	-1,1	-2,3	
9	1884	95,34	0,5	1,0	
10	2810	1021,34	5,2	11,0	Ausreisser / Outlier
11	1776	-12,66	-0,1	-0,1	
12	766	-1022,66	-5,2	-11,0	Ausreisser / Outlier

4.7 Isoleucin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	3450
Median	3544
Robust mean (X)	3500
Robust standard deviation (S^x)	248
Target standard deviation (sigma)	395
Lower limit of target range	2710
Upper limit of target range	4291
Quotient S^x/σ	0,6
Standard uncertainty U^x	98
Quotient U^x/σ	0,2
Results in target range	9
Percent in target range	90

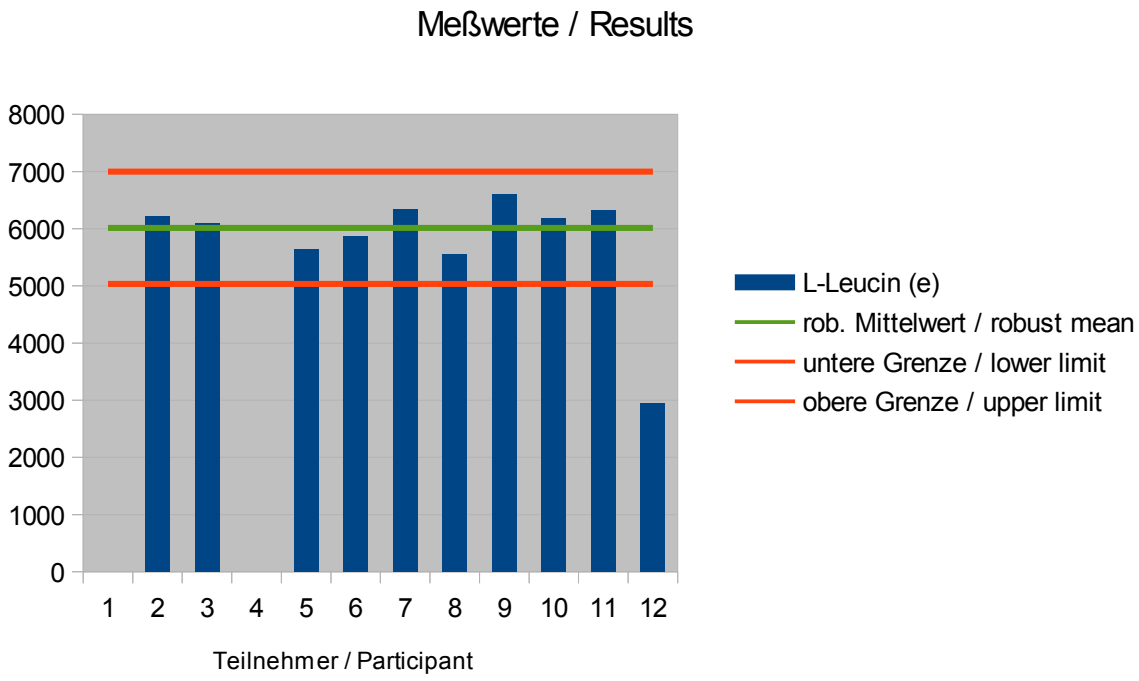


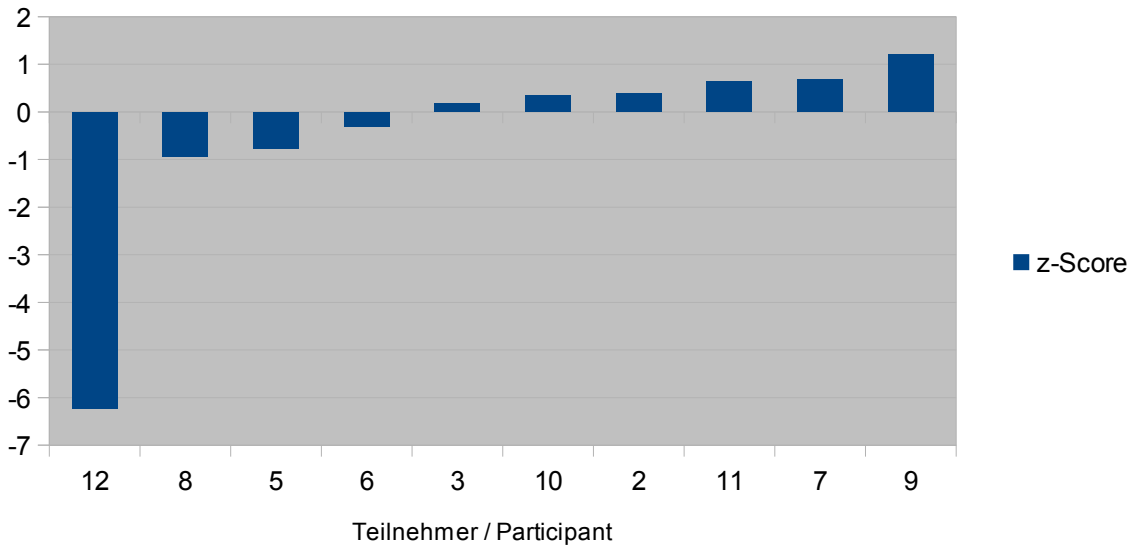


Auswerte nummer / Evaluation number	L-Isoleucin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,658				
2	3575	74,72	0,2	0,5	
3	3500	-0,28	0,0	0,0	
4	0,35				
5	3232,75	-267,53	-0,7	-1,6	
6	3327,5	-172,78	-0,4	-1,1	
7	3604	103,72	0,3	0,6	
8	4210	709,72	1,8	4,3	
9	3676	175,72	0,4	1,1	
10	3520	19,72	0,0	0,1	
11	3567	66,72	0,2	0,4	
12	2290	-1210,28	-3,1	-7,4	Ausreisser / Outlier

4.8 Leucin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	5779
Median	6145
Robust mean (X)	6016
Robust standard deviation (S^x)	462
Target standard deviation (sigma)	492
Lower limit of target range	5033
Upper limit of target range	6999
Quotient S^x/σ	0,9
Standard uncertainty U^x	182
Quotient U^x/σ	0,4
Results in target range	9
Percent in target range	90

