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CIHEAM work group: Nutritive value of feedstuffs and by-products of the Mediterranean area

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SUMMARY - In order to achieve the greatest possible standardization of the analysis techniques, two analysis chains were organized in 1985-1989 in the main laboratories of CIHEAM member countries. The results show that it is desirable the search for the means to decrease the great variability of the results obtained.

RESUME - "Groupe de travail du CIHEAM: Valeur nutritive des fourrages et sous-produits méditerranéens". Afin de favoriser une plus grande harmonisation des techniques d'analyses, deux chaînes d'analyse ont été organisées en 1985-1989 dans les principaux laboratoires des pays constituant le CIHEAM. Les résultats montrent qu'il est souhaitable de rechercher les moyens de diminuer la grande variabilité des résultats obtenus.

Report of the chain analysis 1985-1989

Due to the observed variability between the results of analytical procedures used for the evaluation of feedstuff and by-products, the INTERNATIONAL CENTRE FOR ADVANCED MEDITERRANEAN AGRONOMIC STUDIES (ICAMAS) proposed in their annual meeting of 1983, to carry out a chain analysis between the members of the ICAMAS laboratories.

The objective of this Chain Analysis was to test the analytical methods (chemical and biological) used by the participating laboratories.

A first chain of analysis was carried out in 1985. As a consequence of the great variability found in the results obtained a second chain of analysis was thought to be convenient and it was realized in 1989.

A summary of the methodology, participating laboratories and results obtained in both chains of analysis is shown below.

The following analytic determinations were proposed in 1985:

- DRY MATTER
- ASH
- CRUDE PROTEIN
- CRUDE FIBRE
- GROSS ENERGY

- DRY MATTER DIGESTIBILITY TILLEY & TERRY
- DRY MATTER DIGESTIBILITY PEPSIN - CELLULASE
- NYLON BAG - DM DISAPPEARANCE: 8,12,24,48,72 HOURS
- NITROGEN SOLUBILITY
- NEUTRAL DETERGENT FIBRE
- ACID DETERGENT FIBRE
- ACID DETERGENT LIGNIN,

on eight feed samples:

- ALFALFA HAY
- GRASS HAY
- CEREAL STRAW
- NH₃ - CEREAL STRAW
- BEET PULP
- GRAPE MARC
- OLIVE PULP
- BARLEY

Analytical determinations made on each feed in 1989:

- DRY MATTER
- ASH
- CRUDE PROTEIN
- N DEGRADABILITY by PROTEASES at 1 and 24 HOURS
- DRY MATTER DIGESTIBILITY TILLEY & TERRY

- DRY MATTER DIGESTIBILITY PEPSIN - CELLULASE
- NEUTRAL DETERGENT FIBRE
- ACID DETERGENT FIBRE
- ACID DETERGENT LIGNIN

on five feed samples:

- ALFALFA HAY
- CITRUS PULP
- WHEAT STRAW
- UREA TREATED WHEAT STRAW
- WHEAT BRAN

To avoid any sort of variability, the ICAMAS supplied each laboratory with the samples (ground, screened 0.8-1.0 mm), the nylon bags, the cellulase and the pepsin, as well as the recommended methods. Instructions were sent to the laboratories.

Analytical methods

DETERMINATIONS

DM - ASH - CP
 N DEG. at 1 and 24 h.
 NYLON BAG
 DM Disappearance
 IVDMD
 DMD - CELL
 NDF - ADF - ADL

METHODS

A.O.A.C. (1980)
 AUFREERE, J. (1988)
 ORSKOV, E.R. (1980)
 TILLEY & TERRY (1963)
 AUFREERE, J. (1982)
 Van SOEST, P.J. (1967)

Statistical results

The results for each determination and feed-sample were analysed for the mean, standard deviation(s) and coefficient of variation (CV). In this first analysis all the results from all the laboratories were included but, a critical examination of the data indicated that some results deviated too much from the mean.

In order to identify the extreme values, the Dixon's outlier test* was applied. This test calls a value a *strag-*

gler when the statistical test lies between its 5% and 1% critical values and *outlier* when the statistical test is greater than its 1% critical value.

After applying the Dixon's test, all values of one analytical determination were eliminated when the majority of the values were stragglers and/or outliers (*outlying laboratory*). When the number of stragglers and/or outliers was small, the individual values statistically outliers were discarded but the stragglers were retained (Table 1,2,4 and 5).

Dixon's test was not applied to the Nylon bag technique (8-12,24,48,72 hours) because of very few results.

In the initial protocol it was recommended that each determination should be supported by three replicas of the analysis but only their average was requested in this work, the reproducibility of each determination (inter-laboratory test) was studied but not the repeatability (within-laboratory test).

