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INTER-LABORATORY INVESTIGATIONS ON EVALUATION OF INDUSTRIAL FODDER MIXTURES HOMOGENEITY

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Key words: inter-laboratory investigations, fodder mixtures, homogeneity, study methods

The paper presents results from the comparative studies upon methods of industrial fodder mixtures homogeneity evaluation. Competence of participating laboratories to make a legal control within that issue was confirmed. Achieved results on the mixing level (variability coefficient) of fodder mixtures for turkeys and swine were lower than 10% – limit value. Reproducibility limits of results achieved by two laboratories determining chlorides and calcium within legal control were set for 5% (chlorides) and 3% (calcium). Acceptance of expanded uncertainty at the same level as uncertainty of analytical methods was recommended.

INTRODUCTION

By virtue of the Art. 44, Par. 5.8, Decree from 23 August 2001 on animal feeding means [2004], tasks of Veterinary Inspection also include legal control of fodder referring to homogeneity evaluation (level of mixing). Laboratories belonging to Veterinary Hygiene Institute (ZHW) and Zootechnical Institute (IZ), National Fodder Laboratory (KLP) in Lublin as a reference laboratory have been charged with investigations in this range. However, the legislator has not indicated recommended procedures and investigation methods. In order to enable realization of the task within the monitoring by IW, instruction entitled *Homogeneity evaluation of fodder mixtures on a base of the level of a key component mixing* was elaborated in KLP Lublin. The instruction has been accepted by the Main Veterinarian and has been passed to Regional Veterinary Institutes (WIW) and ZHW as well as verified at inter-laboratory investigations.

The investigations have been aimed at evaluating the competence of laboratories to evaluate the homogeneity levels of fodder mixtures. Results achieved in a given laboratory have been compared to other laboratories' results, which had supplied objective assessment of the work. All information collected form the comparative investigation participants have been considered as secret.

Attached comparative investigation of homogeneity evaluation methods has been organized in accordance to recommendation of a guide ISO/IEC 43-1:1997, using the proficiency testing scheme FAPAS [PN-EN ISO/IEC 17025:2001; Guide ISO/IEC 43 – 1:1997; FAPAS, 2002].

The study has been aimed at verifying the method for evaluating the homogeneity of fodder mixtures and assessing the competence of laboratories taking part in investigations to make the legal control within that range.

MATERIALS AND METHODS

Samples of fodder mixtures. MD (for turkeys) and MS (for piglets) were achieved from standard air-dried granulated fodder mixtures. Serial samples consisting of five primary samples (about 250 g each) were collected. Primary samples were placed in sealed foil bags and marked with subsequent numbers from 1 to 5. A serial sample was then placed in a hermetic foil bag and stored at controlled-temperature of 20°C and moisture content of 40% of the room till the dispatch.

Samples were prepared for shipping on 2 November 2005. Every participant got two serial samples – MD and MS mixtures. Also recommended analytical aliquots for every tested parameter as well as a table to write data characterising method and conditions, the tests were performed with were included.

In total, 10 laboratories entered the comparative investigations. The participants were asked to make homogeneity determinations within the range possible to achieve by a given laboratory and to send back results till 30 November 2005.

Participants supplied single results (in two replications), mean value, standard deviation, and variability coefficient for 5 primary results. This made it possible to estimate the method's reproducibility in investigations of mixing level. Every participant was assigned with the code from L1 through L10.

Statistical result processing allowed for making an objective report serving laboratories to assess their own results and get generalized view to results achieved by all participants taking into account analytical methods, procedures, and devices

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TABLE 1. Variability coefficient CV (%) for key component determination in serial samples.

Analysis	Variability coefficient, CV (%)		
	Min	Max	Mean value
Chlorides *, Chlorides **	0.60	7.50	2.71
Calcium *, Calcium **	1.63	4.54	2.61

applied. The way of calculating the attribute value, statistical processing, and the results evaluation was performed in accordance to the Guide ISO/IEC. Proficiency Testing by Inter-Laboratory Investigations. Part 1. Designing and Realizing the Proficiency Testing Programs [Guide ISO/IEC 43-1:1997].