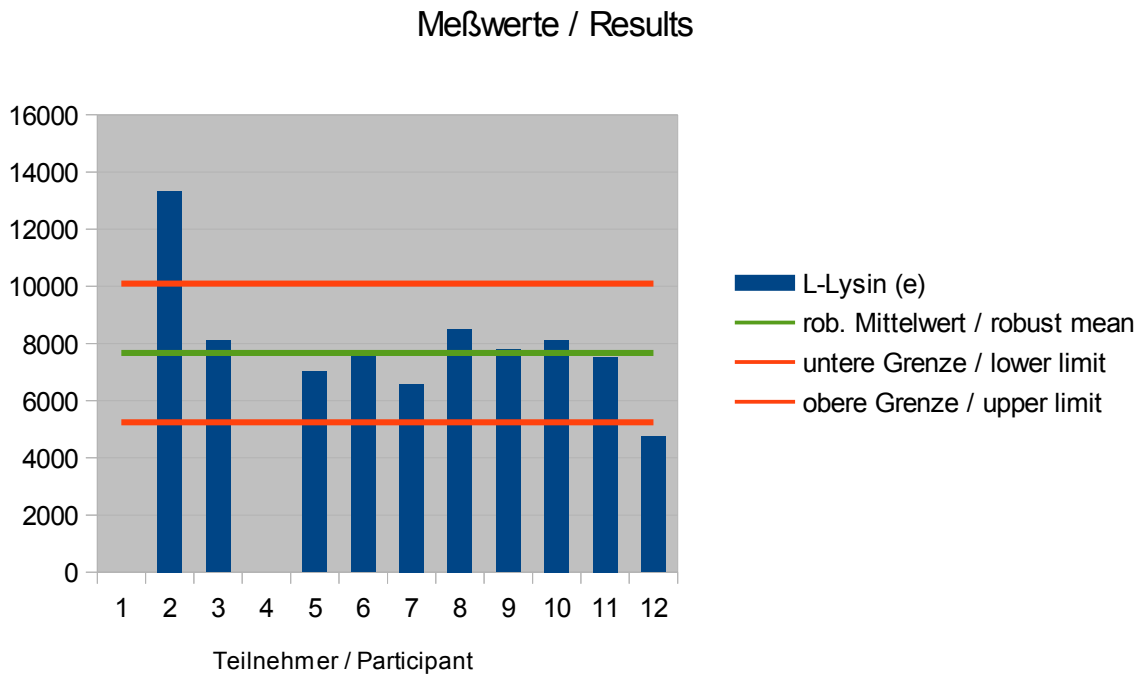


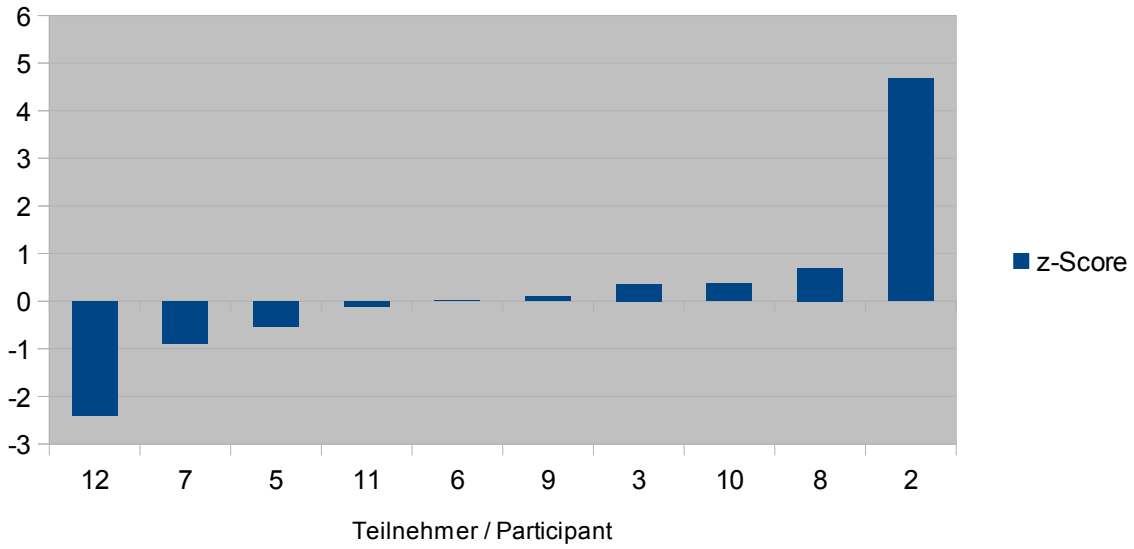


Auswerte nummer / Evaluation number	L-Leucin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	1,11				
2	6210	193,64	0,4	0,7	
3	6100	83,64	0,2	0,3	
4	0,62				
5	5636	-380,36	-0,8	-1,5	
6	5862,5	-153,86	-0,3	-0,6	
7	6345	328,64	0,7	1,3	
8	5556	-460,36	-0,9	-1,8	
9	6606	589,64	1,2	2,3	
10	6190	173,64	0,4	0,7	
11	6334	317,64	0,6	1,2	
12	2950	-3066,36	-6,2	-11,8	Ausreisser / Outlier

4.9 Lysin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	7942
Median	7734
Robust mean (X)	7667
Robust standard deviation (S^x)	1040
Target standard deviation (sigma)	1213
Lower limit of target range	5241
Upper limit of target range	10093
Quotient S^x/σ	0,9
Standard uncertainty U^x	411
Quotient U^x/σ	0,3
Results in target range	8
Percent in target range	80

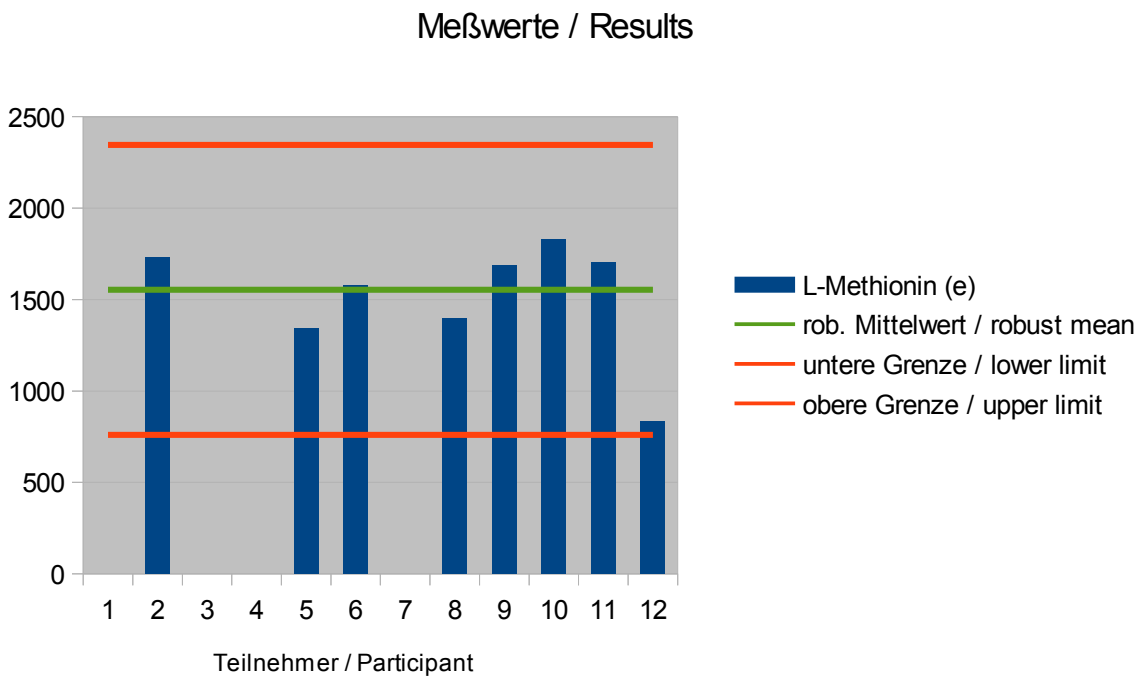


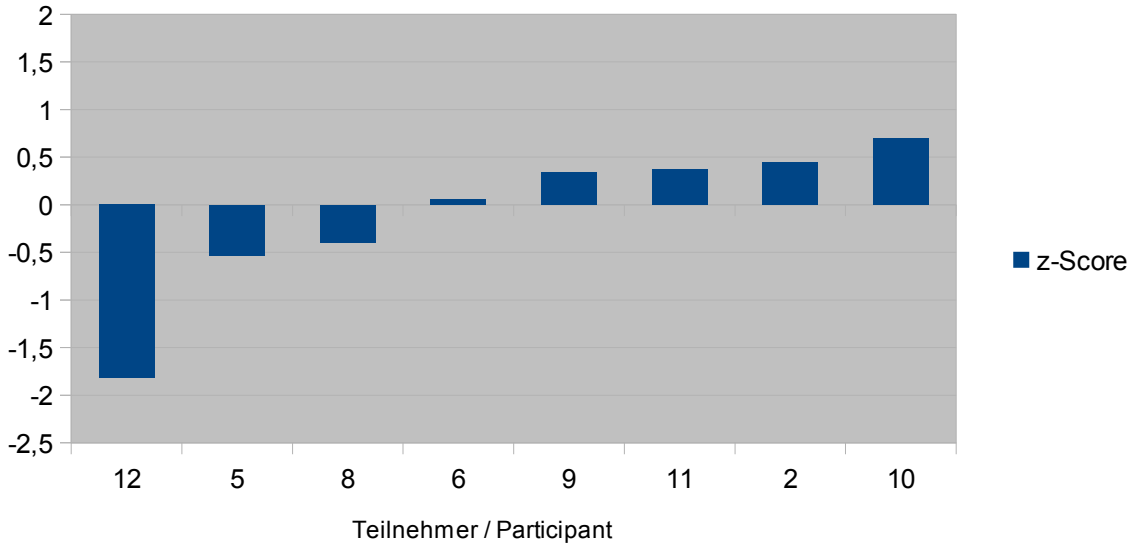


Auswerte nummer / Evaluation number	L-Lysin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	1,64				
2	13340	5673,12	4,7	17,8	Ausreisser / Outlier
3	8100	433,12	0,4	1,4	
4	0,75				
5	7020,5	-646,38	-0,5	-2,0	
6	7687,5	20,62	0,0	0,1	
7	6592	-1074,88	-0,9	-3,4	
8	8508	841,12	0,7	2,6	
9	7780	113,12	0,1	0,4	
10	8120	453,12	0,4	1,4	
11	7527	-139,88	-0,1	-0,4	
12	4740	-2926,88	-2,4	-9,2	

4.10 Methionin(e) in mg/kg

Statistic Data	
Number of the results	8
Number of outliers	0
Mean	1512
Median	1633
Robust mean (X)	1553
Robust standard deviation (S^x)	262
Target standard deviation (sigma)	396
Lower limit of target range	760
Upper limit of target range	2345
Quotient S^x/σ	0,7
Standard uncertainty U^x	116
Quotient U^x/σ	0,3
Results in target range	8
Percent in target range	100

