PREDICTION OF METABOLIZABLE ENERGY CONTENT OF POULTRY FEEDSTUFFS – RESPONSE SURFACE METHODOLOGY *VS.* ARTIFICIAL NEURAL NETWORK APPROACH

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Abstract

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Metabolisable energy (ME) represents portion of energy utilized by the animal. Experiments for determination of ME require test animals, collection of samples and excreta, and determination of total energy content of used material. Therefore, ME determination can be expensive and time consuming. The aim of this study was to investigate the effect of enzymatic digestible organic matter (EDOM) and values of proximate chemical analysis on prediction of true metabolisable energy (TME) of feedstuffs for broilers. The performance of Artificial Neural Networks (ANN) was compared with the performance of second order polynomial (SOP) model, as well as with experimental data in order to develop rapid and accurate method for prediction of TME.

Analysis of variance and post-hoc Tukey's HSD test at 95% confidence limit have been calculated to show significant differences between different samples. Response Surface Method has been applied for evaluation of TME. Second order polynomial model showed high coefficients of determination ($r^2 = 0.927$). ANN model also showed high prediction accuracy ($r^2 = 0.983$). Principal Component Analysis was successfully used in prediction of TME.

Key words: true metabolisable energy, broilers, feedstuffs, SOP, ANN

Abbreviations: ANN – Artificial Neural Network; BFGS – Broyden–Fletcher–Goldfarb–Shanno; CA – crude ash; CFa – crude fat; CFi – crude fibre; CP – crude protein; df – degrees of freedom; DM – dry matter; EDOM – Enzymatic digestible organic matter; F – F test value; ME – Metabolisable energy; Max – maximum; Min – minimum; MLP – multi-layer perceptron models; OM – organic matter, OMD – Organic matter digestibility; PCA – Principal Component Analysis; r^2 – coefficient of determination; RSM – Response Surface Methodology; SD – standard deviation, SOP – second order polynomial model; SOS – Sum of Squares; TME – True metabolisable energy; TME_n – Nitrogen corrected true metabolisable energy; Var – variance

Introduction

For proper utilization of feedstuffs used in preparation of compound feeds, it is necessary to have information about ingredients' nutritional quality. Accurate nutrient composition of the feedstuffs will enable formulation of correct balanced diet and thus provide all required nutrients to the animals (Dale and Batal, 2002; De Leon et al., 2010). Complete information about the nutrient composition includes energy

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value of the ingredients, since only sufficient amount of energy allows performance of metabolic processes and animal activity. Metabolisable energy (ME) represents portion of energy utilized by the animal, i.e. bioavailable energy. Direct determination of ME of the feedstuffs implies *in vivo* experiments (Mohamed, 1984; Girish et al., 2013). These experiments require test animals, collection of samples and excreta, and determination of total energy content of used materials. Therefore, ME determination can be expensive and time consuming. Thus, it is important to develop fast laboratory methods for accurate and inexpensive prediction of ME (Zhang, 1994; Colovic et al., 2011).

Organic matter digestibility (OMD) is a nutritive value parameter which provides information about amount of total digestible organic matter, and for its calculation content of organic matter of feed, as well of faeces is needed. For decreasing of experimental expenses, in vitro experiments with exogenous enzymes can be performed, where the enzymes have aim to mimic the digestive processes in the animal. Multi-enzymatic incubation method was reported by Hvelplund et al. (1990) for estimation of the enzymatic digestibility of organic matter (EDOM) of straws. EDOM method was utilized by Palic and Muller (2006) for prediction of the OMD of a wide range of ruminant feedstuffs. Enzymatic determination of OMD was used by Wilfart et al (2008) to determine hydrolysis kinetics of four feedstuffs for pigs. Narashimha et al (2013) applied in vitro enzymatic assay on commonly used poultry feed ingredients as a tool for formulating customized enzyme mixtures for degradation of non-starch polysaccharides. Recently, mathematical modelling has been increasingly used for the study of the given systems. Developed empirical models show a reasonable fit to experimental data and successfully predict ME (Perai et al., 2010). Nonlinear models are found to be more suitable for real process simulation. Second order polynomial (SOP), using Response Surface Methodology (RSM) and Artificial Neural Network (ANN) models have gained momentum for modelling and control of processes (Priddy and Keller, 2005; Khuri and Mukhopadhyay, 2010).