The attribute value X was calculated on the basis of results from participating laboratories. The following procedure was applied: removal of extreme values (elimination of extreme values – mistakes – using Grubb's test), calculating mean values after removal of extreme values, calculating the standard deviation, and calculating the *z-score*.

Z-score values for participants were calculated on the of the following formula:

$$z = \frac{(x - X)}{s}$$

where: x – participant's result, X – mean value after removal of extreme values, and s – standard deviation (calculated on the basis of study results),

Values of *z-score* were calculated for all results; also those removed as extreme ones.

Result achieved by a laboratory usually differs from attribute value X. Thus, *z-scores* may be negative or positive.

The following criteria for *z-score* assessment were accepted: z 2 satisfactory result, $2<z<3$ doubtful result, and $z\geq3$ unsatisfactory result [Guide ISO/IEC 43-1:1997].

According to the document published by Polish Certifying Center [2004] entitled *PCC's policy referring to use of proficiency testing at laboratory certification and control processes No DA-05*, 20% of doubtful results are permissible in the case of the use of *z-score* for assessing the results achieved.

TABLE 3. Results and *z-score* mixing levels (CV%) on a base of chloride determination in fodder mixture MD.

No. of laboratory	Variability coefficient CV – chlorides attribute value 2.46 (%) SD=1.99	
	result	*z-scores*
L1	9.62	3.60
L2	1.18	-0.64
L3	7.50	2.53
L4	1.04	-0.71
L5	1.90	-0.28
L6	1.70	-0.38
L7	3.19	0.37
L8*	1.65	-0.41
L9	1.85	-0.31
L10	2.11	-0.18

* laboratory L8 – Mohr's method; other laboratories – according to attachment No 2.
Extreme value 9.62%. Number of laboratories – 10; satisfactory results – 8; doubtful results – 1; unsatisfactory results – 1.

RESULTS AND DISCUSSION

The maximum value of variability coefficient as a measure of mixing level of fodder mixture components is 10%, which is in accordance to the Instruction [2002]. All results (variability coefficients calculated on a base of supplied study results) achieved by participating laboratories were lower than that value. It indicated the correct homogeneity of fodder mixtures, and on the other hand, satisfactory quality and reliability of determinations made by participating laboratories. Granulated fodder mixtures were selected as the inter-laboratory investigations objects. In this case, there is no practical risk to separate components, and proper mixing before granulating process guarantees appropriate homogeneity of a product.

Laboratory L8 made chloride determinations applying two methods: Mohr's and Volhard's; laboratory L3 made calcium determinations by means of AAS technique and titrimetric technique. To make these laboratory results comparable, appropriate calculations were performed for both variants.

TABLE 2. Reproducibility standard deviation, reproducibility limits and expanded uncertainty in comparative investigations of methods for fodder homogeneity assessment on a base of mixing level.

Fodder mixture	Determination	Attribute value X (%)	Reproducibility standard deviation SD_R	Reproducibility limit $R=2.8 \times SD_R$	Expanded uncertainty $U=2 \times SD_R$
MD	Chlorides*	2.46	1.99	5.57	3.98
	Chlorides**	3.35	2.91	8.14	5.82
MS	Chlorides*	2.63	1.96	5.49	3.92
	Chlorides**	2.32	1.67	4.67	3.34
MD	Calcium*	2.80	0.98	2.74	1.96
	Calcium**	3.07	1.11	3.11	2.22
MS	Calcium*	2.27	0.42	1.18	0.84
	Calcium**	2.31	0.44	1.23	0.88

Chlorides* – laboratory L8 – Mohr's method; Chlorides ** – laboratory L8 – Volhard's method;
Calcium * – laboratory L3 and other laboratories – AAS technique; Calcium ** – laboratory L3 – titrimetric technique; other laboratories – AAS technique.