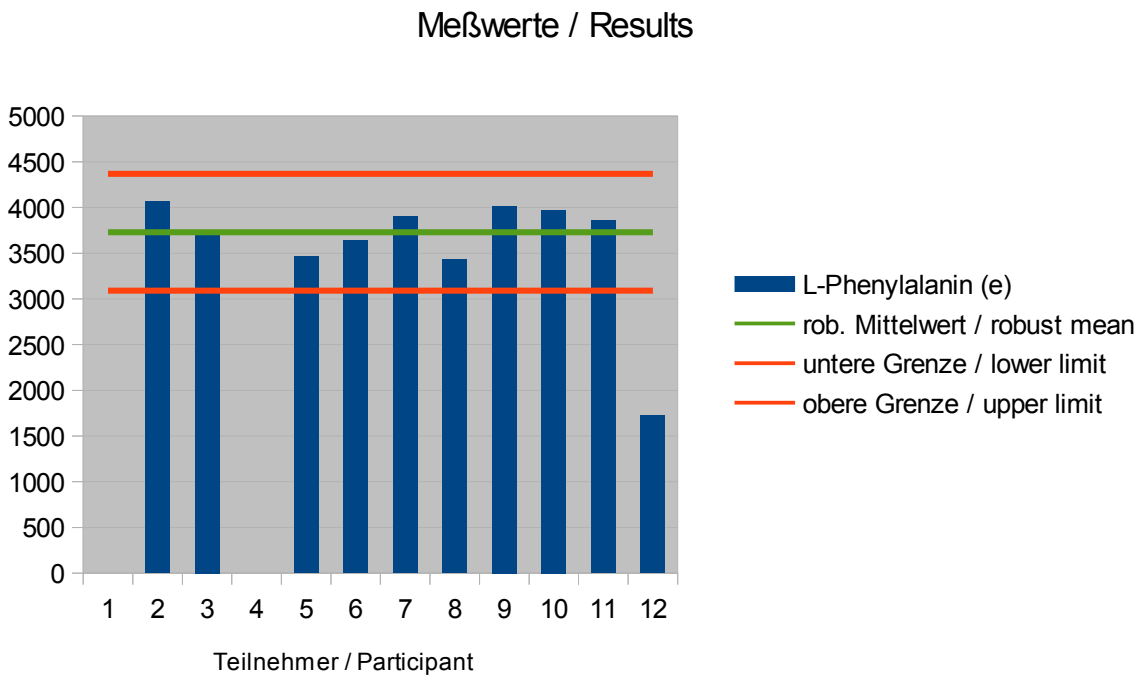


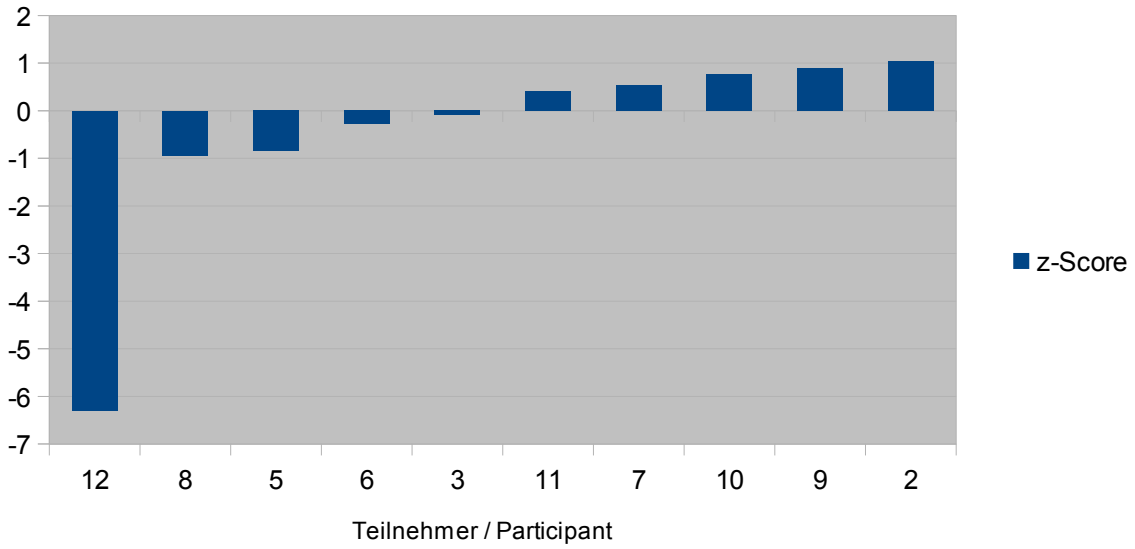


Auswerte nummer / Evaluation number	L-Methionin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,332				
2	1730	177,04	0,4	2,2	
3					
4	0,16				
5	1341	-211,96	-0,5	-2,6	
6	1577,5	24,54	0,1	0,3	
7					
8	1395	-157,96	-0,4	-1,9	
9	1689	136,04	0,3	1,7	
10	1830	277,04	0,7	3,4	
11	1701	148,04	0,4	1,8	
12	832	-720,96	-1,8	-8,8	

4.11 Phenylalanin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	3575
Median	3779
Robust mean (X)	3730
Robust standard deviation (S^x)	312
Target standard deviation (sigma)	319
Lower limit of target range	3091
Upper limit of target range	4368
Quotient S^x/σ	1,0
Standard uncertainty U^x	123
Quotient U^x/σ	0,4
Results in target range	9
Percent in target range	90