The definition of reproducibility given by the International Standard Organization (ISO) was used: the reproducibility (R) is the value below which the absolute difference between two single test results obtained with the same method on identical test materials, under different conditions (different operators, different apparatus, different laboratories and/or different time), may be expected to lie within a specified probability, 95% in our case (ISO Ref No 5725-1981 (E)).

The estimated value of "R" is given by the equation: $R = f \cdot 2 \cdot s$, if the probability level is 95%, the factor f is approximately 2, then $R = 2.83 \cdot s$.

A summary of the results per method is collected in tables 3 and 6, showing the standard deviation (s), the coefficient of variation of the standard deviation (CVs) and the reproducibility (R), expressed as the average of the results of the samples.

(*) Dixon W.J. and Massey F.J. 1969. Introduction to statistical analysis. McGraw Hill, New York.

Table 1: Statistical results after removing critical values for Dixon's outlier test at 5% (stragglers) and 1% (outliers) 1985

	ALFALFA HAY	GRASS HAY	CEREAL STRAW	NH ₃ -CEREAL STRAW	BEET PULP	GRAPE MARC	OLIVE PULP	BARLEY
DRY MATTER								
Mean	94.79	90.14	91.79	91.79	91.15	90.25	94.19	90.98
Minimum	93.10	87.90	90.63	90.67	90.07	88.74	93.45	89.10
Maximum	97.23	92.75	93.76	94.10	93.22	92.38	95.82	94.31
Std. Dev.	1.04	1.07	0.94	0.99	0.95	1.07	0.71	1.27
CVs.	1.10	1.19	1.02	1.08	1.04	1.19	0.75	1.40
n	20	19	20	20	20	20	20	20
ASH								
Mean	11.09	9.57	9.35	8.98	9.59	13.89	6.64	3.09
Minimum	10.10	8.74	8.64	8.10	8.60	6.99	6.61	2.54
Maximum	11.60	10.18	9.90	11.10	10.21	15.39	7.28	3.30
Std. Dev.	0.42	0.38	0.38	0.61	0.49	1.39	0.45	0.19
CVs	3.79	3.97	4.06	6.79	5.11	10.01	6.78	6.15
n	19	19	19	19	19	19	19	19
CRUDE PROTEIN								
Mean	17.31	13.83	3.48	9.49	9.97	11.63	9.53	9.55
Minimum	12.69	10.16	3.13	6.41	8.15	10.02	7.56	8.49
Maximum	19.60	15.90	4.09	11.90	10.80	12.91	10.92	11.50
Std. Dev.	1.47	1.64	0.25	1.17	0.68	0.83	1.05	0.68
CVs	8.49	11.86	7.18	12.33	6.82	7.14	11.02	7.12
n	20	20	20	20	20	20	20	20
CRUDE FIBRE								
Mean	31.87	29.46	43.02	43.31	19.68	34.93	33.56	4.68
Minimum	27.83	23.17	33.73	36.30	16.40	27.60	24.38	2.96
Maximum	37.69	41.09	50.12	49.49	23.19	46.64	42.19	5.76
Std. Dev.	2.57	3.85	3.95	3.55	1.78	4.61	4.03	0.73
CVs	8.06	13.07	9.18	8.20	9.04	13.20	12.01	15.60
n	17	16	17	17	17	17	17	17
GROSS ENERGY								
Mean	4254	4273	4198	4238	3882	4405	4979	4261
Minimum	3968	3847	3760	3894	3507	4044	4667	3777
Maximum	4693	4490	4550	4505	4129	4843	5369	4571
Std. Dev.	185	206	221	176	175	220	198	215
CVs	4.3	4.8	5.3	4.2	4.5	5.0	4.0	5.1
n	11	11	11	11	11	11	11	11

Nylon bag technique was not treated for Dixon's test because of very few results

Table 2: Statistical results after removing critical values for Dixon's outlier test at 5% (stragglers) and 1% (outliers) 1985

