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Full Length Research Paper

Modelling the association between *in vitro* gas production and chemical composition of some lesser known tropical browse forages using artificial neural network

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***In vitro* gas production of four different browse plants (*Azadirachta indica, Terminalia catappa, Mangifera indica and Vernonia amygdalina*) was investigated under different extractions. The relationship between the forage composition parameters (dry matter, organic matter, crude protein, acid detergent fibre, neutral detergent fibre and acid detergent lignin), process parameters (extraction mode and incubation time), and volume of gas production were modelled with artificial neural network (ANN). The ANN model consisted of simple, multi-layered, back-propagation networks with eight input neurons consisting of the composition and process parameters and one output neuron for the gas volume. The networks were trained with different algorithms and varying number of layer and neuron in the hidden layer to determine the optimum network architecture. The network with single hidden layer having 45 'tangent sigmoid' neurons trained with Livenberg-Marquard algorithm combined with 'early stopping' technique was found to be the optimum network for the model with R-value: mean = 0.9504; max. = 0.9618; min. = 0.9343; and std. = 0.0059. The influence of each chemical composition and processing parameters on gas production was simulated. The developed ANN model offers a more cost and time efficient strategy in feed evaluation for ruminant animals**.

Key words: *In vitro* gas production, browse plants, extraction, artificial neural network.

INTRODUCTION

Poor nutrition has long been identified as one of the major constraints to livestock productivity in developing countries (Osuji et al., 1993). This is due to the fact that animals are fed predominantly on low quality feeds, which are deficient in nutrients essential for efficient microbial fermentation (Bamikole and Babayemi, 2004). These deficiencies in the feed quality are mainly reflected in low voluntary intake, digestibility and matabolisable energy in livestock (Babayemi and Bamikole, 2006a). Consequently, both growth and milk yield are grossly below the normal production. In ruminant production systems, the understanding of the factors which affect rumen degradability of forage feeds and microbial protein production is essential to feed scientists in formulating diets that will be utilised more efficiently. The responses or performance of feeds is mainly dependent to its degradability and chemical composition (Nherera et al., 1999). *In vitro* gas production technique is commonly used to determine the relationship between chemical composition, incubation time and digestibility of forage feeds (Tolera et al., 1997; Larbi et al., 1998). This method has been reported to be less expensive, time consuming and allows for proper control experimental conditions as compared with *in vivo* trials and has been wisely used to predict dry matter intake (Blummel and Qrskov, 1993), digestibility (Khazaal et al., 1993) and metabolisable energy (Babayemi and Bamikole, 2006b).

In feed evaluation, many empirical models had been formulated relating the gas production to other feed parameters (Beuvink and Kodut, 1993; Pell and Schofield,

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1993; Pitt and Pell, 1997). Nherera et al. (1999) employed correlation and simple and multiple regression analysis to assess connections that coexist between *in vitro* gas production and chemical composition. As important as these models are in feed evaluation, the complexities associated with detailed and comprehensive study of the various factors affecting gas production are still limiting for thorough interpretation of data and application to feed nutritionist. In the present paper, we pro-posed a new approach using artificial neural network (ANN) to model the complex relationships between the volume of gas production and chemical composition of some selected tropical browse forages (*Azadirachta indica, Terminalia catappa, Mangifera indica and Vernonia amygdalina*).