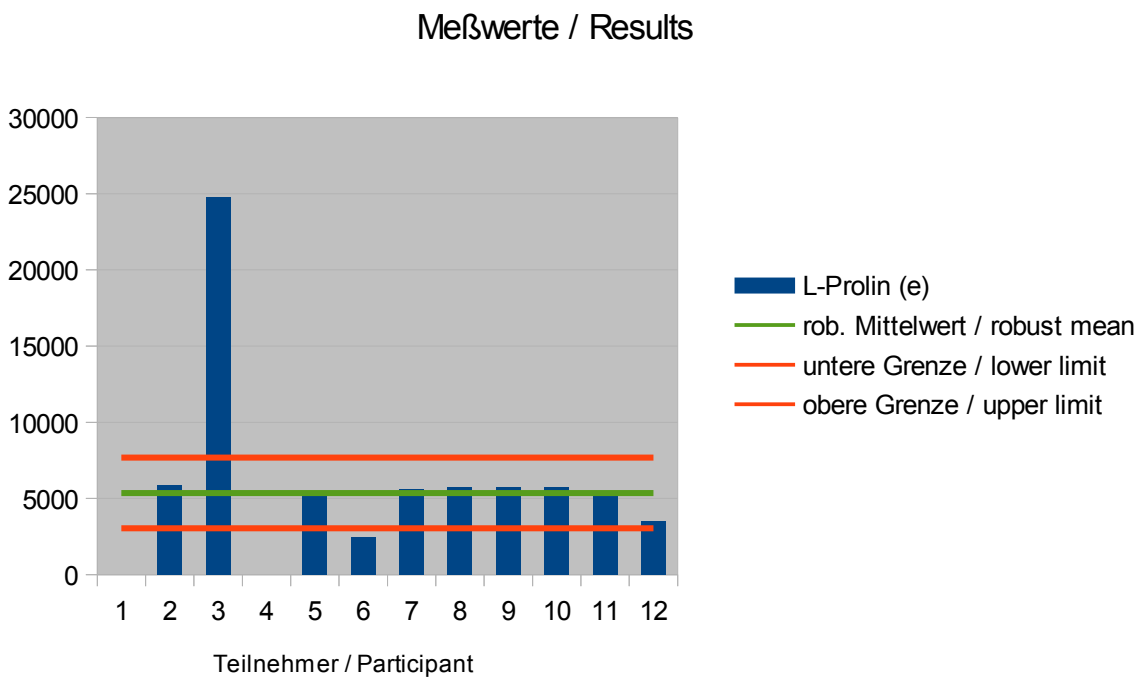


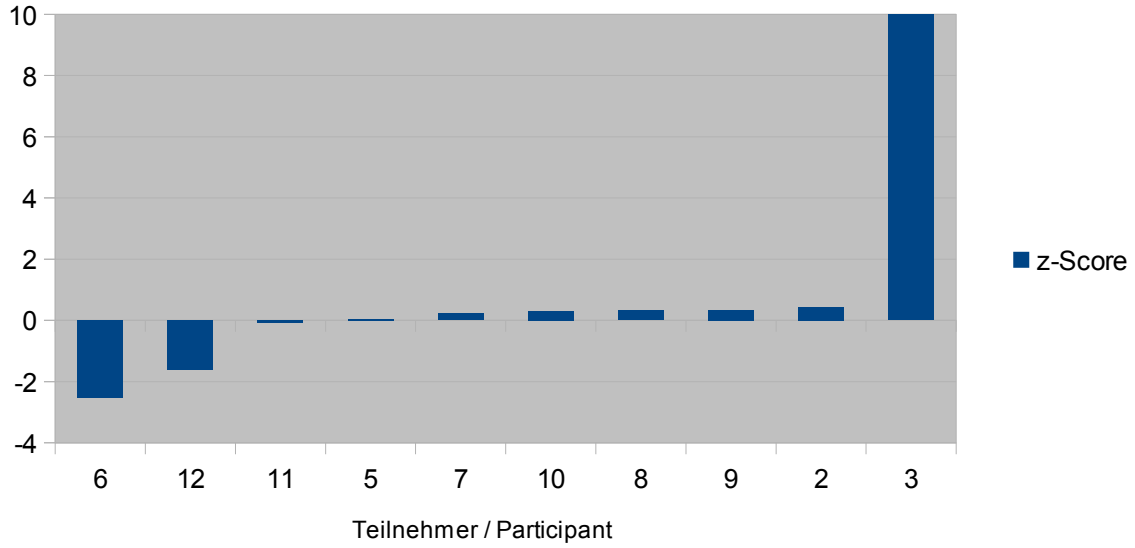


Auswerte nummer / Evaluation number	L-Phenylalanin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,708				
2	4059,95	330,39	1,0	1,9	
3	3700	-29,56	-0,1	-0,2	
4	0,39				
5	3462	-267,56	-0,8	-1,5	
6	3641,5	-88,06	-0,3	-0,5	
7	3899	169,44	0,5	1,0	
8	3430	-299,56	-0,9	-1,7	
9	4014	284,44	0,9	1,6	
10	3970	240,44	0,8	1,4	
11	3858	128,44	0,4	0,7	
12	1720	-2009,56	-6,3	-11,6	Ausreisser / Outlier

4.12 Prolin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	7013
Median	5681
Robust mean (X)	5362
Robust standard deviation (S^x)	1313
Target standard deviation (sigma)	1161
Lower limit of target range	3040
Upper limit of target range	7685
Quotient S^x/σ	1,1
Standard uncertainty U^x	519
Quotient U^x/σ	0,4
Results in target range	8
Percent in target range	80

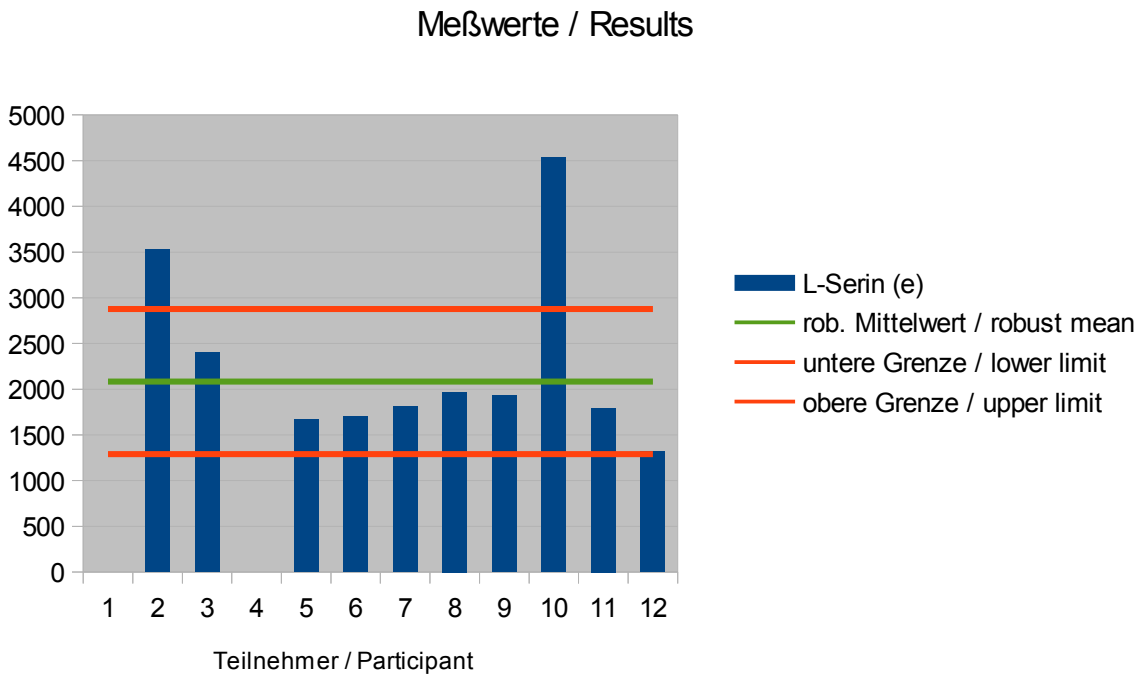


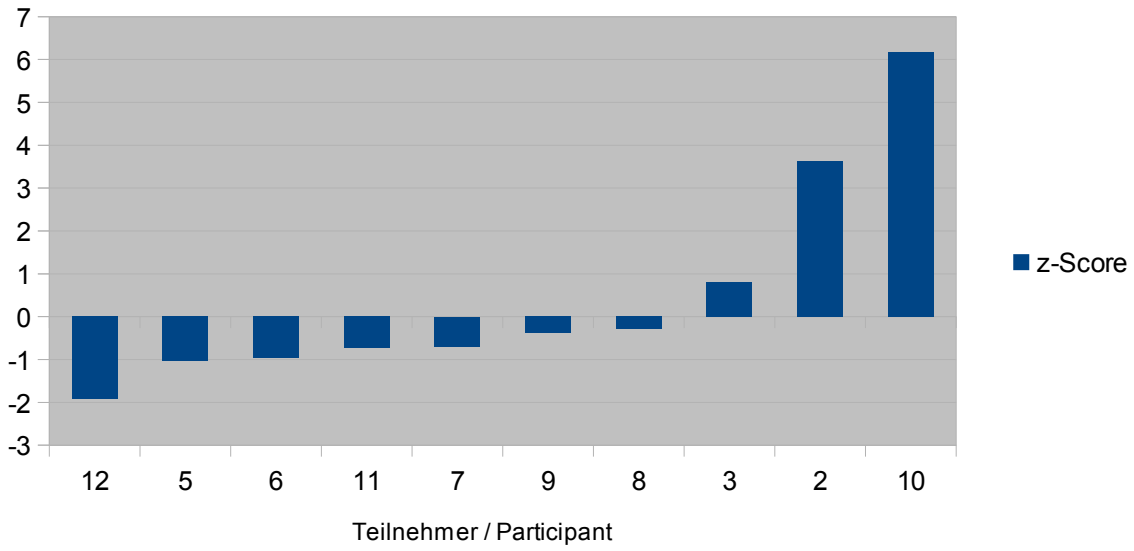


Auswerte nummer / Evaluation number	L-Prolin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	1,11				
2	5875	512,78	0,4	2,2	
3	24800	19437,78	16,7	82,5	Ausreisser / Outlier
4	0,52				
5	5406,75	44,53	0,0	0,2	
6	2427,5	-2934,72	-2,5	-12,5	
7	5631	268,78	0,2	1,1	
8	5743	380,78	0,3	1,6	
9	5765	402,78	0,3	1,7	
10	5730	367,78	0,3	1,6	
11	5267	-95,22	-0,1	-0,4	
12	3480	-1882,22	-1,6	-8,0	

4.13 Serin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	2264
Median	1867
Robust mean (X)	2082
Robust standard deviation (S^x)	691
Target standard deviation (sigma)	397
Lower limit of target range	1288
Upper limit of target range	2876
Quotient S^x/σ	1,7
Standard uncertainty U^x	273
Quotient U^x/σ	0,7
Results in target range	8
Percent in target range	80

