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AN INTERPRETATION SYSTEM OF FEED ANALYSES BASED ON INTERLABORATORY COMPARISONS

TUMAČENJE SUSTAVA ANALIZE KRMIVA NA TEMELJU MEĐULABORATORIJSKIH USPOREDBI

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SUMMARY

The aim of the present paper was to evaluate the uncertainty of feed analysis results based on inter-laboratory comparisons (ILC) and proficiency testing (PT), as well as to use the obtained uncertainties to create an interpretation system for feed analysis results and feed quality evaluation. The ILC and PT were conducted with the participation of Polish laboratories, including laboratories for feed control and supervision (Regional Veterinary Laboratories). The results of four PTs and seven ILCs performed in 2004-2007 were evaluated. The expanded uncertainty was calculated as a double standard deviation of reproducibility. The expanded uncertainty for method analysis of nutrients (moisture, crude ash, crude protein, crude fat, crude fat after acidic hydrolysis, crude fiber, starch and sugar), minerals (chlorides, phosphorus, calcium, magnesium, sodium and potassium), some feed additives (iron, manganese, zinc, copper, selenium, vitamin A, vitamin E, tryptophan and ethoxyquin) were calculated. The obtained expanded uncertainties were in most cases similar to those used by German laboratories in feed control and supervision. The method proposed to calculate the uncertainty can be used to interpret analytical results and to evaluate the permitted levels of undesirable substances in feeds, as well as to evaluate nutrients and feed additives in feedstuffs.

Key words: inter-laboratory comparison, result of feed analysis, uncertainty, interpretation

INTRODUCTION

Chemical analyses are widely applied in assessing the content of nutrients in feed materials and mixtures. The results of such analyses are used to compose the recipes for new feed mixtures or to confirm the nutrients and energy content in feed mixtures and concentrates, declared by the manufacturers. Hence, it is important that the results of chemical analyses should be accurate and precise enough to enable a reliable assessment of a diet. The most effective method of evaluating research methods and improving the laboratory research quality and competence in a particular analytical range is participating in inter-laboratory comparisons (ILC) and proficiency testing (PT) organized in accordance with the accepted principles (International

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Standard, 1997; Thompson and Wood, 1993; FAPAS, 2002; Horwitz, 2000).

PTs and ILCs provide a lot of valuable information which can be used to assess the uncertainty of metabolisable energy, on the basis of the results of chemical analyses and regression equations. One of the experimental approaches adopted to evaluate the expanded uncertainty (U) is using the standard deviations (SD_R) of PT/ILC results ($U= 2 \times SD_R$), (VDLUFA, 2004; Korol et al., 2006; Korol, 2008).

The work aimed at analyzing the data recorded in PT and ILC investigations performed in the years 2004-2007 by the National Research Institute of Animal Production, National Feed Laboratory in Lublin, to evaluate the uncertainties of the results of feed analyses for the needs of research and supervision, as well as to use these uncertainties in developing a system of result interpretation.

MATERIAL AND METHODS

National Feed Laboratory organized in 2004-2007 four PTs examining basal nutrients (moisture, crude ash, total protein, crude fat, hydrolyzed fat, crude fiber, starch, sugars), macro elements (P, Cl, Ca, Mg, Na, K), and microelements (Fe, Mn, Zn, Cu), as well as seven ILCs for feed additives (Se, vitamins A and E, ethoxyquin, tryptophan).

Sixteen RVLs, two National Feed Laboratories, and several laboratories of feed producers entered the tests. In total, 21 feed samples were tested (feed components, feed mixtures, and premixes), as well as 2064 analyses were made. The range of PT/ILC and detailed data are presented in Table 1. The laboratories used their own protocols to determine the nutrients and feed additives but usually all these protocols were based on standard methods of feed analyses (VDLUFA, 2004).

