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LONG-TERM MONITORING OF HOMOGENEITY OF COMPOUND FEED IN THE GOVERNMENT SUPERVISION

DUGOROČNO PRAĆENJE HOMOGENOSTI KRMNIH SMJESA POD NADZOROM VLADE

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SUMMARY

The paper presents instruction concerning homogeneity evaluation of compound feed based on the level of the key component (chloride or calcium) mixture, which was elaborated in the National Research Institute of Animal Production, National Feed Laboratory (NFL) in Lublin. The instruction was accepted by competent authority and was verified at inter-laboratory comparisons ILC from 2005 to 2007. Several licensed laboratories took part in the investigations project in the frame of official supervision as well as reference laboratory – NFL. The laboratories determined the calcium and chlorides content in samples of serial feed mixtures. Feeds were estimated for poultry, pigs and cattle in the form of powder, granules and crumbles. All laboratories achieved results of mixing levels (coefficient of variation) below 10%, which is a critical value. Mean values of coefficient of variation of results were not higher than 4% for chlorides and 3% for calcium. Expanded uncertainty was estimated. Competence of laboratories participating in the testing and referring to the legal control of compound feed homogeneity was confirmed. A research homogeneity program for compound feed was conducted in Poland from 2006 to 2007. Inspection results confirmed acceptable quality of compound feed in this scope.

Key words: inter-laboratory comparison, compound feed, homogeneity

INTRODUCTION

Compound feeds should contain feed materials and additives in appropriate proportions depending on the species, age, and performance of animals, to which they are intended. It is important to mix thoroughly the feed components thus to form uniform mixture. Incomplete mixing of components, namely feed additives and active substances in medicated feeds, may result in negative effects. Uneven distribution of mixture components, segregation

during transfer in technological lines or in transport may be the reason for negative side effects and favor the carry-over (Neuman, 2000).

Homogeneity of the compound feed depends on its constituent's physical properties such as bulk density, particle size, electrostatic features, as well as technological factors like mixer type and mixing

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time. A producer, when preparing the optimum mixing procedure, pays attention to mixing time, electric energy utilization, and labor inputs necessary to reach the goals, i.e. to achieve mixing coefficient of variation for a given component of compound feed not greater than 10% ($CV \leq 10\%$). Values of $CV > 10\%$ may indicate insufficient components mixing and improper homogeneity of the compound feed (Mc Ellhiney, 1994, Putier, 2001, Mann, 2004).

Regulation 183/2005 enforced the obligation to produce safe feed that would guarantee required production effects and qualitative traits of animal products. The aims of official control are to check the safety and quality of feeds in accordance to Regulation 882/2004. However, no standard methods for homogeneity assessment were have been worked. In order to make possible the control of compound feed homogeneity by official supervising units, the instruction for testing the homogeneity of compound feeds on the basis of chlorides or calcium mixing was worked out at NFL in Lublin. The instruction was implemented by Regional Veterinary Laboratories (RVL) to make surveys of feeds within the frames of official control.

The paper was aimed at evaluating the competence of RVL licensed to test homogeneity of compound feeds based on results of participation in proficiency testing as well as evaluating the test uncertainty on the basis of chloride and calcium analyses. Homogeneity of medicated feeds was also assessed on based on testing calcium and chlorides as well as active substances in applied medicine. Moreover, results of homogeneity tests of domestic feeds the frame of national plan of official supervision are presented in the paper.

MATERIAL AND METHODS

Homogeneity of six loose and granulated compound feeds for broiler chickens, hatching hens, cattle, piglets, and oysters was controlled during proficiency tests (PT) carried out in 2005-2007. In total, 41 laboratories took part in the PT: about 14 laboratories per single PT, on average, including selected RVL and 2 laboratories of NFL in Lublin. Proficiency tests were performed in accordance with Guide ISO/IEC 43-1:1997. Homogeneity was assessed according to the instruction of homogeneity

assessment for compound feeds on the basis of chlorides or calcium determination (Instruction, 2005).

A series of five incremental samples of about 250 g each was collected from tested feed. Samples were taken randomly according to the Regulation of Minister of Agriculture and Rural Development from 17 June 2004 on detailed rules of animal feeding means and medicated feed sampling for tests and handling with samples within official supervision (Dz.U. No 156 from 2004, pos. 1654 with subsequent changes). Incremental samples were placed in hermetic containers and marked with numbers from 1 to 5, then sent to PT participants.