MATERIALS AND METHODS

Artificial neural networks

An application of artificial neural network (ANN) is becoming increasingly popular in modelling of complex system parameters. ANN is essentially a "black box" approach, in which complex relationships of system parameters can be correlated without full understanding of the system mechanisms. ANN models have been found to demonstrate excellent self-organization and generalization capabilities, which make them better alternative to conventional statistical method like multiple regressions in modelling of systems with complex and non-linear variables (Picton, 2000). ANN is a parallel processing architecture in which knowledge is represented in the form of weights between a set of highly connected processing elements (PE), which are called nodes or neurons, which consists generally of five basic components: (1) input, (2) weight and biases, (3) summing junction, (4) transfer function, and (5) output. ANN is designed similar to the biological neuron to mimic the functions of the human brain in learning, assimilation and reproduction of knowledge. In neural networks, knowledge is acquired during the training or learning process by updating or adjusting the weights in the network through different algorithms. The network weights are upgraded literary until the network reproduces the desired output or target from a given set of input. The network is trained with either supervised learning when both input and the desired targets are presented to the network or unsupervised learning when the expected targets is not used in the training. The back-propagation algorithm is a supervised training rule with multiple-layer networks, in which the network weights are moved along the negative of the gradient of the mean squared error (MSE) so as to minimize the difference between the network's output and the desired target. There are generally four steps in the training process: (1) assembling the training data, (2) designing the network object, (3) training of the network, and (4) simulation of the network response with new input data sets. After a sufficient training session, which may require considerable computational resources such as memory and time of the computer, the trained network has adequate capabilities to perform non-linear pattern association between input and output variables and can easily predict the output when a new input data set that is not used in the training is presented to the network. ANN models are known to be efficient and less time-consuming in modelling of complex systems compared to other known mathematical models. They are particularly used in real time or on-line monitoring of complex system variables (Bishop, 1995; Patterson, 1996; Picton, 2000).

Collection of browse shrubs

The shrubs (*A. indica, T. catappa, M. indica* and *V. amygdalina*) were obtained from the botanical garden of the University of Ibadan after being identified by the technical team of the Department of Botany and Microbiology of the same University. Known weights of the leaves from the various shrubs were taken for the determination of dry matter by oven drying at 65°C until a constant weight was obtained.

Chemical analysis

Crude protein, crude fibre, ether extract and ash contents of the leaves were determined according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assessed according to VanSoest et al. (1991).

In vitro gas production

Rumen fluid was obtained from three West African dwarf female goats. The method for collection was as described by Babayemi et al. (2006) using suction tube from goats were previously fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% *Panicum maximum* at 5% body weight.. The rumen liquor was collected into the thermo flask that had been pre-warmed to a temperature of 39ºC from the goats before they were offered the morning feed. Incubation procedure was as reported by Menke and Steingass (1988) using 100 ml calibrated transparent plastic syringes with fitted silicon tube. The sample weighing 200 mg (n = 3) was carefully dropped into the syringes and thereafter, 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (Tables 1 and 2). The rumen liquor and buffer were in 1:4 (v/v) respectively. The mixture was handled under continuous flushing with CO_2 and immediately dispensed using another 50 ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 39 ±1ºC and the volume of gas production was measured at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Extraction of the leaves for saponin, phenols and steroids

Saponin, phenols and steroids were determined as reported by Babayemi and Bamikole (2006). Briefly, 2 g each of leaf of the shrubs were extracted with 30 ml of petroleum ether (PE) and 25 ml of methanol water (MW, 9/1, v/v). The mixture was shaken at 250 revolutions per minute for 1½ h, filtered and separated by a funnel. The lower (MW) and upper layers were emptied into 50 ml volumetric flasks. From the MW fraction, 1.67 ml was dispensed in 9 ml distilled water, filtered and out of it; 1 ml was taken into a test tube. The test tube was shaken for 30 s and left to stand for 15 min. Saponin content was evaluated from the height of the foam layer as negative (< 5 mm), low (5 – 9 mm), medium (10 – 14 mm) and high (> 15 mm). For phenol analysis, 1 ml from the MW fraction was dispensed into bottles (n = 5) with 1% iron III chloride (w/v) added at 0.6 ml. Phenols form complexes with ferric iron, resulting in a blue solution and hence, their presence was scored as: no phenols (no colour change), hydrolysable (dark-blue) and condensed tannins (dark-green). For steroids, 10 ml from PE fraction was evaporated in a water bath at 45ºC and 0.5 ml chloroform; 0.25 ml acetic anhydride and 0.125 ml concentrated tetraoxosulphate IV were added. The mixture was agitated briefly and the colour reac-

Table 1. Chemical composition (g/100 g DM) of browse forages.