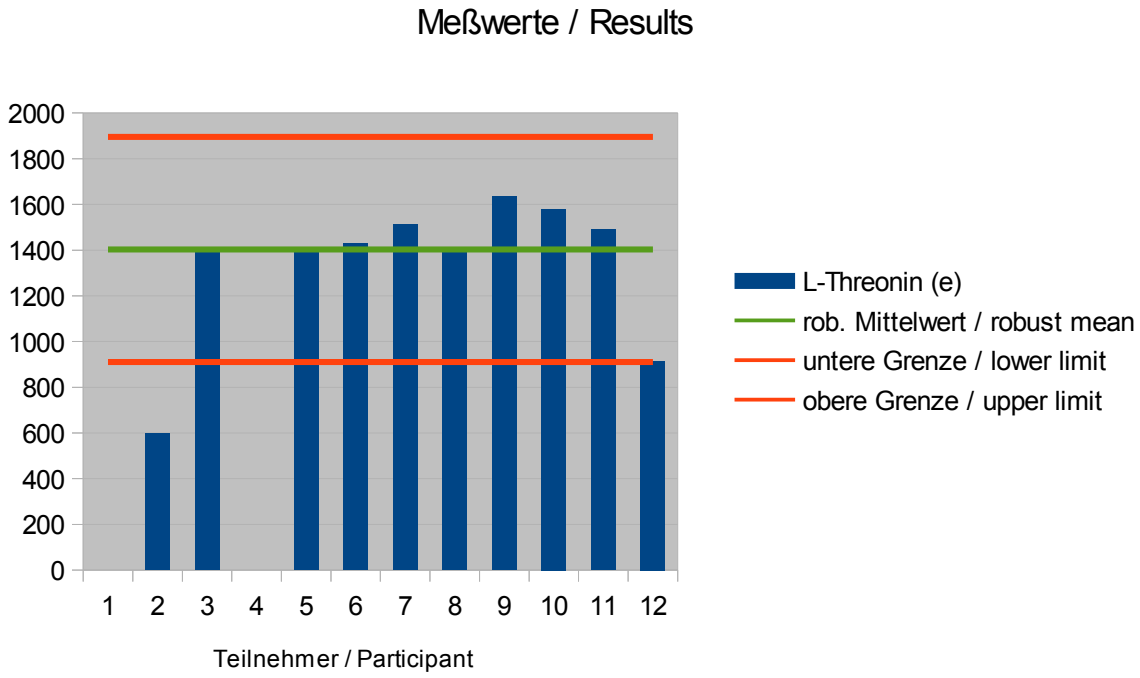


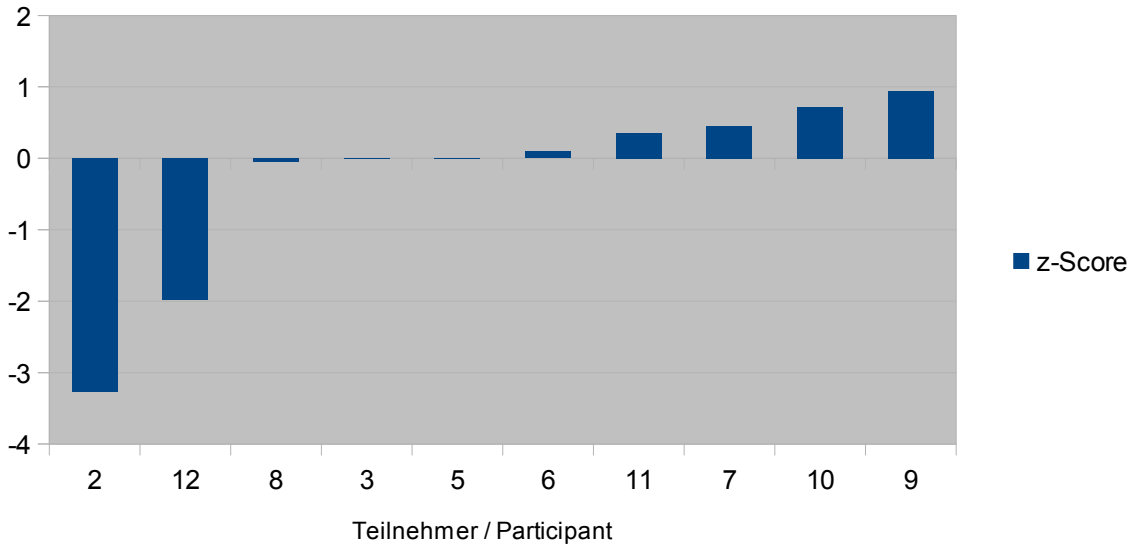


Auswerte nummer / Evaluation number	L-Serin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,838				
2	3524	1441,56	3,6	13,7	
3	2400	317,56	0,8	3,0	
4	0,18				
5	1672	-410,44	-1,0	-3,9	
6	1700	-382,44	-1,0	-3,6	
7	1806	-276,44	-0,7	-2,6	
8	1968	-114,44	-0,3	-1,1	
9	1928	-154,44	-0,4	-1,5	
10	4530	2447,56	6,2	23,2	Ausreisser / Outlier
11	1795	-287,44	-0,7	-2,7	
12	1318	-764,44	-1,9	-7,2	

4.14 Threonin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	1335
Median	1415
Robust mean (X)	1403
Robust standard deviation (S^x)	205
Target standard deviation (sigma)	246
Lower limit of target range	910
Upper limit of target range	1895
Quotient S^x/σ	0,8
Standard uncertainty U^x	81
Quotient U^x/σ	0,3
Results in target range	9
Percent in target range	90

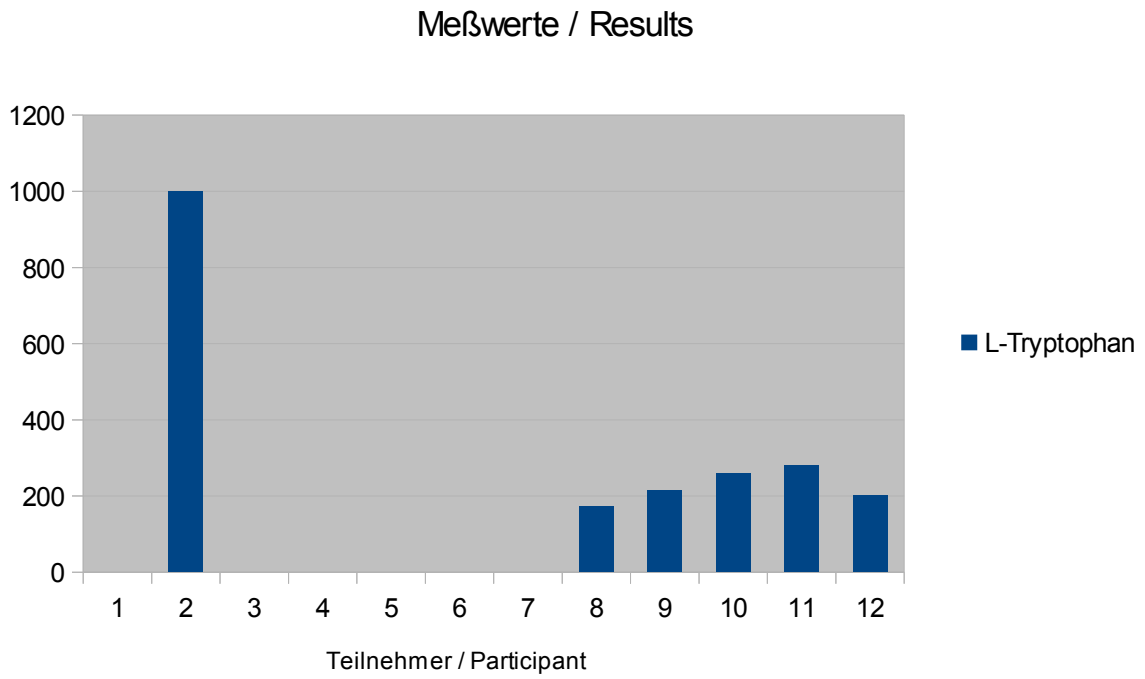




Auswerte nummer / Evaluation number	L-Threonin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,421				
2	598	-804,83	-3,3	-10,7	Ausreisser / Outlier
3	1400	-2,83	0,0	0,0	
4	0,15				
5	1401,75	-1,08	0,0	0,0	
6	1427,5	24,67	0,1	0,3	
7	1513	110,17	0,4	1,5	
8	1390	-12,83	-0,1	-0,2	
9	1635	232,17	0,9	3,1	
10	1580	177,17	0,7	2,3	
11	1490	87,17	0,4	1,2	
12	915	-487,83	-2,0	-6,5	