Table 1. Selected proficiency tests and inter-laboratory comparisons performed in 2004-2007
Tablica 1. Odabrani stručni testovii međulaboratorijske usporedbe 2004-2007

No Br.	PT/ILC symbol, year PT/ILC simbol. god.	Tested nutrients and feed additives Testirana hraniva i dodaci krmivima	Number of labs Broj lab	Number of samples Broj uzoraka	Number of analyses Broj analiza
1	PT 2004	Moisture content, crude ash, ash insoluble in HCl, total protein, crude fiber, crude fat, hydrolyzed crude fat, starch, sugars, chlorides, phosphorus, calcium, magnesium, sodium, potassium, iron, manganese, zinc, copper in feed materials and mixtures – Sadržaj vlage, sirovi pepeo, pepeo topiv u HCl, ukupne bjelančevine, sirova vlaknina, sirova masnoća, hidrolizirana sirova masnoća, škrob, šećeri,kloridi, fosfor, kalcij, magnezij, soda, kalij, željezo, mangan, cink, bakar u materijalu za hranu i smjesama	19	2	314
2	PT 2005		19	2	448
3	PT 2006		19	2	536
4	PT 2007		23	2	670
5	ILC Vit A and E 2006	Vitamin A, vitamin E (tocopherole acetate)	5	2	20
6	ILC Vit A and E 2007		7	2	28
7	ILC Try 2006	Tryptophan - Triptofan	3	2	6
8	ILC Try 2007		4	1	4
9	ILC EQ 2006	Ethoxyquin - Etoksikvin	3	2	6
10	ILC Se 2006	Selenium in selenium premixes (2006), premixes and feed mixture (2007) - Selen u premiksima selena (2006) premiksi i krmne smjese (2007)	9	2	18
11	ILC Se 2007		7	2	14
Total PT and ILC - Ukupno PT i ILC (13)			118	21	2064

PTs were organized and performed in accordance with International Standard (1997). The homogeneity of feed products was tested on the basis of chloride content, determined by a biamperometric titration method (Korol and Matyka, 1982) and the procedure recommended in FAPAS protocol (FAPAS, 2002).

The assigned values were defined as mean or median of the results of participating laboratories after removing the extreme values (elimination of outliers applying Grubbs's test). Then, standard deviation, *z-score* and Horrat values (Hor) were calculated (International Standard, 1997; Horwitz and Albert, 2006). The Hor is the ratio of the reproducibility standard deviation SD_R, calculated from the data, to the target standard deviation σ_p, calculated from the Horwitz formula in Thompson modification $\sigma_p=0.023\ C^{0.826}$, where C means concentration expressed as denominated mass fraction (*e.g.* 1 mg/kg = 10^{-6}). Acceptable Hor values, which describe the precision of measurements, are 0.5 < Hor < 2 (Thompson, 2004; Horwitz and Albert, 2006).

RESULTS AND DISCUSSION

The detailed results of PTs and ILCs organized in 2004-2007, including z-scores and Hor values, are presented in the research reports and can be found in published materials (Korol et al., 2006; Bielecka, 2008; Rubaj, 2008; Korol, 2008).

The values of standard deviations of the elements and feed additives examined in PTs and ILCs were used to develop the value of the expanded uncertainty. The results of expanded uncertainty estimation for basal nutrients testing (moisture content, crude ash, total protein, crude fat, crude fiber) and Horrat values equal to 0,8 appeared to be similar to those applied by German and Hungarian laboratories (VDLUFA, 2004; Permissible deviations, 2003; Bielecka, 2008), (Table 2). However, improved precision is required in the case of starch and sugar analyses (Hor=2.0), because these components enter the regression equations applied to estimate metabolisable energy of feed materials and compound feeds for poultry and swine (Smulikowska and Rutkowski, 2005; Pig

Table 2. Comparison of expanded uncertainties for methods of basal nutrients testing in feeds on a base of PT 2004-2007 results (Bielecka, 2008)

Tablica 2. Usporedba proširenih kolebljivosti za postupke testiranja bazičnih hraniva u krmivima na temelju rezultata PZ 2004-2007 (Bielecka, 2008)