Nutrient composition	Forage species			
	Azadirachta indica	***Terminalia catappa***	***Mangifera indica***	***Vernonia amygdalina***
Dry matter	37.1	29.4	46.5	16.8
Organic matter	94.0	91.5	89.0	88.0
Crude protein	14.8	11.6	10.5	21.8
Neutral detergent fibre	9.5	26.0	15.5	5.0
Acid detergent fibre	34.5	38.5	37.0	45.0
Acid detergent lignin	33.3	6.5	23.0	48.9

Table 2. Constituents of McDougall's buffer solution for the *in vitro* gas production.

Compound	g/l
Sodium bicarbonate ($NaHCO_2$)	9.8
Sodium phosphate dibasic (Na_2HPO_4)	2.77
Potassium chloride (KCl)	0.57
Sodium Chloride (NaCl)	0.47
Magnesium sulphate ($MgSO_4.7H_2O$)	0.12
Calcium chloride ($CaCl_2.2H_2O$)	0.16
Urea (NH_3O)	1.0

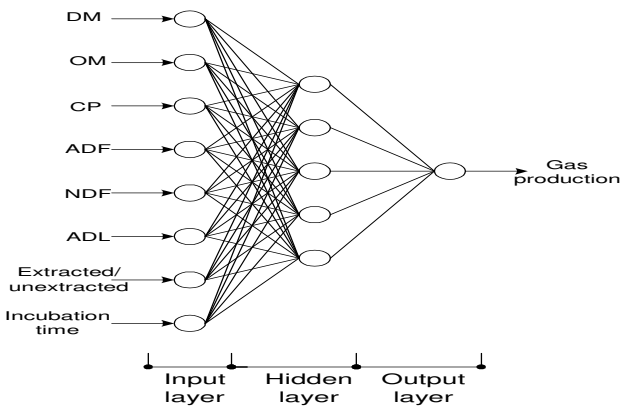


Figure 1. The multilayer network architecture to predict *in vitro* gas production.

tion was assessed being steroids (blue or green), triterpenoids (read, pink or purple) or saturated steroids (light yellow).

Design and statistics

Multi-layer feed-forward back-propagation hierarchical networks with different architecture were designed using the MATLAB Neural Network Toolbox (Howard and Beale, 2000). The networks consist of three layers: input layer; hidden layer; and output layer (Figure 1). There are eight input parameters to the network, which consist of the chemical composition parameters of the browse plants (dry matter, organic matter, crude protein, acid detergent fibre, neutral detergent fibre, and acid detergent lignin), processing mode and incubation time; and one output parameter corresponding to the volume of the gas produced. Different networks with single or double hidden layer topologies were used and the number of neurons was varied from 5 to 45, at interval of 5 neurons to enhance the generalisation capability of the network. Neurons with tangent sigmoid (tansig) transfer function (Equation 1) were used in the hidden layer, linear transfer function (purelin) was used in the output layer.

$$f(z) = \frac{1}{1 + e^{-z}} \qquad (1)$$

Where z is the weighed sum of the input.

In order to train the networks, experimental data acquired during the *in vitro* gas production of the four browse plants (A. *indica, T. catappa, M. indica and V. amygdalina*) were used. The chemical composition of the browse plants, processing mode (extracted or unextracted) and the incubation time (2 to 24 h) were used as input dataset, while the gas production was used as target dataset. A total of 192 input/target datasets were acquired from the experiments. Since neural network requires numerical input, the processing mode was coded with numerical values. Thus, 1 and 0 were used for extracted and unextracted samples respectively. Prior to the training, both input and target datasets were scaled so that they fall approximately in the range (-1, 1). Standard back-propagation training algorithms used in the study were scaled conjugate gradient (SCG) and Levenberg-Marquardt (LM).

In order to avoid 'overfitting' of the data and hence improve generalisation of the network the '*early stopping'* technique was used in conjunction with the training algorithms. The input/target datasets was divided randomly into three subsets: training; validation; and test datasets. The training set, which consists of half of the dataset, was used for computing the gradient and updating the network weights and biases, while one-quarter of the dataset was used as validation and test set respectively. The error on the validation set was monitored during the training process. The training was stopped when the network began to overfit when the data and the validation error began to increase. The maximum number of 100 iterations and minimum gradient of 10^{-10} was used in the training process. The performance of the different networks with variant architecture and training algorithms are evaluated based on the correlation coefficient (R-value) between the predicted and the experimental values. The R-value is a measure of how well the variation in the output is explained by the targets. For perfect correlation between targets and outputs the number is equal to 1.