TABLE 4. Results and *z-score* mixing levels (CV%) on a base of chloride determinations in MS fodder mixture.

No. of laboratory	Variability coefficient CV – chlorides attribute value 3.45 (%) SD=2.91	
	result	*z-scores*
L1	9.62	2.12
L2	1.18	-0.78
L3	7.50	1.39
L4	1.04	-0.83
L5	1.90	-0.53
L6	1.70	-0.60
L7	3.19	-0.09
L8*	4.44	0.34
L9	1.85	-0.55
L10	2.11	-0.46

* laboratory L8 – Volhard's method. Number of laboratories – 10; satisfactory results – 9; doubtful results – 1.

Tables 3 and 4 present the examples of result processing.

Achieved data confirm the assumptions put at working out the instruction for the way of mixing level evaluation. The selection of key components, chlorides, or calcium should be considered as justified. In the case of evaluation of mixing level on the basis of chlorides content determination, similar variability coefficients were achieved (0.60% to 7.50%, with mean value of 2.71%) against 2.61% in the case of calcium as an indicator (from 1.63% to 4.54%). Calculated variability coefficients for chlorides and calcium determinations in serial samples that characterise the level of fodder mixture mixing, were below 10% value accepted as the limit. It is noteworthy that the range of recorded results (variability) was wider in the case of chlorides (Table 1).

Table 2 lists the comparative results of mixing level investigations to assess the homogeneity of fodder mixtures on a base of achieved data. Among results derived from chlorides analysis presented in Table 3, Grubb's test eliminated the extreme value of 9.62% (L1). The same result 9.62% (L1) was not eliminated as extreme one from result population in Table 4. Therefore, the mean mixing level of 3.35% and other parameters in Table 2 appeared to be apparently different from the remaining. In this case, it would be useful to apply strong statistics for evaluating the attribute value X. Thus, in order to estimate the reproducibility limit in interlaboratory investigations as well as expanded uncertainty, determination results (MD, chlorides**) were not taken into considerations.

CONCLUSIONS

1. All laboratories achieved results of mixing levels (variability coefficients) in fodder mixtures for turkeys (MD) and piglets (MS) below 10%, and mean values for two applied methods appeared to be similar to results from chlorides (2.71%) and calcium determination (2.61%).

2. Variability of results referring to mixing level on the basis of chlorides analysis appeared to be higher than that for calcium determination. It probably results from higher variability (lower precision) of chloride than calcium determination method applied by laboratories as well as physical properties of additives containing chlorides and calcium.

3. Reproducibility limit for results of mixing level from two laboratories determining chlorides within the legal monitoring program should not be higher than 5%.

4. Reproducibility limit for results of mixing level from two laboratories determining calcium within the legal monitoring program should not be higher than 3%.

5. Laboratories performing the mixing level investigations within the legal monitoring program should accept expanded uncertainty at such levels as uncertainty for analytical methods.

6. Competence of laboratories participating in the testing and referring to the legal control of industrial fodder mixtures homogeneity was confirmed.

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BADANIA KOMPETENCJI LABORATORIÓW W OCENIE HOMOGENICZNOŚCI PRZEMYSŁOWYCH MIESZANEK PASZOWYCH

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Przedstawiono wyniki badań porównawczych metod oceny homogeniczności przemysłowych mieszanek paszowych. Potwierdzono kompetencje laboratoriów uczestniczących do wykonywania zadań kontroli urzędowej w tym zakresie. Uzyskane wyniki badania stopnia wymieszania mieszanek paszowych (współczynnika zmienności) dla indyków i prosiąt były niższe od 10% – wartości granicznej. Granicę odtwarzalności wyników otrzymywanych przez dwa laboratoria oznaczające chlorki i wapń na potrzeby urzędowego nadzoru ustalono na poziomie 5% (chlorki) i 3% (wapń). Zalecono przyjęcie niepewności rozszerzonej na takim poziomie jak niepewności metod analitycznych.