Component Sastojak	Content range Raspon sadržaja	U=2 x CV_R or 2 x SD_R PT 2004-2007 Poland - Poljska	U=2 x CV_R or 2 x SD_R VDLUFA Germany - Njemačka	U=2 x CV_R or 2 x SD_R Hungarian data Mađarska
Moisture content Sadržaj vlage	5-10 g/100g	±5-6%	-	±8%
		±0,46 g/100g	±0,3 g/100g	-
Total protein Ukupne bjelančevine	10-20 g/100g	±2,5-3,0%	±2,5%	±4%
	> 20 g/100g	±0,8 g/100g	±0,8 g/100g	±0,8 g/100g
Fat - Masnoća	do 5 g/100g	±0,35 g/100g	±0,4 g/100g	±0,4 g/100g
Fiber - Vlaknina	do 6 g/100g	±0,3 g/100g	±0,4 g/100g	±0,6 g/100g
Ash - Pepeo	5-10 g/100g	±6,0%	-	±8%
		±0,4 g/100g	±0,4 g/100g	-
Starch - Škrob	>20 g/100g	±1,0-1,6 g/100g*	±1 g/100g	±2 g/100g
Sugars - Šećeri	<20 g/100g	±0,8 g/100g*	±0,5 g/100g	-

SD_R - standard deviation; CV_R - coefficient of variation

SD_R - standardno odstupanje, CV_R - koeficijent varijacije

Feeding Standards, 1993). The expanded uncertainty of metabolisable energy evaluation in feeds for poultry, calculated from regression equations (Smulikowska and Rutkowski, 2005) and following PT results, amounted to 0.4 MJ/kg, whereas German laboratories use the expanded uncertainty of 0.3 MJ/kg (Korol et al., 2008; VDLUFA, 2004).

The results from PT and expanded uncertainty values referring to macroelements (Cl, P, Ca, Mg, Na, K, Hor = 1.2) and microelements (Fe, Mn, Zn, Cu, Hor = 1.0) were also consistent with German (VDLUFA, 2004) and Hungarian data (Permissible deviations, 2003), (Table 3). In the case of feed additives, including vitamins A (Hor = 1.0) and E (Hor = 1.5) as well as selenium (Hor = 1.2), acceptable Horrat values were obtained (Rubaj, 2008). However, an improvement in the laboratory precision and accuracy is needed, compared to German and Hungarian data (Table 4).

The PTs and ILCs performed allowed us to accept values of expanded uncertainties for tested nutrients and feed additives and to apply them to interpret results for official supervision needs (Tables 5-7). A legal obligation to apply expanded uncertainty to estimate feed testing results was introduced by EU feed law in 2005 concerning undesirable substances in feeds. In this case the result should be expressed as $X \pm U$, where X is the analysis result, and U is expanded uncertainty at expanding coefficient k = 2. However, official feed law does not exclude the application of the same rules to estimate nutrients and feed additives in reference to exceeded the maximum or minimum content and to confirm their consistence with the manufacturer's declaration. This interpretation is recommended by European Association of Feed Additives Producers (FEFANA) regarding active substances in feed additives (Bertin, 2005).

Table 3. Comparison of expanded uncertainties for methods of macroelements and microelements analysis in feeds on a base of PT 2004-2007 results (Bielecka, 2008; Rubaj 2008)

Tablica 3. Usporedba proširenih kolebljivosti za postupke analize makroelemenata i mikroelemenata u krmivima na temelju rezultata PT 2004-2007 (Bielecka, 2008; Rubaj 2008)