RESULTS AND DISCUSSION

Artificial neural network performance

Table 3 shows the statistics of the performance of the networks for 30 different trials, where different random

Table 3. Statistics of the R-values of network performance for the training and entire dataset.

Training algorithm	No of hidden layer	No. of neurons in hidden layer	Correlation coefficient (R-value)							
			Training dataset				Entire dataset			
			Mean	Max	Min	Standard deviation	Mean	Max	Min	Standard deviation
Levenberg-Marquardt (LM)	1	5	0.9623	0.9911	0.8683	0.0270	0.9307	0.9526	0.8136	0.0271
		10	0.9738	0.9944	0.8444	0.0340	0.9438	0.9613	0.8656	0.0225
		15	0.9748	0.9938	0.8736	0.0261	0.9411	0.9567	0.8864	0.0195
		20	0.9774	0.9940	0.8908	0.0206	0.9458	0.9599	0.8774	0.0164
		25	0.9818	0.9937	0.9292	0.0147	0.9440	0.9582	0.9009	0.0146
		30	0.9749	0.9938	0.9076	0.0226	0.9410	0.9571	0.8579	0.0203
		35	0.9777	0.9950	0.8953	0.0230	0.9449	0.9602	0.9065	0.0126
		40	0.9778	0.9927	0.9419	0.0169	0.9407	0.9594	0.8831	0.0204
		45	0.9853	0.9961	0.9627	0.0088	0.9504	0.9618	0.9343	0.0059
	2	5	0.9540	0.9941	0.5634	0.0864	0.9207	0.9569	0.5090	0.0868
		10	0.9724	0.9968	0.8935	0.0275	0.9349	0.9617	0.8376	0.0295
		15	0.9768	0.9970	0.8895	0.0210	0.9394	0.9575	0.8474	0.0222
		20	0.9635	0.9986	0.6630	0.0644	0.9302	0.9549	0.7100	0.0511
		25	0.9779	0.9963	0.8433	0.0306	0.9404	0.9610	0.7912	0.0310
		30	0.9808	0.9976	0.9344	0.0176	0.9438	0.9624	0.8927	0.0173
		35	0.9613	0.9968	0.8033	0.0508	0.9259	0.9592	0.7699	0.0475
		40	0.9690	0.9975	0.7652	0.0458	0.9363	0.9560	0.7810	0.0334
		45	0.9680	0.9974	0.7963	0.0476	0.9319	0.9602	0.7918	0.0439
Scaled conjugate gradient (SCG)	1	5	0.9368	0.9815	0.7876	0.0448	0.9110	0.9520	0.8410	0.0306
		10	0.9535	0.9889	0.8735	0.0307	0.9210	0.9488	0.8802	0.0227
		15	0.9417	0.9889	0.8071	0.0434	0.9115	0.9490	0.7856	0.0367
		20	0.9423	0.9860	0.8540	0.0399	0.9122	0.9530	0.8254	0.0313
		25	0.9507	0.9903	0.8228	0.0372	0.9186	0.9512	0.8451	0.0302
		30	0.9425	0.9888	0.7476	0.0534	0.9129	0.9491	0.7411	0.0455
		35	0.9398	0.9892	0.7372	0.0576	0.9103	0.9498	0.7420	0.0457
		40	0.9659	0.9895	0.8624	0.0282	0.9312	0.9518	0.8590	0.0216
		45	0.9483	0.9892	0.8040	0.0386	0.9170	0.9517	0.8110	0.0319
	2	5	0.9161	0.9800	0.3704	0.1104	0.8909	0.9483	0.3274	0.1109
		10	0.9607	0.9899	0.9211	0.0212	0.9270	0.9517	0.8869	0.0183
		15	0.9373	0.9897	0.6590	0.0641	0.9087	0.9514	0.7024	0.0507
		20	0.9693	0.9912	0.8761	0.0236	0.9359	0.9524	0.9023	0.0130
		25	0.9494	0.9889	0.6474	0.0659	0.9153	0.9521	0.6684	0.0552
		30	0.9585	0.9915	0.7682	0.0511	0.9248	0.9516	0.7814	0.0421
		35	0.9548	0.9915	0.8327	0.0381	0.9198	0.9514	0.8327	0.0340
		40	0.9642	0.9904	0.8775	0.0286	0.9300	0.9510	0.8523	0.0244
		45	0.9645	0.9906	0.8687	0.0299	0.9303	0.9522	0.8596	0.0228