4.15 Tryptophan in mg/kg

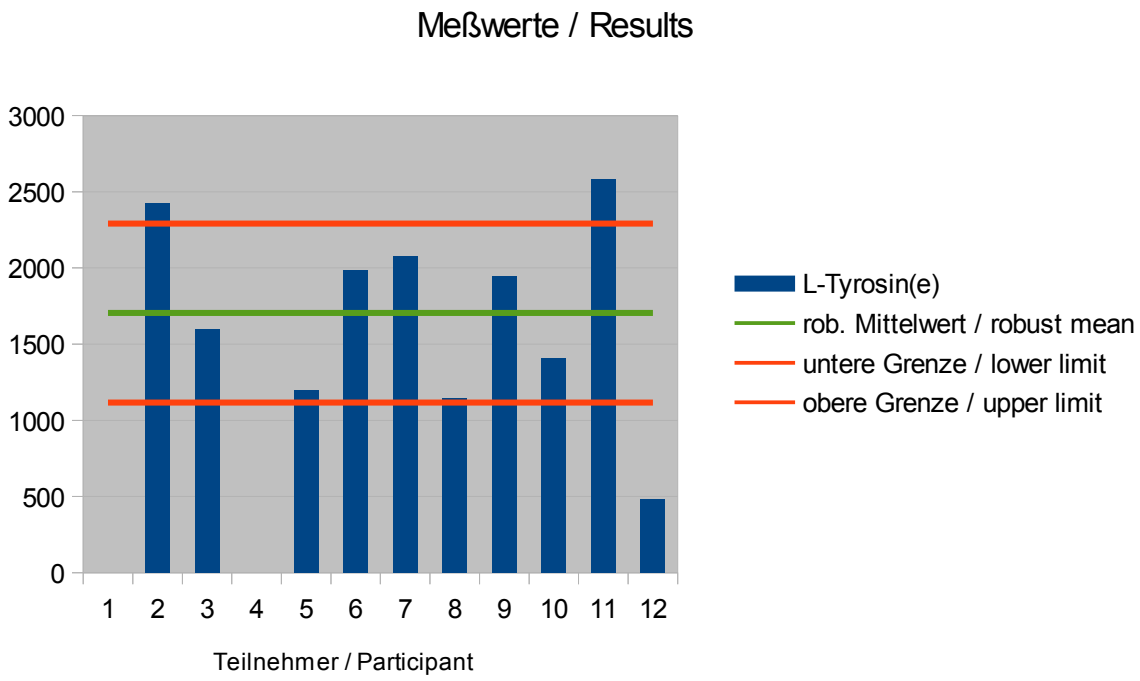
Statistic data	
Number of the results	6
Number of outliers	1
Mean	nicht ausgewertet / not evaluated
Median	nicht ausgewertet / not evaluated
Robust mean (X)	nicht ausgewertet / not evaluated
Robust standard deviation (S^x)	nicht ausgewertet / not evaluated
Target standard deviation (sigma)	nicht ausgewertet / not evaluated
Lower limit of target range	nicht ausgewertet / not evaluated
Upper limit of target range	nicht ausgewertet / not evaluated
Quotient S^x/sigma	nicht ausgewertet / not evaluated
Standard uncertainty U^x	nicht ausgewertet / not evaluated
Quotient U^x/sigma	nicht ausgewertet / not evaluated
Results in target range	nicht ausgewertet / not evaluated
Percent in target range	nicht ausgewertet / not evaluated

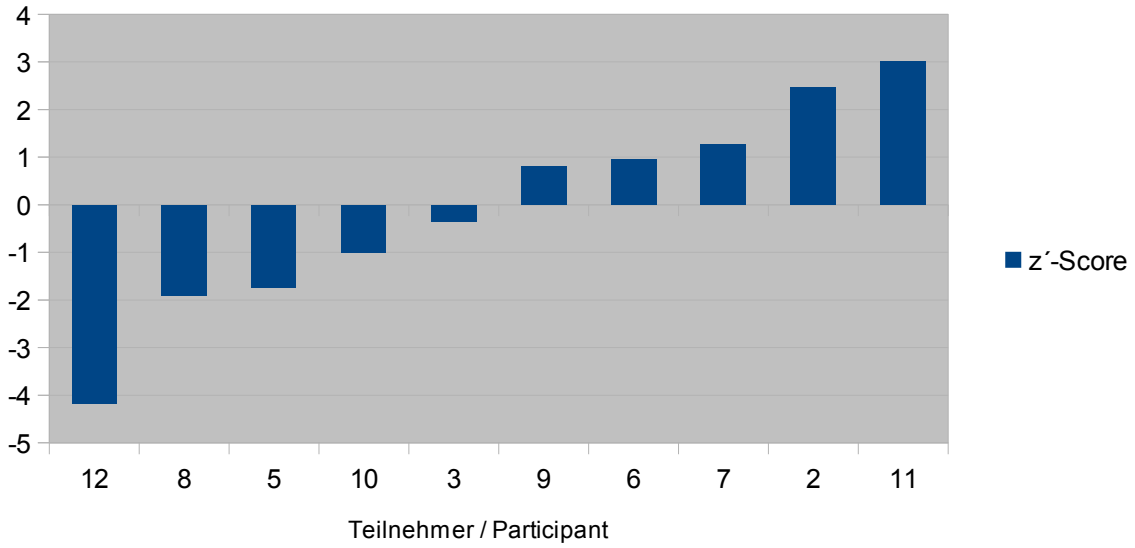


Auswerte nummer / Evaluation number	L-Tryptophan
1	Nicht analysiert / Not tested
2	1000,5
3	
4	
5	nicht kalibriert / not calibrated
6	
7	
8	173
9	214
10	260
11	281
12	201

4.16 Tyrosin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	1685
Median	1772
Robust mean (X)	1704
Robust standard deviation (S^x)	687
Target standard deviation (sigma)	293
Lower limit of target range	1118
Upper limit of target range	2291
Quotient S^x/σ	6,2
Standard uncertainty U^x	272
Quotient U^x/σ	2,4
Results in target range	7
Percent in target range	70