Every PT participant received 2 series of samples ($2 \times 5 \times 250$ g). In addition, recommended weight of analytical amount for each parameter, form for results, and table to put data characterizing methods and conditions used, were enclosed.

The participant had to provide single results, mean values, standard deviations, and variation coefficients for five incremental samples.

The way of assigned value determination, statistical calculations, and assessment results were described in details in Guide ISO/IEC 43-1 (1997). The assigned value was defined as arithmetic mean from all participating laboratories. Parameter *z-score* for particular PT participants was calculated on the basis of the formula:

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

where: *x* – participant's result, *X* – assigned value for all participants after extreme results removing, *s* – standard deviation calculated on the basis of results. Values of *z-score* were calculated for all results, including the ones removed as extreme. Criteria for indices assessment were accepted according to Guide ISO/IEC 43-1:

- $z \leq 2$ satisfactory result,
- $2 < z < 3$ questionable result,
- $z \geq 3$ unsatisfactory result.

Results from proficiency test were delivered in a form of a report to every participant. Expanded uncertainty $U = 2 \cdot SD_R$ was estimated on the basis of standard deviation SD_R from homogeneity test results. Based on the verified instruction, laboratories

of RVL performed homogeneity tests for almost 300 compound feeds in 2006-2007 in the frame of official supervision.

mixture structure on results of calcium and chlorides determination as well as homogeneity assessment. Statistical analysis did not prove significant influence of compound feed form on achieved results.

RESULTS AND DISCUSSION

According to the Instruction (2005), acceptable value of compound feed homogeneity level, the measure of which is coefficient of variation CV for chlorides or calcium in serial sample, amounts to $CV \leq 10\%$. All sent analyses results serving for calculation of variation coefficients met the above criterion. Results indicated the correctly performed mixing process and sufficient testing quality at participating laboratories (Table 1). Loose, crushed, and granulated compound feeds were subjected to assessment, which was aimed at verifying the influence of

Table 1. Variation coefficients CV (%) for chlorides and calcium determination within proficiency tests organized in 2005-2007

Tablica 1. Koeficijenti varijacije CV (%) za određivanje klorida i kalcija u testovima sposobnosti organiziranim 2005-2007.

Parameter / years Parametar / godina	Variability coefficient - Koeficijent varijacije, CV%		
	Min	Max	Mean
Chlorides - Kloridi:			
2005	1.18	7.50	2.71
2006	0.60	7.60	3.86
2007	0.64	5.84	2.30
Calcium - Kalcij:			
2005	1.63	4.54	2.61
2006	0.36	8.45	2.95
2007	0.43	4.91	2.49

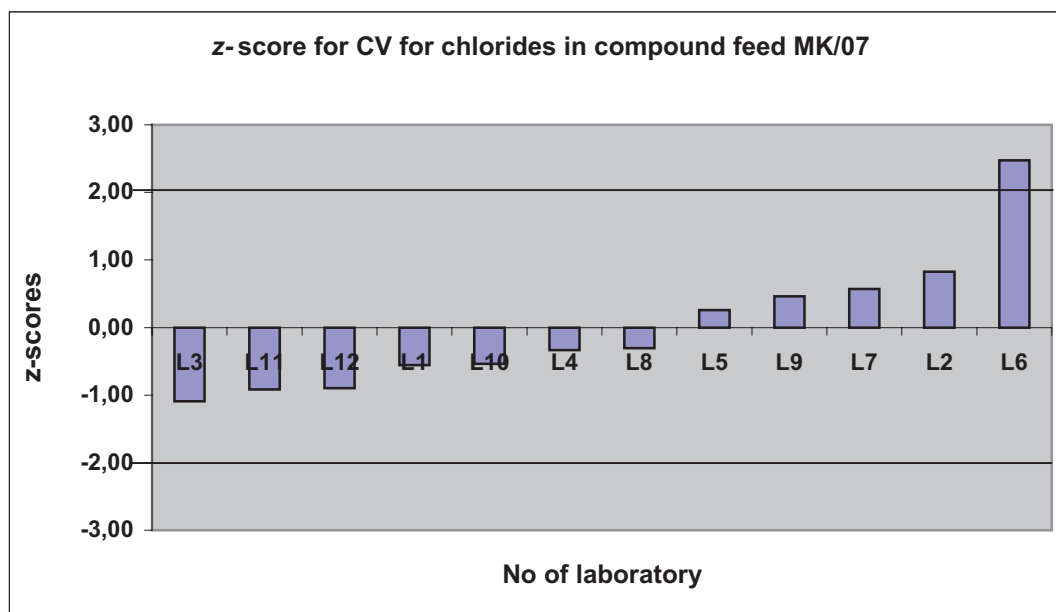


Figure 1. Histogram of homogeneity assessment on the basis of chlorides determination in compound feed for poultry MK/07 (assigned value $CV = 2.67\%$; standard deviation $SD_R = 1.28\%$)