initial weights were used in each trial, for the training and entire datasets. The performance analysis showed that the networks investigated had elevated generalisation capacity. The fastest learning was obtained with SCG algorithm, but the performance was subordinate when compared to LM. The network with 45 neurons in a single hidden layer trained with the LM, which provided the most accurate prediction (that is, with R-value: mean = 0.9504; max. = 0.9618; min. = 0.9343; and std. = 0.0059), was chosen for the model. Figure 2 compares the values obtained from the experimental and the predicted *in vitro* gas production volume from ANN model using test data-

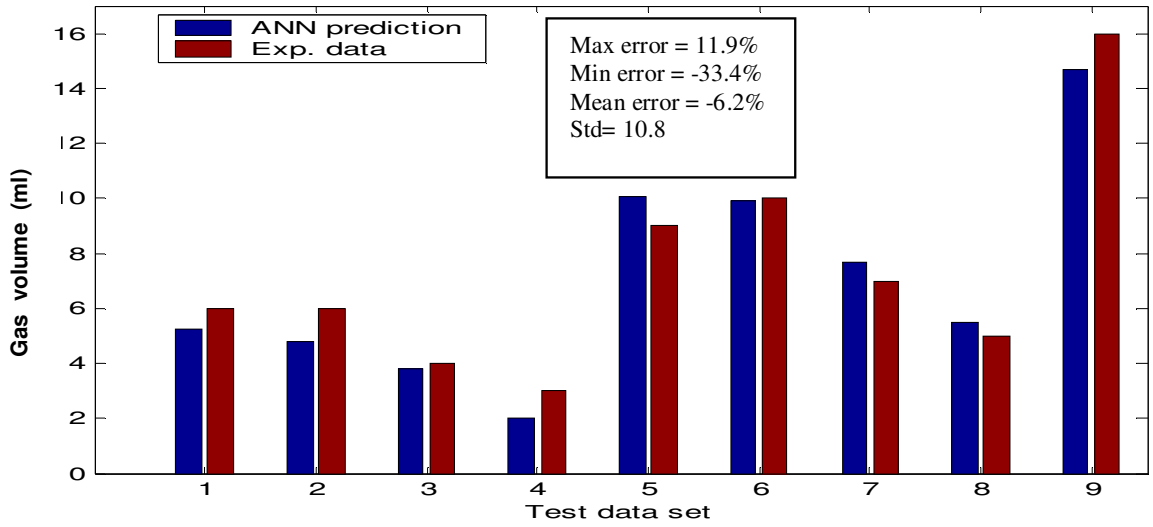


Figure 2. Comparison between the experiment data and the neutral network preditions.