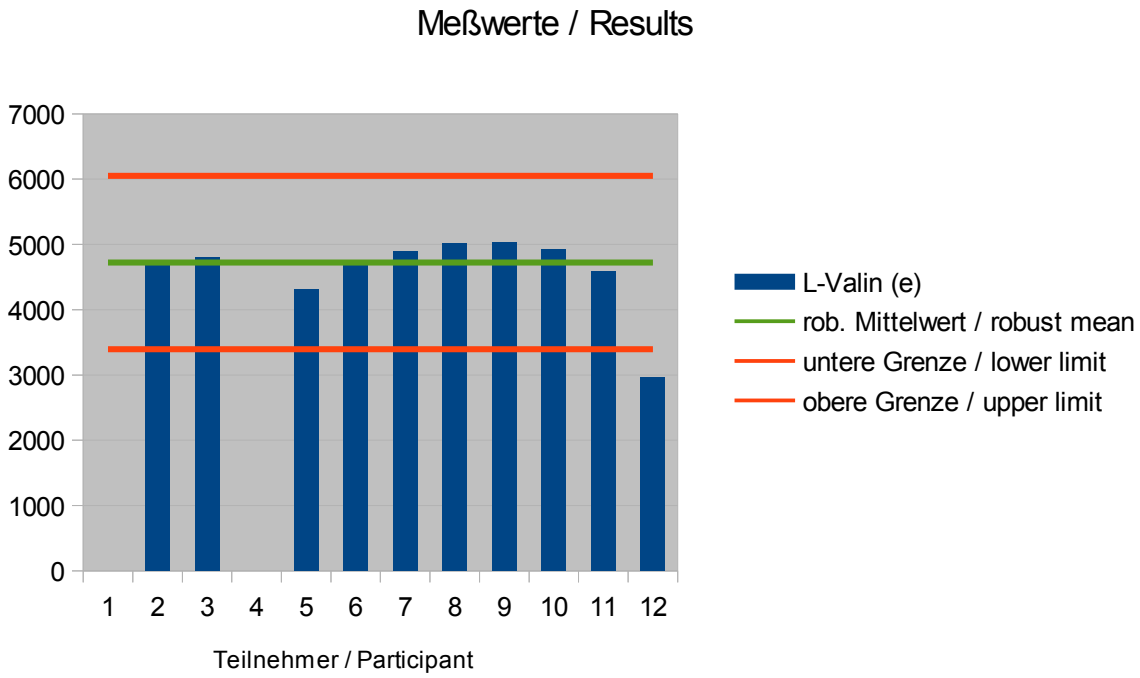


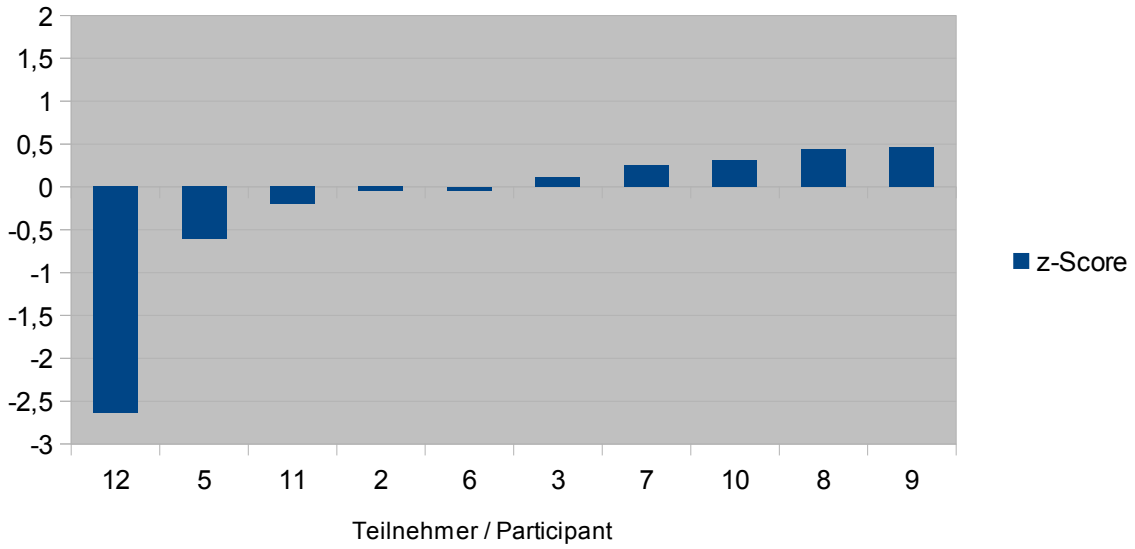


Auswerte nummer / Evaluation number	L-Tyrosin(e)	Abweichung / Deviation	z´-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,401				
2	2425	720,69	2,5	8,1	
3	1600	-104,31	-0,4	-1,2	
4	0,21				
5	1196,25	-508,06	-1,7	-5,7	
6	1985	280,69	1,0	3,2	
7	2078	373,69	1,3	4,2	
8	1145	-559,31	-1,9	-6,3	
9	1944	239,69	0,8	2,7	
10	1410	-294,31	-1,0	-3,3	
11	2586	881,69	3,0	9,9	
12	480	-1224,31	-4,2	-13,8	Ausreisser / Outlier

4.17 Valin(e) in mg/kg

Statistic Data	
Number of the results	10
Number of outliers	1
Mean	4593
Median	4749
Robust mean (X)	4723
Robust standard deviation (S^x)	303
Target standard deviation (sigma)	664
Lower limit of target range	3394
Upper limit of target range	6051
Quotient S^x/σ	0,5
Standard uncertainty U^x	120
Quotient U^x/σ	0,2
Results in target range	9
Percent in target range	90





Auswerte nummer / Evaluation number	L-Valin (e)	Abweichung / Deviation	z-Score	z-Score (Horwitz) zur info	Hinweis / Remark
1	0,906				
2	4690	-32,59	0,0	-0,2	
3	4800	77,41	0,1	0,4	
4	0,48				
5	4319,75	-402,84	-0,6	-1,9	
6	4697,5	-25,09	0,0	-0,1	
7	4886	163,41	0,2	0,8	
8	5015	292,41	0,4	1,4	
9	5027	304,41	0,5	1,4	
10	4930	207,41	0,3	1,0	
11	4592	-130,59	-0,2	-0,6	
12	2970	-1752,59	-2,6	-8,3	Ausreisser / Outlier

5 Documentation

5.1 *Primary data*

5.1.1 *Free amino acids in mg/kg*

Teilnehmer / Participant	DLA-Nr Probe A / Sample A	DLA-Nr Probe B / Sample B	Alanin(e)	Ergebnis / Result A	Ergebnis / Result B	Arginin(e)	Ergebnis / Result A	Ergebnis / Result B
1	24	35	0,314	0,306	0,322	0,022	0,02	0,023
2	4	32	1525	1520	1530	151	160	143
3 *	14	47	0,16	0,16	0,16			
4	12	48	0,16	0,156	0,160			
5	10	44	1444,25	1462,5	1426	<100	<100	<100
6	7	33	1578	1630	1525			
7	15	40	1371	1384	1358	61	63	60
8	13	34	1029	948	1109	44,6	43	46,1
9	3	25	1634	1645	1623	70	73	67
10	6	28	1620	1620	1620	<200	<200	<200
11	18	30	1541	1547	1534	50	< 50	55
12	21	45	1005	1039	971			

* Einheit / Unit: g/100g

Teilnehmer / Participant	Asparagin säure / Aspartic Acid	Ergebnis / Result A	Ergebnis / Result B	Cystein(e)	Ergebnis / Result A	Ergebnis / Result B	Cystin(e)	Ergebnis / Result A	Ergebnis / Result B
1	0,304	0,297	0,311	N/A	N/A	N/A	0,061	0,056	0,065
2	1435	1420	1450	ND	ND	ND	ND	ND	ND
3 *	0,16	0,16	0,16				0,04	0,04	0,04
4	0,15	0,148	0,150						
5	nicht auswertbar / not evaluable	nicht auswertbar / not evaluable	nicht auswertbar / not evaluable	nicht kalibriert / not calibrated	nicht kalibriert / not calibrated	nicht kalibriert / not calibrated	<100	<100	<100
6	1790	1865	1715						
7	1598	1615	1582						
8	1085	1050	1120	990	800	1180			
9	1688	1701	1674						
10	1500	1470	1530	<200	<200	<200	<200	<200	<200
11	1513	1527	1499	< 200	< 200	< 200	< 50	< 50	< 50
12	972	943	1001						

* Einheit / Unit: g/100g

Teilnehmer / Participant	Glutaminsäure / Glutamic Acid	Ergebnis / Result A	Ergebnis / Result B	Glycin(e)	Ergebnis / Result A	Ergebnis / Result B	Histidin(e)	Ergebnis / Result A	Ergebnis / Result B
1	1,95	1,91	1,98	0,323	0,314	0,331	0,531	0,519	0,542
2	9105	9160	9050	1795	1870	1720	1744	1743	1745
3 *	1,06	1,06	1,06	0,17	0,17	0,17	0,19	0,19	0,19
4	1,05	1,045	1,065	0,17	0,163	0,167	0,17	0,168	0,173
5	9664	9846	9482	1502,5	1530	1475	1773,75	1764,5	1783
6	10688	11045	10330	1593	1645	1540	1728	1785	1670
7	11045	11121	10968	1619	1626	1612	1931	1952	1911
8	8285	8345	8225	1600	1485	1715	1573	1490	1655
9	11908	11748	12068	1726	1738	1714	1884	1895	1872
10	11700	12300	11100	1730	1730	1720	2810	2790	2820
11	10226	10254	10198	1591	1602	1580	1776	1787	1764
12	6660	6662	3943	1142	1135	1148	766	773	759

* Einheit / Unit: g/100g

Teilnehmer / Participant	Isoleucin(e)	Ergebnis / Result A	Ergebnis / Result B	Leucin(e)	Ergebnis / Result A	Ergebnis / Result B	Lysin(e)	Ergebnis / Result A	Ergebnis / Result B
1	0,658	0,649	0,667	1,11	1,09	1,13	1,64	1,62	1,66
2	3575	3600	3550	6210	6230	6190	13340	13540	13140
3 *	0,35	0,36	0,34	0,61	0,62	0,6	0,81	0,81	0,81
4	0,35	0,343	0,363	0,62	0,605	0,626	0,75	0,746	0,762
5	3232,75	3282,5	3183	5636	5576	5696	7020,5	7272	6769
6	3328	3465	3190	5863	6130	5595	7688	7955	7420
7	3604	3627	3580	6345	6381	6310	6592	6651	6533
8	4210	3695	4725	5556	4736	6375	8508	8260	8755
9	3676	3602	3750	6606	6445	6766	7780	7663	7897
10	3520	3540	3500	6190	6250	6120	8120	8120	8130
11	3567	3526	3608	6334	6247	6420	7527	7559	7494
12	2290	2291	1612	2950	2954	2264	4740	4744	2692

* Einheit / Unit: g/100g

Teilnehmer / Participant	Methionin(e)	Ergebnis / Result A	Ergebnis / Result B	Phenyl alanin(e)	Ergebnis / Result A	Ergebnis / Result B	Prolin(e)	Ergebnis / Result A	Ergebnis / Result B
1	0,332	0,324	0,339	0,708	0,705	0,71	1,11	1,08	1,13
2	1730	1740	1720	4060	4140	3980	5875	5960	5790
3 *				0,37	0,37	0,37	2,48	2,49	2,47
4	0,16	0,156	0,165	0,39	0,382	0,393	0,52	0,516	0,518
5	1341	1359	1323	3462	3449	3475	5406,75	5365,5	5448
6	1578	1640	1515	3642	3760	3523	2428	2625	2230
7				3899	3941	3857	5631	5636	5626
8	1395	1200	1590	3430	3235	3625	5743	5370	6115
9	1689	1700	1677	4014	3993	4034	5765	5571	5959
10	1830	1850	1820	3970	3950	3990	5730	5760	5710
11	1701	1678	1723	3858	3826	3890	5267	5281	5252
12	832	800	865	1720	1719	1727	3480	3484	2609