ANN models are recognized as a good modelling tool since they provide the empirical solution to the problems from a set of experimental data, and are capable of handling complex systems with nonlinearities and interactions between decision variables (Almeida, 2002). The specific objective of this study was to investigate the effect of EDOM and values of proximate chemical analysis on TME of feedstuffs for broilers. The performance of ANNs was compared with the performance of SOP, as well as to experimental data in order to develop rapid and accurate method for prediction of TME.

Materials and Methods

Chemical analysis

Fifty seven feedstuffs commonly used in broiler diets were used in this study. AOAC official methods (AOAC, 2000) were used for proximate analysis, i.e. determination of dry matter (DM), crude protein (CP), crude fat (CFa), crude fibre (CFi) and ash (CA) of the feedstuffs. Organic matter (OM) was calculated by subtracting CA content from total dry matter content.

For determination of the enzymatic digestibility of organic matter (EDOM) of the feedstuffs, a three-step method of Boisen and Fernandez (1997) developed for pigs, was modified to two consecutive incubation steps corresponding to digestion in the stomach and the small intestine. In the first step, samples were incubated with pepsin at pH 2 and 40°C for the duration of 75 min. In the second step, samples were incubated with pancreatin at pH 6.8 and 40°C during 18 h. In each series of samples, a blank was included. For precipitation of solubilized protein sulphosalicylic acid was used. Liquid medium was filtrated and precipitated materials were collected, dried and ashed. Enzymatic digestibility of organic matter was calculated based on the results of DM and CA in the sample and residue, respectively.

Determination of true metabolisable energy

The nitrogen corrected true metabolisable energy (TME_n) content of the feedstuffs was determined *in vivo* according to the procedure described by McNab and Blair (1988) using adult roosters. Each feedstuff was replicated among six roosters.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a mathematical procedure used as a central tool in exploratory data analysis (Brlek et al., 2013). It is a multivariate technique in which the data are transformed into orthogonal components that are linear combinations of the original variables. PCA is performed by Eigenvalue decomposition of a data correlation matrix (Abdi and Williams, 2010). This transformation is defined in such a way that the first component has the largest possible variance. This analysis is used to achieve maximum separation among clusters of parameters (Pezo et al., 2013). This approach, evidencing spatial relationship between processing parameters, enabled a differentiation between the different samples.

Second order polynomial model (SOP)

According to general recommendations, prior to ANN modelling analysis of variance (ANOVA) was performed, in order to check the significant effect of the input variables over the output, as well to justify the later use of ANN model by coefficient of determination (r^2). Analysis and mathematical modelling was performed using StatSoft Statistica 10.0 software (Statistica, 2010).

The SOP model was used for estimation of the main effect of the process variables on responses. The independent variables used for modelling were DM, CP, CF, CF, CA, OM, and EDOM, while TME_n was response variable. SOP model was fitted to data collected by experimental measurements:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=1}^i \beta_{ij} X_i X_j , \quad (1)$$

where: β_0 , β_i , β_{ii} , β_{ij} are constant regression coefficients; *Y* is response variable; while X_i and X_j are independent variables. The significant terms in the model were found using ANOVA for each dependent variable.

Artificial Neural Network (ANN) modelling

The database for ANN was randomly divided to: training data (60%), cross-validation (20%) and testing data (20%). The cross-validation data set was used to test the performance of the network, while training was in progress as an indicator of the level of generalization and the time at which the network has begun to over-train. Testing data set was used to examine the network generalization capability.