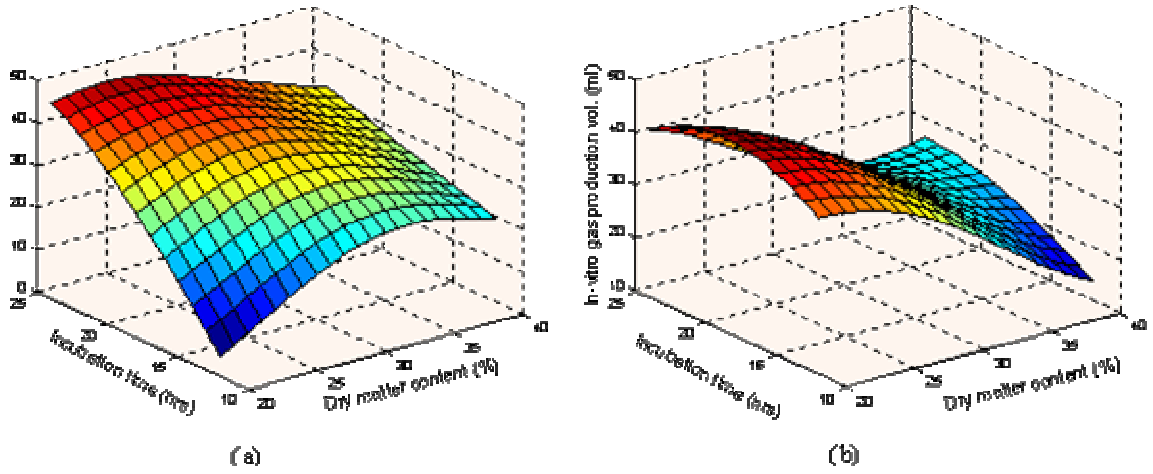


Figure 3. Effect of dry matter content and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at OMC=94%, CP=14.8%, ADF=34.5%, NDF=9.5% and ADL=33.3%.

set that were not used during the training process.

Artificial neural network simulation

The system's parameter interactions involved in the *in vitro* gas production are known to be complex and non-linear. The ANN model allows for the analysis of the effect of any of the variables involved in the system. The relative influence of each parameter (chemical: dry matter content, organic matter content, crude protein, acid detergent fibre, neutral detergent fibre and acid detergent lignin; and process: extraction mode and incubation time) on volume of gas produced were simulated with the ANN model keeping others parameters constant. The effect of the system parameters on gas production are shown in 3D plots in Figures 4 to 6

Effect of dry matter content

Figure 3 shows the influence of dry matter content (from 20 to 40%) on the *in vitro* gas production at constant condition (OM = 94%; CP = 14.8%; ADF = 34.5%; NDF = 9.5%; ADL = 33.3%) for both extracted and unextracted forage of the browse plants. It can be observed that gas production increased significantly in accordance with the incubation time, while gas production tended to increase with dry matter content (from 20 to 30%) and then decreased with further increase in dry matter content above

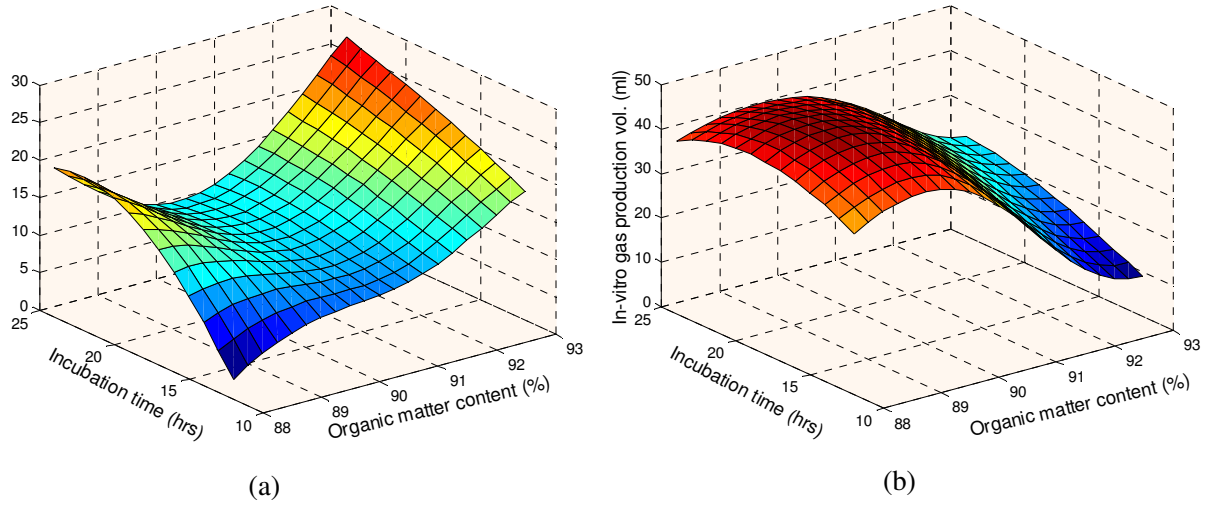


Figure 4. Effect of organic matter content and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at DMC=40%, CP=14.8%, ADF=34.5%, NDF=9.5% and ADL=33.3%.