* Einheit / Unit: g/100g

Teilnehmer / Participant	Serin(e)	Ergebnis / Result A	Ergebnis / Result B	Threonin(e)	Ergebnis / Result A	Ergebnis / Result B	Tryptophan	Ergebnis / Result A	Ergebnis / Result B
1	0,838	0,827	0,848	0,421	0,412	0,429	Nicht analysiert / Not tested	Nicht analysiert / Not tested	Nicht analysiert / Not tested
2	3524	3587	3461	598	667	529	1001	1019	982
3 *	0,24	0,24	0,24	0,14	0,14	0,14			
4	0,18	0,178	0,180	0,15	0,148	0,151			
5	1672	1676	1668	1401,75	1416,5	1387	nicht kalibriert / not calibrated	nicht kalibriert / not calibrated	nicht kalibriert / not calibrated
6	1700	1760	1640	1428	1480	1375			
7	1806	1805	1807	1513	1523	1504			
8	1968	1895	2040	1390	1220	1560	173	168	179
9	1928	1949	1907	1635	1650	1619	214	214	213
10	4530	4540	4530	1580	1550	1620	260	250	270
11	1795	1803	1787	1490	1501	1479	281	267	294
12	1318	1320	1316	915	919	911	201	194	208

* Einheit / Unit: g/100g

Teilnehmer / Participant	Tyrosin(e)	Ergebnis / Result A	Ergebnis / Result B	Valin(e)	Ergebnis / Result A	Ergebnis / Result B	Teilnehmer / Participant	Verbindung / Compound	Ergebnis / Result A	Ergebnis / Result B
1	0,401	0,451	0,351	0,906	0,893	0,918	2	L-Asparagin	3530	3390
2	2425	2650	2200	4690	4790	4590				
3 *	0,16	0,16	0,16	0,48	0,47	0,47	9	L-Asparagin	3604	3556
4	0,21	0,196	0,222	0,48	0,476	0,488	9	L-Citrullin	1189	1191
5	1196,25	1250,5	1142	4319,75	4346,5	4293	9	L-Glutamin	1090	1132
6	1985	1765	2205	4698	4865	4530				
7	2078	2110	2045	4886	4935	4838	11	Citrullin	1152	1161
8	1145	1021	1270	5015	4580	5450	11	L-Glutamin	1117	1109
9	1944	1943	1945	5027	4955	5098	11	Ornithin	1803	1777
10	1410	1390	1430	4930	4930	4940				
11	2586	2285	2886	4592	4576	4607				
12	480	494	466	2970	2971	2086				

* Einheit / Unit: g/100g

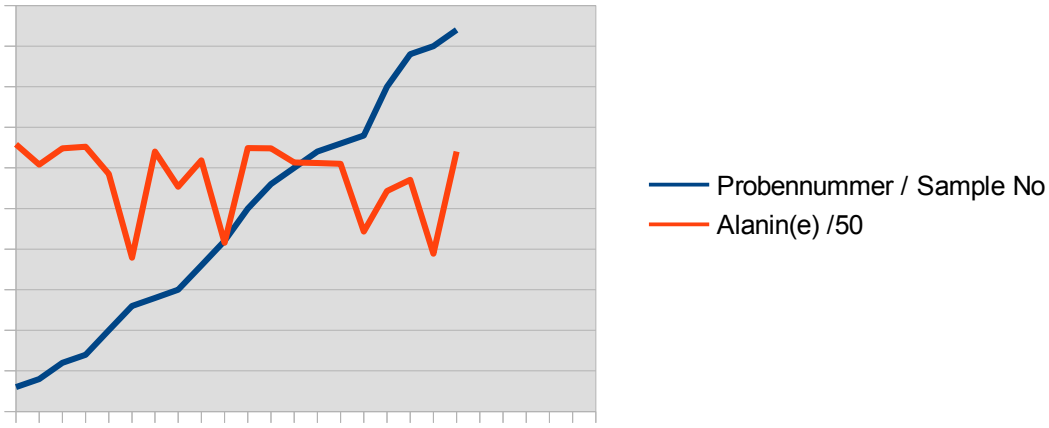
5.2 Homogeneity

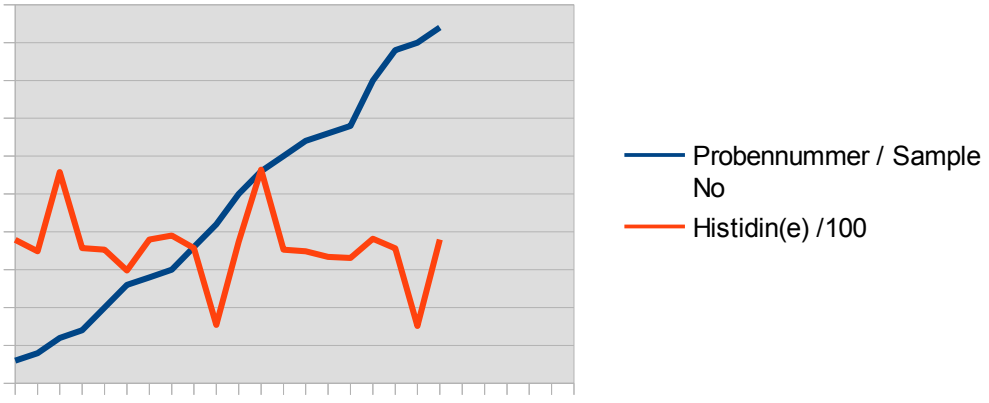
5.2.1 Repeatability standard deviation of duplicate tests of the participants

The repeatability standard deviation was calculated with the data documented in 5.1. It is 4,3% of X (Alanine) in comparison to 4,2 %, which is documented in ASU L 07.00-64.

And it is 3,5 % of X (Histidine) in comparison to 5,8%, which is documented in ASU L 07.00-64.

5.2.2 Comparison of sample number / test result





5.3 Analytical methods

Participant	Method	Homogenization	Digestion	Weight	Derivatization	Reference material	Calibration	Method is accredited	Remarks
								Yes / no	
1	Free amino acids	Yes	No	1g	Yes	No	Yes	No	Ser = Ser+Asp; His= His+Glu; Arg=Arg+Taurin; unable to resolve above analytes.
2	Yes	Yes	No	0.2 g	Yes	No	Yes	No	
3	Determination of free amino acids in food with amino acid analyser with ninhydrine according to ASU L 49-07-2	none	Extraction with 0,1 M HCl	1-3 g	Postcolumn-derivatisation with ninhydrine	intern	Intern standard multi point-calibration	Yes	
4	Free amino acids (analogue (EU/152/2009)			app. 5 g		Feed (intern RM)	internal std., 1-point-calibration	Yes	
5									
6	IC with post column derivatisation				Ninhydrine		intern	Yes	
7	Amino acids after acidic hydrolysis with amino acids analyser (post column derivatization)	mortar	protein precipitation with sulfosalicylic acid	0,5 g - 1 g	Ninhydrine	Milk protein	external standard, linear regression	Yes	
8	EZ:faast-Kit Phenomenex, LC-MS/MS	none	acidic extraction	0,05g	Chloroformate-Derivates	IST	Internal standard multi point calibration	Yes	
9	Free amino acids after protein precipitation	mixed well	none	1-3 g	Ninhydrine	No	internal calibration	Yes	protein precipitation with trichloro acetic acid (10 %)
10	HPLC/FLD after derivatisation			100mg	Yes with AQC	No	External calibration with internal standard	Yes	
11	Determination of free amino acids in food with amino acid analyser	mortar	none	app.1g	Ninhydrine	amino acid standard AAS18, single reference substances	One point calibration	Yes	Sample was defatted with PE
12	Phenomenex EZ:faast			1g/100ml	Phenomenex EZ faast	DLA Material	4-point	Yes	

6 Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		ENGLAND
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		TAIWAN
		GERMANY
		GERMANY
		GERMANY
		GERMANY

[The address data of the participants were deleted for publication of the evaluation report.]

7 Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs;W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
12. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
13. ASU §64 LFGB : L07.00-64 Bestimmung von frei vorliegenden Aminosäuren in Fleischerzeugnissen (August 2014)
14. ASU §64 LFGB : L49.07-1; Bestimmung der Aminosäuren in Aminosäurengemischen.
15. ASU §64 LFGB : L49.07-2; Bestimmung der Aminosäuren in diätetischen Lebensmitteln auf Basis von Proteinhydrolysaten
16. ASU §64 LFGB : L49.07-3; Bestimmung des Tryptophangehaltes in diätetischen Lebensmitteln auf Basis von Proteinhydrolysaten
17. VERORDNUNG (EG) Nr. 152/2009 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Untersuchung von Futtermitteln.