Component Sastojak	Content range Raspon sadržaja	U=2 x CV_R or 2 x SD_R PT 2004-2007 Poland - Poljska	U=2 x CV_R or 2 x SD_R VDLUFA Germany - Njemačka	U=2 x CV_R or 2 x SD_R Hungarian data Mađarska
Chlorides - Kloridi	< 1 g/100g	±1-1,1 g/kg	±1 g/kg	±3 g/kg
Phosphorus - Fosfor	< 1 g/100g	±0,4-0,7 g/kg	±0,6 g/kg	±1 g/kg
Calcium - Kalcij	1-2 g/100g	±5-12%	±10%	±10%
Magnesium - Magnezij	< 1 g/100g	±7-8%	±10%	-
Sodium - Soda	< 1g/100g	±10-14%	±12,5%	±12,5%
Potassium - Kalij	<1 g/100g	±7-12%	±10%	±12,5%
Iron - Željezo	>100 mg/kg	±8-30% (20%)	±15%	±15%
Manganese - Mangan	>100 mg/kg	10-14%	±15%	±15%
Zinc - Cink	>100 mg/kg	±8-11%	±15%	±15%
Copper - Bakar	> 50 mg/kg	±14-22%	±25%	±30%

SD_R - standard deviation; CV_R - coefficient of variation

SD_R - standardno odstupanje, CV_R - koeficijent varijacije

Table 4. Comparison of expanded uncertainties for methods of some feed additives testing in feeds on a base of PT 2004-2007 results (Rubaj, 2008)

Tablica 4. Usporedba proširenih kolebanja za postupke testiranja nekih dodataka u hrani na temelju rezultata PT testiranja 2004-2007 (Rubaj, 2008)

Component Sastojak	Content range Raspon sadržaja	U=2 x CV_R or 2 x SD_R PT 2006 Poland - Poljska	U=2 x CV_R or 2 x SD_R PT 2004-2007 Poland - Poljska	U=2 x CV_R or 2 x SD_R VDLUFA Germany Njemačka	U=2 x CV_R or 2 x SD_R Hungarian data Mađarska
Vitamin A*	from - od 4000 to 20000 jm/kg >1000000 jm/kg	±42,0% ±10,0%	±14,9% ±29,8%	±25,0% ±10,0%	±2500 jm/kg ±10,0%
Vitamin E*	from 50 to 150 mg/kg > 750 mg/kg	±16,0% ±6,0%	±41,0% ±15,2%	±20,0% ±10,0%	±20,0% ±10,0%
Tryptophan Triptofan	< 20 g/kg	±15%	±12%	-	±15%
Ethoxyquin Etoksifin	from - od 10 to 100 mg/kg from 100 to 125 mg/kg	±20% ±12%	-	-	±15% ±15 mg/kg
Selenium* Selen*	< 0,5 mg/kg > 20 mg/kg	- ±12-15%	28% 30%	±50,0% ±15,0%	±30,0% ±15,0%

SD_R - standard deviation; CV_R - coefficient of variation
SD_R - standardno odstupanje, CV_R - koeficijent varijacije

Table 5. Expanded uncertainties for methods of basal nutrient analyses for the purpose of official feed supervision (Bielecka, 2008)

Tablica 5. Proširena kolebanja za postupke analiza bazalnih hranuiva u svrhu službenog nadzora hrane (Bielecka, 2008)

Component Sastojak	Content range Raspon sadržaja	Permissible analytical deviations = expanded uncertainty Dozvoljena analitička odstupanja = nesigurno proširenje (2 x SD_R or CV_R)
Moisture - Vlaga	c ≤ 50 g/kg 50 g/kg < c ≤ 100 g/kg 100 g/kg < c ≤ 150 g/kg	±3 g/kg ±5% ±5 g/kg
Ash - Pepeo	c ≤ 50 g/kg 50 g/kg < c ≤ 100 g/kg 100 g/kg < c ≤ 200 g/kg	4 g/kg ±8% ±8 g/kg
Total protein Ukupne bjelančevine	c ≤ 100 g/kg 100 g/kg < c ≤ 200 g/kg 200 g/kg < c ≤ 300 g/kg c > 300 g/kg	±4 g/kg ±4% ±8 g/kg ±3%
Crude fat Sirova masnoća	c ≤ 50 g/kg 50 g/kg < c ≤ 100 g/kg	±4 g/kg ±8%
Crude fiber Sirova vlaknina	c ≤ 60 g/kg 50 g/kg < c ≤ 120 g/kg c > 120 g/kg	±4 g/kg ±6% ±8 g/kg
Starch - Škrob	c ≤ 100 g/kg 100 g/kg < c ≤ 200 g/kg c > 200 g/kg	±8 g/kg ±8% ±16 g/kg
Sugars - Šećeri	c ≤ 100 g/kg c > 100 g/kg	±6 g/kg ±6%