To improve the behaviour of the ANN, both input and output data were normalized. In order to obtain good network behaviour, it is necessary to make a trial and error procedure and also to choose the number of hidden layers, and the number of neurons in hidden layer(s). A multi-layer perceptron model (MLP) consisted of three layers (input, hidden and output). Such a model has been proven as a quite capable of approximating nonlinear functions (Hu and Weng, 2009) giving the reason for choosing it in this study. In this work the number of hidden neurons for optimal network was ten. Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm was used for ANN modelling.

Training, testing and system implementation

Table 1

After defining the architecture of ANN, the training step was initiated. The training process was repeated several times in order to get the best performance of the ANN, due to a high degree of variability of parameters. It was accepted that the successful training was achieved when learning and cross-validation curves – Sum of Squares (SOS) vs. training cycles – approached zero. Testing was carried out with the best weights stored during the training step. Coefficient of determination (r^2) and SOS were used as parameters to check the performance (i.e. the accuracy) of the obtained ANNs.

After the best behaved ANN was chosen, the model was implemented using an algebraic system of equations to predict TME_a content of feedstuffs.

Sensitivity analysis

Sensitivity analysis is a sophisticated technique which is necessary to use for studying the effects of observed input variables and also the uncertainties in obtained models and general network behaviour. Neural network were tested using sensitivity analysis, to determine whether and under what circumstances obtained model might result in an illconditioned system (Taylor, 2006). On the basis of developed ANN model, sensitivity analysis was performed in order to more precisely define the influence of processing variables on the observed outputs. The infinitesimal amount (+ 0.0001%) has been added to each input variable, in 10 equally spaced individual points encompassed by the minimum and maximum of the train data. These signals were normally distributed with a constant intensity and frequency. It was used to test the model sensitivity and measurement errors.

Results and Discussion

Results of proximate analysis, EDOM and TME_n content of poultry feedstuffs are presented using descriptive statistics in Table 1. DM, CP, CFa, CFi, CA, OM, EDOM, and TME_n varied significantly, implying that fitting of the experimental data can be performed using SOP and ANN modelling.

Principal Component Analysis (PCA)

Preliminary performed calculation for estimation of effects, using RSM of experimental data, showed that only EDOM, CFa, CFi, and CA variables influenced TME_n at sta-

Results of proximate analysis, EDOM and TME_n content of poultry feedstuffs (n = 57)								
	DM,	CP*,	CFa*,	CFi*,	CA*,	OM*,	EDOM,	TME
	%	%	%	%	%	%	%	MJ/Kg DM
Average	89.18	32.76	6.07	8.08	6.08	93.92	72.64	13.85
SD	1.81	21.88	5.62	8.84	4.38	4.38	15.51	3.58
Min	85.72	8.07	0.78	0.19	0.99	82.78	28.42	4.68
Max	93.82	75.45	20.96	34.70	14.88	99.01	95.49	18.17
Var	3.27	478.93	31.60	78.21	19.22	19.22	240.46	12.82

Results expressed on dry matter basis, SD - standard deviation, Min - minimum, Max - Maximum, Var. - variance

tistically significant level. Therefore DM, CP and OM were excluded from further calculation.

The PCA applied to the given data set has shown a differentiation between the samples according to used process parameters, and it was used as a tool in exploratory data analysis to characterize and differentiate neural network input parameters (Figure 1). As it can be seen, there is a neat separation of the observed samples according to used assays. Quality results show that the first two principal components, accounting for 82.60% of the total variability for TME_n, can be considered sufficient for data representation. CFi content, TME_n and EDOM had been more influential for the first factor coordinate calculation (accounting 27.8, 30.8 and 29.3% contribution, respectively), while CFa and CA content had been more influential for the second factor coordinate calculation (24.1 and 72.4%, respectively).