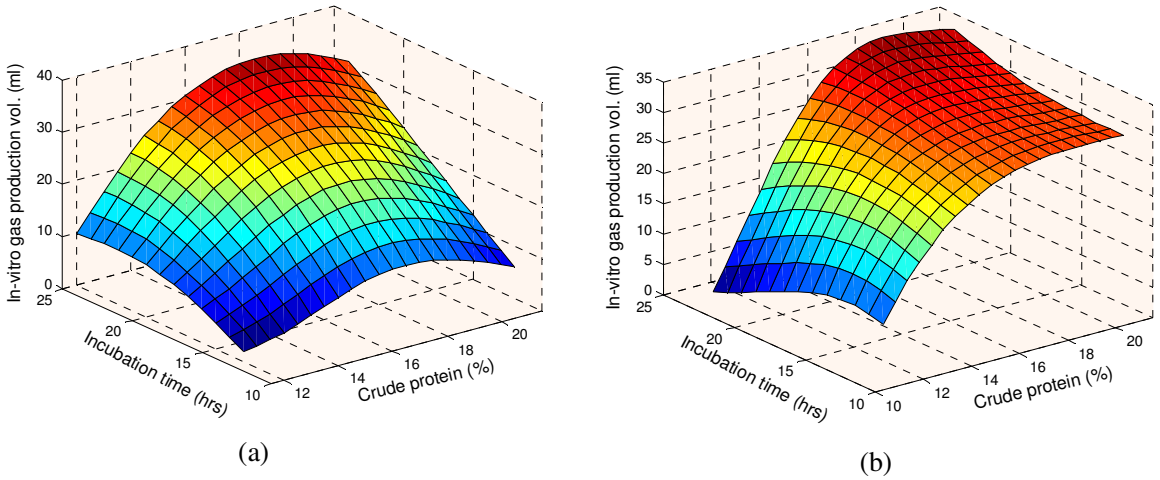


Figure 5. Effect of crude protein and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at DMC = 30%, OMC=92%, ADF = 34.5%, NDF = 9.5% and ADL = 33.3%.

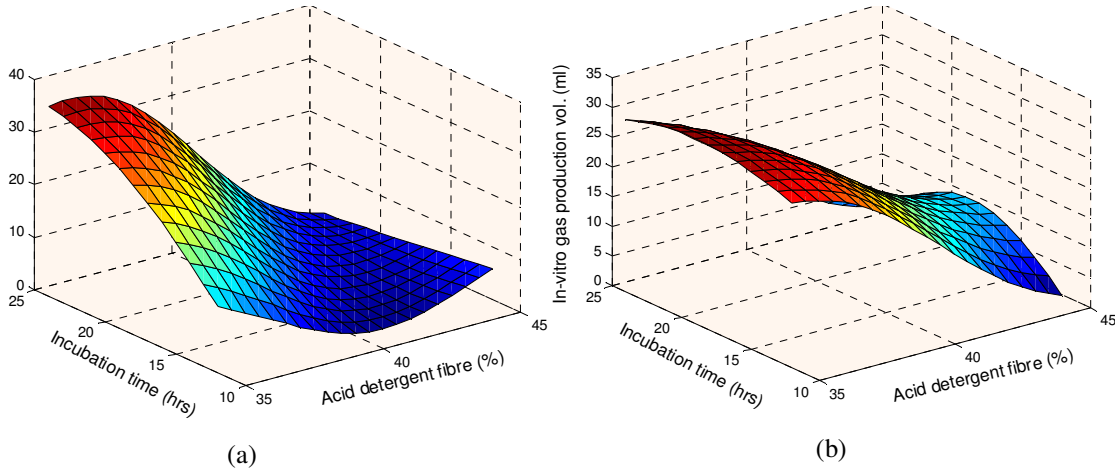
30% for extracted sample (Figure 3a). For unextracted sample (Figure 3b), incubation time had no significant effect on gas production, while increase in dry matter content showed a remarkable tendency to reduction in gas productions.