SD_R - standard deviation; CV_R - coefficient of variation
SD_R - standardno odstupanje, CV_R - koeficijent varijacije

Table 6. Expanded uncertainties for methods of macroelements analyses for the purpose of official feed supervision (Bielecka, 2008)

Tablica 6. Proširena kolebanja za postupke analiza makroelemenata u svrhu službenog nadzora hrane (Bielecka, 2008)

Component Sastojak	Content range Raspon sadržaja	Permissible analytical deviations = expanded uncertainty Dozvoljena analitička odstupanja = nesigurno proširenje (2 x SD_R or CV_R)
Sodium chloride (as Cl) - Klorid sode	c ≤ 10 g/kg	±1 g/kg
	10 g/kg < c ≤ 50 g/kg	±10%
Phosphorus - Fosfor	c ≤ 10 g/kg	±0,7 g/kg
	10 g/kg < c ≤ 150 g/kg	±7%
Calcium - Kalcij	c ≤ 5 g/kg	±0,5 g/kg
	5 g/kg < c ≤ 50 g/kg	±10%
	50 g/kg < c ≤ 100 g/kg	±5 g/kg
Magnesium - Magnezij	c ≤ 2 g/kg	±0,2 g/kg
	2 g/kg < c ≤ 50 g/kg	±10%
Sodium - Soda	c ≤ 2 g/kg	±0,2 g/kg
	2 g/kg < c ≤ 50 g/kg	±10%
Potassium - Kalij	c ≤ 2 g/kg	±0,2 g/kg
	2 g/kg < c ≤ 50 g/kg	±10%

SD_R - standard deviation; CV_R - coefficient of variation

SD_R - standardno odstupanje, CV_R - koeficijent varijacije

Feed producers are legally obliged to place labels and declare some feed additives content. Permissible deviations from the declared content are determined by EU feed law and national regulations. It is important to distinguish tolerance limits of a feed component in a feed product (e.g. ±10%) determined by EU feed law, which is a permissible technical deviation resulting from the nature of feed production process, from analytical deviation of the result which is known as an uncertainty of measurement. In the case of undesirable substance analysis, the result should be considered as inconsistent with requirements when the lower limit of uncertainty range measurement is above the permissible level.

The results from the basal nutrient and feed additive components in feed materials, premixes, and feed mixtures should be interpreted as inconsistent with the producer's declaration when the upper limit of uncertainty range is lower than the lower limit of tolerance range, or inversely, when the lower limit of uncertainty range is higher than the upper limit of tolerance range.

In the case of exceeding the maximum feed additives content in a compound feed (e.g. Fe, Mn, Zn, Cu, Se; vitamin A; ethoxyquin), the result should be interpreted as inconsistent with regulations when the lower limit of uncertainty range is higher than its maximum content.

Table 7. Expanded uncertainties for methods of selected feed additives testing for the purposes of official feed supervision (Rubaj, 2008)

Tablica 7. Proširena kolebanja za postupke testiranja odabranih dodataka hrani u svrhu službenog nadzora hrane (Rubaj, 2008)