PCA (Figure 1) showed quite good discrimination between samples. Alfalfa samples are grouped at the left side of the graph, while various soya samples are grouped at the right side of the graph. Fish meal samples are located in the upper right corner, while white and yellow maize samples are placed in the lower right corner. Sunflower meal and wheaten bran are located in the middle of graph. Position of chemical analysis parameters, EDOM and TME_n is showing that EDOM and TME_n are positively correlated, while CFi and TME_n are negatively correlated, meaning that when CFi of the raw material is decreasing and EDOM is increasing, TME_n will increase. Presented influence of CFi on ME of the feedstuffs is in accordance with the results of Zhang et al. (1994) who showed that increase in neutral detergent fibre



Fig. 1. Biplot graph of feedstuffs for broilers with the results of proximate analysis, EDOM and TME_n content

reduces ME of barley samples. Palic et al. (2012) proposed linear equations for predicting TME_n using 23 samples of complete diets and the same combination of feedstuffs used in this study, and they also showed that TME_n is in positive correlation with EDOM. However, proposed linear equations had considerably poor prediction of experimental data (r^2 ranged from 0.689 to 0.844).

Analysis of variance and SOP models

Analysis of variance (ANOVA) was conducted for obtained SOP model, and output were tested against the impact of input variables (Table 2). Analysis revealed that linear, quadratic, as well as interchange terms considerably influenced forming of SOP model for TME_n calculation.

According to ANOVA results, TME_n was mostly affected by quadratic term of EDOM, which was statistically significant at p < 0.05 limit. Linear term of CFa was also very influential, as well as quadratic terms of CA and CFi content (all these terms were statistically significant at p < 0.05 limit). Nonlinear, interchange terms of CFi × EDOM and CFa × CFi were also statistically significant, showing the importance of CFa, CFi and EDOM in TME_n calculation. Most of statistically significant terms in SOP calculation are of non-linear nature, which leads to the conclusion that using ANN calculation would improve the validity of the model.

Table 2				
Analysis of variance ((ANOVA)	of feedstuffs	for	broilers

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Factor	df	SOS	F	р
CFa	1	11.99*	12.87	0.00
CFa ²	1	0.89	0.95	0.33
CFi	1	1.09	1.17	0.29
CFi ²	1	7.90^{*}	8.48	0.01
CA	1	0.00	0.00	0.96
CA^2	1	8.37*	8.99	0.00
EDOM	1	0.18	0.19	0.66
EDOM ²	1	22.72*	24.40	0.00
CFa × CFi	1	4.46*	4.79	0.03
CFa × CA	1	2.24	2.40	0.13
CFa × EDOM	1	1.47	1.58	0.22
$CFi \times CA$	1	0.39	0.42	0.52
$CFi \times EDOM$	1	13.05*	14.01	0.00
$CA \times EDOM$	1	0.86	0.92	0.34
Error	42	39.11		
r ²		0.936		

*Significant at p<0.05 level, 95% confidence limit

df - degrees of freedom, SOS - sum of squares, F - F test value

The residual variance, marked as 'Error' in Table 2, presents the model disagreement with the experimental values i.e. contributions of terms that are not described in the SOP model. Developed model showed statistically insignificant deviation from the experimental values of the model, which confirmed their suitability. Therefore, it was confirmed that obtained model was statistically significant and in agreement with experimental results.

Neurons in the ANN hidden layer

All variables considered in the RSM, were also used for the ANN modelling. Determination of the appropriate number of hidden layers and number of hidden neurons in each layer is one of the most critical tasks in ANN design. The number of neurons in a hidden layer depends on the complexity of the relationship between inputs and outputs. As this relationship becomes more complex, more neurons should be added (Curcic et al., 2014).

The optimum number of hidden neurons was chosen upon minimizing the difference between predicted ANN values and desired outputs, using Sum of Squares (SOS) during testing as performance indicator. Used multi-layer perceptron models (MLPs) were marked according to StatSoft Statistica's notation. MLP was followed by number of inputs, number of neurons in the hidden layer, and the number of outputs. According to ANN performance (Table 3), it was noticed that the optimal number of neurons in the hidden layer for TME_n calculation was 10 (network MLP 4-10-1), when obtaining high values of r^2 (0.983 for ANN during training period, compared to 0.936 for SOP model) and low values of SOS.