Slika 1. Histogram ocjene homogenosti na temelju određivanja klorida u smjesama za perad MK/07 (dodijeljena vrijednost $CV = 2,67\%$; standardna devijacija $SD_R = 1,28\%$)

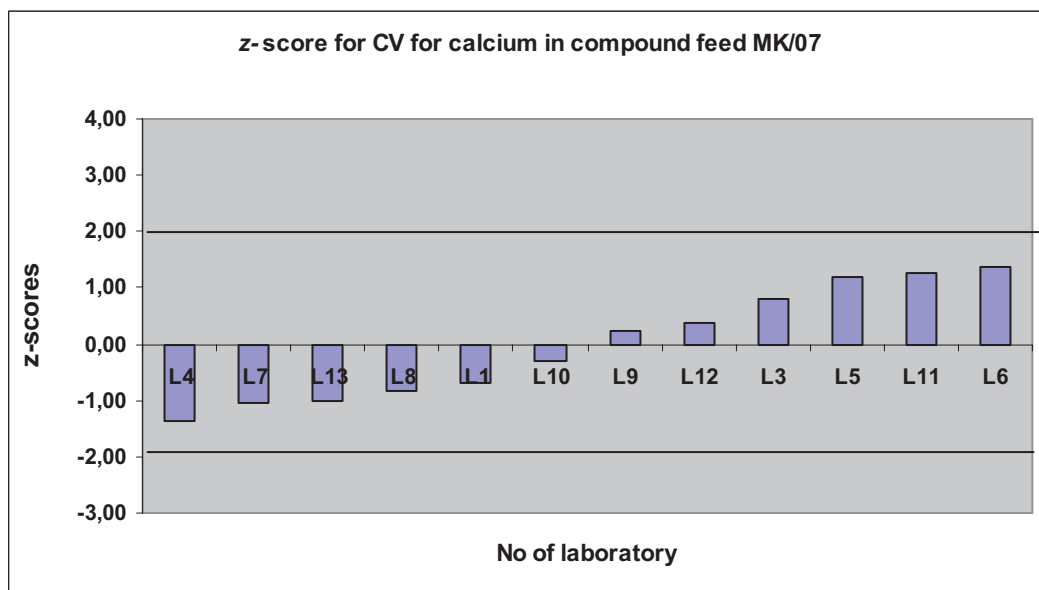


Figure 2. Histogram of homogeneity assessment on the basis of calcium determination in compound feed for poultry MK/07 (assigned value CV = 2.51%; standard deviation $SD_R = 0.96\%$)

Slika 2. Histogram ocjene homogenosti na temelju određivanja kalcija u krmnoj smjesi za perad MK/07 (dodijeljena vrijednost CV = 2,51 standardna devijacija $SD_R = 0,96$)

Table 2. Reproducibility standard deviations and expanded uncertainties in comparative studies up feed homogeneity assessments on the basis of uniformity level in 2007.

Tablica 2. Standardna odstupanja reproduktivnosti i proširena kolebanja u usporedbi s proučavanjem procjene homogenosti krmiva na temelju razine ujednačenosti u 2007.

Compound feed for Krmna smjesa za	Tested component Testirani sastojci	Assigned value Određena vrijednost CV (%)	Standard deviation of reproducibility Standardno odstupanje od reproduktivnosti SD_R	Expanded uncertainty Nesigurno proširenje $U=2 \times SD_R$
Poultry - crumble Perad - mrvice	Chlorides Kloridi	2.67	1.28	2.56
Oysters - granulate Kamenice - zrnca	Chlorides Kloridi	1.93	0.85	1.70
Poultry - crumble Perad - mrvice	Calcium Kalcij	2.51	0.96	1.92
Oysters - granulate Kamenice - zrnca	Calcium Kalcij	2.46	1.51	3.02

Similar mean variation coefficients for chlorides and calcium determinations aiming at assessing the compound feed homogeneity were achieved in 2007 (Figures 1 and 2). Mean values differed by 0.19%. In the case of chlorides, one questionable result was present (CV 5.84%, z-score 2.48). Moreover, using

the multiple comparisons test (NIR test or planned comparison), the significance of mean CV values differences calculated for chlorides and calcium in feeds tested in 2007 was compared (Table 2). The test results did not confirm considerable differences between mean values.