Component - Sastojak	Content range - Raspon sadržaja	Permissible analytical deviations = expanded uncertainty Dozvoljena analitička odstupanja = nesigurno proširenje (2 x SD_R or CV_R)
Iron - Željezo	c ≤ 50 mg/kg 50 mg/kg < c ≤ 100 mg/kg c > 100 mg/kg	±25% ±10 mg/kg ±15%
Manganese - Mangan	c ≤ 50 mg/kg 50 mg/kg < c ≤ 100 mg/kg c > 100 mg/kg	±25% ±10 mg/kg ±15%
Zinc - Cink	c ≤ 50 mg/kg 50 mg/kg < c ≤ 100 mg/kg c > 100 mg/kg	±25% ±10 mg/kg ±15%
Copper - Bakar	c ≤ 50 mg/kg 50 mg/kg < c ≤ 100 mg/kg c > 100 mg/kg	±25% ±10 mg/kg ±15%
Selenium - Selen	0,1 mg/kg < c ≤ 50 mg/kg 1 g/kg < c ≤ 50 g/kg	±30% ±15%
Vitamin A	1000 jm/kg < c ≤ 4000 jm/kg 4000 jm/kg < c ≤ 100000 jm/kg 100000 jm/kg < c ≤ 150000 jm/kg c > 150000 jm/kg	±1000 jm/kg ±25% ±25000 jm/kg ±20%
Vitamin E (tocopherol acetate)	c ≤ 150 mg/kg 150 mg/kg < c ≤ 200 mg/kg c > 200 mg/kg	±20% 30 mg/kg ±15%
Tryptophan - Triptofan	c ≤ 20 g/kg	±15%
Ethoxyquin - Etoksifin	c ≤ 100 mg/kg 100 mg/kg < c ≤ 150 mg/kg c > 150 mg/kg	±20% 20 mg/kg ±15%

SD_R – standard deviation; CV_R – coefficient of variation

SD_R - standardno odstupanje, CV_R - koeficijent varijacije

CONCLUSIONS

The presented data lead to the following conclusions:

1. Standard deviations of results within PT and ILC are useful to estimate the expanded uncertainties ($U = 2 \times SD_R$) of basal nutrients including starch and sugars, macroelements, microelements, vitamins A and E, tryptophan and ethoxyquin.

2. The results of expanded uncertainties estimation on the basis of PT and ILC may be used to create a consistent system based on accepting uniform values of expanded uncertainties for analyses and matrices tested in PT or ILC by the laboratories of official supervision (Regional Veterinary Laboratories) and reference laboratories.

3. Regardless of the decision of a competent unit referring to implementing a uniform system of feed testing results interpretation, estimated uncertainties may be accepted by laboratories that have not performed a complete validation process and have not determined the uncertainty by means of the model approach.

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SAŽETAK

Cilj ovog rada je procijeniti kolebljivosti rezultata analize krmiva na temelju međulaboratorijskih usporedbi (ILC) i testiranja sposobnosti (PT) kao i upotrijebiti dobivene kolebljivosti kako bi se stvorio sustav za tumačenje rezultata analize krmiva i procjenu kakvoće krmiva. ILC i PT obavljeni su uz suradnju poljskih laboratorija, uključujući laboratorije za kontrolu i nadzor krmiva (Regionalni veterinarski laboratoriji). Ocijenjeni su rezultati četiriju PT-a i sedam ILC-a provedenih od 2004. do 2007. godine. Proširena kolebljivost izračunata je kao dvostruko standardno odstupanje reproduktivnosti. Izračunata je proširena kolebljivost za postupak analize hraniva (vlaga, sirova vlaknina, pepeo, sirove bjelančevine, sirova masnoća nakon kiselinske hidrolize, škrob i šećer), minerala (kloridi, fosfor, kalcij, magnezij, soda i kalij), nekih dodataka hrani (željezo, mangan, cink, bakar, selen, vitamin A, vitamin E, triptofan i etoksiikvin). Dobivene proširene kolebljivosti bile su u većini slučajeva slične onima koje upotrebljavaju u njemačkim laboratorijima u kontroli i nadzoru hrane. Predloženi postupak za izračunavanje kolebljivosti može se upotrijebiti za tumačenje analitičkih rezultata i procjenu dozvoljenih razina nepoželjnih tvari u hrani i dodacima hrani.

Ključne riječi: međulaboratorijska usporedba, rezultat analize hrane, kolebljivost, tumačenje