	ALFALFA HAY	GRASS HAY	CEREAL STRAW	NH ₃ -CEREAL STRAW	BEEET PULP	GRAPE MARC	OLIVE PULP	BARLEY
DMD in vitro								
Mean	61.23	55.89	33.02	43.07	74.84	13.21	22.47	83.12
Minimum	56.97	26.11	7.05	9.78	46.63	3.88	12.78	62.80
Maximum	63.12	64.61	45.09	55.58	88.10	28.30	26.90	90.50
Std. Dev.	1.59	12.07	11.42	14.05	14.31	6.80	4.29	7.39
CVs	2.59	21.60	34.59	32.62	19.12	51.48	19.09	8.89
n	12	14	14	14	13	13	13	13
DMD pep. cel.								
Mean	59.94	57.38	31.48	36.52	81.86	19.61	30.31	89.65
Minimum	53.01	45.20	22.43	27.21	59.66	9.23	23.26	77.50
Maximum	66.40	71.50	49.70	49.70	90.70	37.50	41.38	93.80
Std. Dev.	3.65	8.10	8.08	6.85	8.97	7.89	5.27	4.57
CVs	6.09	14.12	25.67	18.76	10.96	40.23	17.39	5.09
n	12	12	12	12	12	12	12	11
NDF								
Mean	50.54	62.55	79.29	75.75	49.35	70.80	66.39	27.57
Minimum	45.15	56.86	75.28	66.14	38.53	58.62	55.37	19.59
Maximum	63.79	77.28	83.10	83.00	65.10	78.62	82.90	54.89
Std. Dev.	4.33	5.24	1.99	3.49	6.60	6.60	6.68	9.19
CVs	8.57	8.38	2.51	4.61	13.37	9.32	10.06	33.33
n	17	16	16	17	17	17	17	15
ADF								
Mean	36.82	34.27	51.42	51.43	26.22	70.41	53.07	6.09
Minimum	28.31	32.12	43.85	42.67	22.54	58.10	46.67	4.99
Maximum	40.36	36.65	56.90	55.70	30.20	77.21	57.41	7.53
Std. Dev.	2.79	1.45	3.78	3.19	1.89	5.31	3.34	0.66
CVs	7.58	4.23	7.35	6.20	7.21	7.54	6.29	10.84
n	18	15	17	18	18	17	17	15
ADL								
Mean	8.34	5.16	7.99	6.70	4.63	47.48	31.09	1.59
Minimum	6.30	3.35	6.05	4.27	0.66	41.17	26.71	0.52
Maximum	9.60	9.63	11.36	9.17	10.24	49.93	34.41	5.50
Std. Dev.	1.07	1.79	1.62	1.26	2.89	2.94	1.96	1.28
CVs	12.83	34.69	20.28	10.81	62.42	6.19	6.30	80.50
n	13	14	14	14	15	15	13	14
N-SOLUBILITY								
Mean	30.34	29.58	39.87	64.16	19.05	5.21	8.54	29.06
Minimum	2.07	18.61	29.57	53.19	16.33	3.50	6.19	3.69
Maximum	46.00	44.40	53.30	79.10	23.50	7.69	9.90	32.50
Std. Dev.	12.04	7.19	8.32	8.61	2.67	1.35	1.33	3.43
CVs	39.68	24.31	20.87	13.42	14.02	25.91	15.57	11.80
n	9	9	9	9	9	8	8	8

Table 4: Statistical results after removing values for Dixon's outlier test at 5% (stragglers) and 1% (outliers). 1989.

	ALFALFA HAY	CITRUS PULP	WHEAT STRAW	UREA WHEAT STRAW	WHEAT BRAN
DRY MATTER					
Mean	89.2888	87.1456	92.5731	90.9486	89.6481
Minimum	87.9000	85.4600	91.6900	90.2500	88.0000
Maximum	91.3100	88.9500	94.8800	92.1800	93.4300
Standard Dev.	1.0055	.9968	.8318	.4973	1.2134
C.V. %	1.1	1.1	0.9	5.5	1.4
Laboratory N.	16	16	16	14	16
ASH					
Mean	10.1569	5.6250	8.0569	10.8153	5.2337
Minimum	8.7000	4.6000	7.3000	9.7500	4.6000
Maximum	11.1900	6.3100	8.6000	11.6300	5.7500
Standard Dev.	.7783	.5201	.4440	.6600	.3343
C.V. %	7.7	9.2	5.5	6.1	6.4
Laboratory N.	16	16	16	15	16
CRUDE PROTEIN					
Mean	18.8006	9.2325	2.1560	13.1013	16.6244
Minimum	16.9000	7.5000	1.8000	11.3000	15.0000
Maximum	21.5000	10.5700	2.5000	14.1000	17.7000
Standard Dev.	1.2587	.9302	.2091	.7207	.9070
C.V. %	6.7	10.1	9.7	5.5	5.5
Laboratory N.	16	16	15	16	16
DMD in vitro					
Mean	62.0438	86.6986	37.2621	48.1786	71.9057
Minimum	45.4800	58.9500	19.7000	30.0100	61.7000
Maximum	70.7400	97.0000	48.0600	59.4000	76.7000
Standard Dev.	5.8593	11.3783	8.4278	8.9913	4.2953
C.V. %	9.4	13.1	22.6	18.7	5.9
Laboratory N.	13	14	14	14	14
DMD pep. cel.					
Mean	63.3371	89.1523	29.3693	34.8867	73.0200
Minimum	56.8200	84.6100	20.8000	29.4900	57.1200
Maximum	70.3000	94.2000	42.6000	39.9000	84.4000
Standard Dev.	3.1010	2.7317	7.0599	2.7946	6.6682
C.V. %	4.9	3.1	24.0	8.0	9.1
Laboratory N.	14	13	14	12	14

Table 5: Statistical results after removing values for Dixon's outlier test at 5% (stragglers) and 1% (outliers). 1989.