Sodium chloride added into compound feeds is characterized by various refinement levels (salt). The density of sodium chloride (at bulk density state) significantly differs from that of other compound feed components. Sodium chloride has a tendency to segregate. Variability of the chloride contents in a series of measurements of the same feed is considered as a critical homogeneity parameter. Thus, positive result of homogeneity testing on the basis of chlorides determination may be accepted as a proof of sufficient uniformity of tested compound feed.

Results achieved in performed tests confirmed the competence of RVL laboratories in assessing the homogeneity of compound feeds within the frame of official supervision. The expanded uncertainty for homogeneity determination was estimated at 3% (per cent scores – Table 2). To check how homogeneity tests of medicated feeds on a basis of calcium or chlorides determination are consistent with the homogeneity assessment on the basis of a medicine active substance assay, was very interesting. In general, taking into account the measurement uncertainty, confirmation of proper medicines and chlorides mixing was achieved (Table 3). Variation coefficients for the medicine

content were usually higher than for chlorides. However, medicines were tested using microbial assays that are characterized by higher coefficient of variation than chemical methods (Walczyński et al., 2007). Therefore, it can be suggested that positive homogeneity assessment on the basis of the chlorides content indirectly proved the appropriate mixing of medicine in the medicated feed.

Results of the proficiency test performed in 2005-2007 served for construing the list of RVL laboratories competent for evaluating the homogeneity of compound feeds on the basis of the chlorides or calcium content determination in serial samples (5 samples in each series). Tests allowed for confirming the competence of 11 RVL laboratories. Competent RVL laboratories made homogeneity tests of compound feeds within the frame of annual plans of official supervision in 2006-2007. Results from the supervision confirmed sufficient homogeneity of domestic compound feeds tested in accordance with the instruction (Table 4). Within 2-year supervision, only 4 cases of 287 (1.4%) did not meet the requirements. Insufficient homogeneity (CV > 10%) was found most often in compound feeds for ruminants (16.7%). In the opinion of Hancock (1999),

Table 3. Medicated feeds homogeneity assessment results on the basis of active substance determination by means of microbial assays and bioluminescent titration technique (Walczyński et al., 2007)

Tablica 3. Rezultati procjene homogenosti lijekovitih krmiva na temelju određivanja aktivnog sastojka pomoću mikropskih proba i tehnike bioluminescentne titracije (Walczyński i sur., 2007)

Active substance Aktivna tvar	Declared medicine / chlorides content Sadržaj deklariranog lijeka/klorida (g/kg)	Determined content Određeni sadržaj \bar{x} (g/kg)	Standard deviation, SD Standardno odstupanje (g/kg)	Variability coefficient Koeficijent varijacije CV (%)
Chlorotetracycline	0.2	0.228	0.008	4.0
Chlorides	2.6	2.62	0.045	1.7
Tylozine	0.1	0.110	0.013	12.0
Chlorides	3.0	3.10	0.071	2.3
Tiamuline	2.5	2.89	0.262	9.0
Chlorides	2.4	2.40	0.100	4.2
Tylozine	0.1	0.105	0.008	8.0
Chlorides	2.60	2.66	0.152	5.7
Linkomycine	0.022	0.024	0.002	8.0
Chlorides	2.4	2.46	0.055	2.2

providing required homogeneity of a compound feed ($CV < 10\%$) affects the improvement of animal feeding efficiency to a lower extent than it is commonly thought. According to that author, mixture homogeneity is important, namely at feeding young animals, e.g. piglets, and feed homogeneity for broiler chickens at the level of $CV 12\%$ ensured optimum bird growth. Taking into account recommended expanded uncertainty of homogeneity measurement as $\pm 3\%$, the homogeneity testing results with $12 \pm 3\%$ uncertainty should be interpreted as sufficient, because lower uncertainty limit (9%) would be lower than permissible limit (10%). Industrial feed producers, in general, perform supervision upon the homogeneity of their feeds, namely those containing feed additives of medicines (medicated feeds) by applying recommended indices and procedures (Heidenreich and Strauch, 2000; Putier, 2001).

ratio. However, these recommendations range within wide limits – from 50 g (EMEA, 1997) up to 250 g (Instruction, 2005). Also recommended number of primary samples per series may vary from 5 to 10 (EMEA, 1997; Instruction, 2005). Therefore, there is a need to consider boundary conditions for assessing the homogeneity of compound feeds within the official supervision and to introduce appropriate legal regulations (or recommended procedures) to assure comparability of test results achieved in different UE countries (results from official supervision are sent to European Commission). Furthermore, introducing minimum requirements referring to permitted variability of feed products homogeneity testing, weight of incremental sample, and number of samples per series, would allow for improving the efficiency of official supervision of feed products homogeneity assessment.