Simulation of the ANN

Optimal network, used for prediction of TME_n was able to predict reasonably well the output for a broad range of the process variables (coefficients of determination reached 0.983 for TME_n prediction). The predicted values were very close to

Table 3	
Performance of the optimal ANN	

Network name	MLP 4-10-1				
Training	Testing	Validation			
$r^2 = 0.9834$	$r^2 = 0.9605$	$r^2 = 0.9109$			
Training error	Testing error	Validation error			
0.0012	0.0060	0.0046			
Training algorithm: BFGS 52					
Error function: SOS					
Hidden activation: Exponential					
Output activation: Exponential					

the experimental (target) values in most cases, in terms of r^2 value for both SOP and ANN models.

It can be seen that the r^2 value for ANN model is greater than this associated with the SOP model. This agrees with Perai et al. (2010) who compared different statistical approaches for prediction of ME of meat and bone meal. These authors obtained the highest r^2 value (0.94) when ANN was used. Generally, ANN model is more complex (61 weights-biases for ME calculation) than SOP, and it has performed better fitting of experimental data due to the high nonlinearity of the developed system (Kalovic et al., 2013; Chattopadhyaya and Rangarajana, 2014) (Figure 2).

The mean and the standard deviation of residuals have also been analysed. The mean of residuals for ANN model was -0.143, while the standard deviation of residuals was 0.897. These results showed a good approximation to a normal distribution around zero with a probability of 95% ($2 \times$ SD), which means a good generalization ability of ANN model for the range of observed experimental values.

Sensitivity analysis

In order to or assess the effect of changes in the outputs due to the changes in the inputs, a sensitivity analysis was performed. The greater effect observed in the output imply that greater sensitivity is presented with respect to the input (Pezo et al., 2013). Sensitivity analysis has been performed to test an infinitesimal change in an input value in 10 equally spaced individual points, ranged by the minimum and maximum of the observed assay, in order to explore the changes in observed outputs. It is also used to test the model sensitivity and measurement errors (Figure 3).



Fig. 2. Comparison of experimentally obtained TME_n with ANN and SOP predicted values

20 □CFa ■ CFi 15 Δ ▲ CA ۸ 10 ∆EDOM Δ Δ П ₪ п 5 Δ Δ 0 ŵ -5 Δ -10 -15 Min Max Inputspace



The influence of the input over the output variables, i.e. calculated changes of output variables for infinitesimal changes in input variables, is shown on Figure 3. Obtained values corresponded to level of experimental errors, and also showed the CFa, CFi, CA, and EDOM influence on TME, According to Figure 3, TME, was mostly influenced by EDOM, which was also confirmed by ANOVA analysis of SOP model and PCA analysis.

Sensitivity analysis is used to show the influence of the inputs, but it also shows the importance of an input variable at a given point in the input space (Saltelli, 2010). As can be seen from Figure 3 that TME_n was affected more strongly at infinitesimal changes of average EDOM values, while the influence of CFi and CA content was more observable at the minimum of input range. The influence of CFa remained the same through the whole input range. These findings are in accordance with PCA and ANOVA analysis, as well as with experimental measurements.

Conclusion

This paper presents the influence of crude protein, crude fat, crude fibre, ash and enzymatic digestible organic matter on prediction of true metabolisable energy of feedstuffs for broilers. The observed samples were characterized by the results of proximate analysis, EDOM and in vivo TME_ content of poultry feedstuffs. SOP and ANN-based models were developed for prediction of TME_n for a wide range of input variables. Both models are easy to implement and could be effectively used for predictive purposes, modelling and optimization. As compared to RSM, ANN model yielded a better fit of experimental data. Taking into account that a considerable amount and wide variety of data were used in the present work to obtain the ANN model, and considering that the model turned out to yield a sufficiently good representation of the experimental results, it can be expected that it will be useful in practice.

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