	ALFALFA HAY	CITRUS PULP	WHEAT STRAW	UREA WHEAT STRAW	WHEAT BRAN
PROTEIN DEGRADABILITY AFTER 1 H.					
Mean	38.7300	50.3518	53.8675	67.5580	41.3864
Minimum	27.3900	29.7000	29.4000	64.9000	15.4000
Maximum	44.7800	79.8700	72.8200	71.1000	54.7500
Standard Dev.	5.5879	15.0087	14.6863	2.4086	11.7082
C.V. %	14.4	29.8	27.3	3.6	28.3
Laboratory N.	11	11	8	10	11
PROTEIN DEGRADABILITY AFTER 24 H.					
Mean	66.6127	72.2755	66.3570	68.3927	58.4627
Minimum	44.3700	49.0000	50.0000	34.3400	24.0000
Maximum	82.4000	87.7900	87.0500	82.0000	75.8000
Standard Dev.	19.9291	10.3937	11.4245	14.3102	13.5421
C.V. %	16.4	14.4	17.2	20.9	23.2
Laboratory N.	11	11	10	11	11
NDF					
Mean	43.0800	26.5987	78.3456	72.0119	47.4931
Minimum	35.4600	24.1000	72.7300	64.9300	44.5100
Maximum	50.0000	29.1000	86.1000	82.2000	49.7000
Standard Dev.	3.2725	1.4547	3.3409	3.9973	1.8350
C.V. %	7.6	5.5	4.3	5.6	3.9
Laboratory N.	16	15	16	16	16
ADF					
Mean	33.2838	21.2931	52.2756	50.2038	13.5406
Minimum	24.9300	15.5000	45.2000	45.6000	12.1700
Maximum	38.0000	24.7000	58.7000	57.4000	14.7000
Standard Dev.	3.1974	2.3444	3.3375	3.3314	.6685
C.V. %	9.6	11.0	6.4	6.6	4.9
Laboratory N.	16	16	16	16	16
ADL					
Mean	7.2113	3.1725	8.2447	8.0313	3.9131
Minimum	6.0800	1.0000	6.5300	6.8000	3.2000
Maximum	8.1100	8.4300	9.8000	9.5000	4.7000
Standard Dev.	.6047	2.4656	.9808	.7003	.4450
C.V. %	8.4	77.7	11.9	8.7	11.4
Laboratory N.	15	16	15	15	16

Table 3: Statistical results of analytical determinations: Standard deviation (Sx), variation coefficient (C.V.s%) and reproducibility (R). (average of the 8 feedstuffs). 1985

DETERMINATION	Sx	C.V.s	R=Sx x 2.83
Dry Matter	1.01	1.09	2.84
Ash	0.54	5.83	1.53
Crude Protein	0.97	8.99	2.75
Crude Fibre	3.13	11.05	8.87
Gross Energy (Kcal/Kg DM) "In vitro"	199	4.64	564
Dry Matter Dig. "Pepsin cellulase"	8.99	23.75	25.44
Dry Matter Dig. NYLON BAG	6.67	17.29	18.88
8-12 Hours	4.39	13.66	12.41
24 Hours	7.66	20.62	21.67
48 Hours	6.01	14.02	17.01
72 Hours	13.49	29.35	38.17
N-SOLUBILITY	5.62	20.69	15.89
NDF	5.52	11.27	15.62
ADF	2.80	7.16	7.93
ADL	1.85	29.25	5.24

Table 6: Statistical results of analytical determinations: Standard deviation (Sx), variation coefficient (C.V.s%) and reproducibility (R). (average of the 5 feedstuffs). 1989

DETERMINATION	Sx	R=Sx x 2.83	C.V.s
Dry Matter	0.92	2.60	2.00
Ashes	0.54	1.53	6.98
Crude Protein	0.81	2.29	7.50
"In vitro"			
Dry Matter Dig. "Pepsin cellulase"	7.79	22.05	13.94
Dry Matter Dig.	4.47	12.65	9.82
N.D.F.	2.78	7.87	5.38
A.D.F.	2.56	7.24	7.70
A.D.L.	1.04	2.94	23.62
Prot. degr. 1h	9.87	27.93	20.68
Prot. degr. 24h	12.12	34.30	18.42