Table 4. Evaluation of feed components homogeneity in compound feeds achieved during official supervision of feeds in Poland in 2006-2007

Tablica 4. Ocjena homogenosti sastojaka krmiva u krmnim smjesama postignuta za vrijeme službenog nadzora krmiva u Poljskoj 2006-2007.

Compound feeds for Krmne smjese za	Years - Godine			
	2006		2007	
	Total Ukupno	Not meeting requirements Ne odgovaraju uvjetima	Total Ukupno	Not meeting requirements Ne odgovaraju uvjetima
Ruminants - Preživači	30	1 (3.3%)	12	2 (16.7%)
Swine - Svinje	66	0	11	0
Poultry - Perad	96	1 (1.0%)	58	0
Other stock animals for slaughter Ostale životinje za klanje	6	0	8	0
Total - Ukupno	198	2 (1.0%)	89	2 (2.2%)

Presented results of proficiency tests along with results of compound feeds and medicated feeds homogeneity assessments within the frame of official supervision refer to particular procedure accepted in the Instruction (2005). The Instruction assumes weight of primary sample of 250 g and number of samples per series of 5. Weight of primary sample collected to homogeneity tests should provide with the same level of components uniformity as at daily

CONCLUSIONS

1. All laboratories participating in proficiency tests performed in 2005-2007 achieved results of mixing level (variability coefficients) in feed mixtures below 10%, and mean values for two applied methods appeared to be similar to results to chlorides (2,96%) and calcium determination (2,68%).

2. The test results allowed for confirming the competence of 11 Polish Regional Veterinary Laboratories (RVL) in assessing the homogeneity of compound feeds on the basis of the chlorides or calcium content in serial samples (5 samples per 250 g in each series) within the frame of official supervision.

3. Competent RVL laboratories made homogeneity tests of compound feeds in accordance with the Instruction within the frame of annual plans of official supervision in 2006-2007. Results of these supervisions confirmed sufficient homogeneity of domestic compound feeds.

4. The expanded uncertainty in homogeneity determination was estimated for 3% (per cent scores).

5. There is a need to introduce appropriate legal regulations (or recommended procedures) to assure comparability of test results achieved in different UE countries.

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SAŽETAK

Rad daje temeljne upute u vezi procjene homogenosti krmnih smjesa na temelju razine glavnog sastojka (klorida ili kalcija) obrađene u Nacionalnom istraživačkom institutu proizvodnje životinja, Nacionalnog laboratorija za krmiva (NFL) u Lublinu. Upute je potvrdio nadležan stručnjak i provjerene su međulaboratorijskim uspoređivanjem - ILC od 2005. do 2007. godine. Nekoliko ovlaštenih laboratorija sudjelovalo je u projektu istraživanja u okvirima službenog nadzora kao i priručnih laboratorija - NFL. Laboratoriji su odredili sadržaj kalcija i klorida u uzorcima serijskih krmnih smjesa. Krmiva su procjenjivana za perad, svinje i stoku u obliku praška, zrnaca i mrvica. Svi su laboratoriji postigli rezultate raznih razina (koeficijent varijacije) ispod 10%, što je kritična vrijednost. Srednje vrijednosti rezultata koeficijenta varijacije nisu bile više od 4% za kloride i 1% za kalcij. Procijenjeno je prošireno kolebanje. Potvrđena je nadležnost laboratorija koji sudjeluju u testiranju u vezi sa zakonskom kontrolom homogenosti krmnih smjesa. Program istraživanja homogenosti krmnih smjesa provodio se u Poljskoj od 2006. do 2007. godine. Rezultati pregleda potvrdili su prihvatljivu kakvoću krmnih smjesa u okviru ovog rada.

Ključne riječi: međulaboratorijsko uspoređivanje, krmna smjesa, homogenost