Effect of organic matter content

The effect of organic matter content and incubation time on the *in vitro* gas production is shown in Figure 4. In Figure 4a, for extracted sample, the gas production increased with incubation time and organic matter content at lower values of incubation time, while at higher values, gas production assumed a noticeable decrease with organic matter (from 88 to 90%) and invariably amplified with further increase in organic matter above 90%. In Figure 4b, a different trend was observed for unextracted sample; the gas production remained somewhat invariable with incubation time and organic matter (from 88 to 90%) and diminished exponentially with further augmentation in organic matter exceeding 90%.

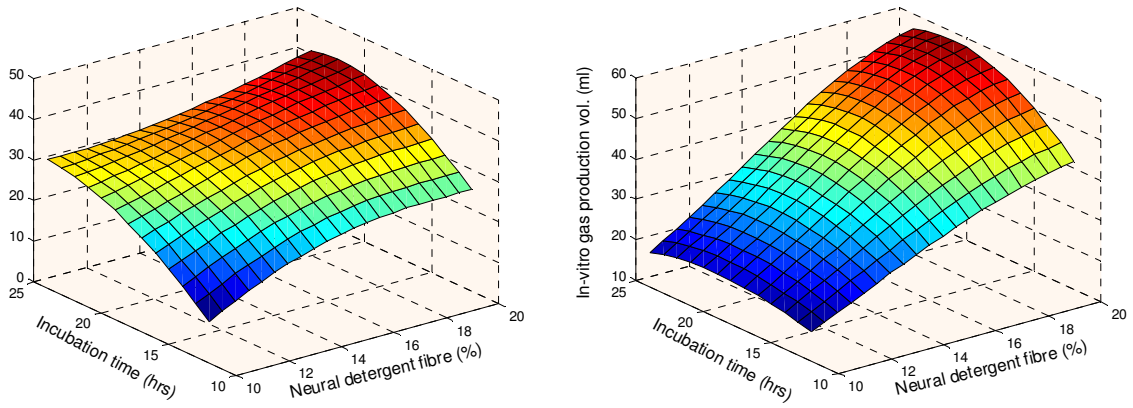
Effect of crude protein

The crude protein content varied over the range of 12 to 21% and the effect on the *in vitro* gas production for extracted and unextracted samples are shown in Figure 5. From Figure 5a, it was conspicuously noticed that the



(a) (b)

Figure 6. Effect of acid detergent fibre and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at DMC=30%, OMC=92%, CP=15%, NDF=9.5% and ADL=33.3%.



(a) (b)

Figure 7. Effect of neural detergent fibre and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at DMC = 30%, OMC = 92%, CP = 15%, ADF = 35% and ADL = 33.3%.

amount of gas produced increased slightly with incubation time and crude protein. The artificial neural net work revealed that the gas production and crude protein maintained a close relationship. From 12 to 15% crude protein, for example, there was a persistent increase in gas production and then decreased with further increase in crude protein above 15%. The variation in gas production became more pronounced at higher incubation time and crude protein. This trend suggests an optimum crude protein of 15% for extracted forage digestion. For unextracted sample (Figure 5b) a slight reduction in gas production was observed with increase in incubation time, while gas production tended to increase exponenttially with crude protein.

Effect of acid detergent fibre

Figure 6 depicts the *in vitro* gas production responses to variation in acid detergent fibre. Figure 6a showed that for extracted forage, gas production increased rapidly with incubation time at lower values of acid detergent fibre (ADF), while at higher values of ADF gas production remained constant. For the unextracted forage, as shown in Figure 6b, the gas production remained constant with incubation time, while gas production decreased with higher acid detergent fibre.

Effect of neutral detergent fibre

The influence of neutral detergent fibre (NDF) on *in vitro* gas production is presented in Figure 7. For extracted forage, gas production increased generally with the presence of NDF and incubation time (Figure 7a). In Figure 7b for unexctracted forage, gas production increased with neutral detergent fibre, while gas production remained the same with incubation time at lower values

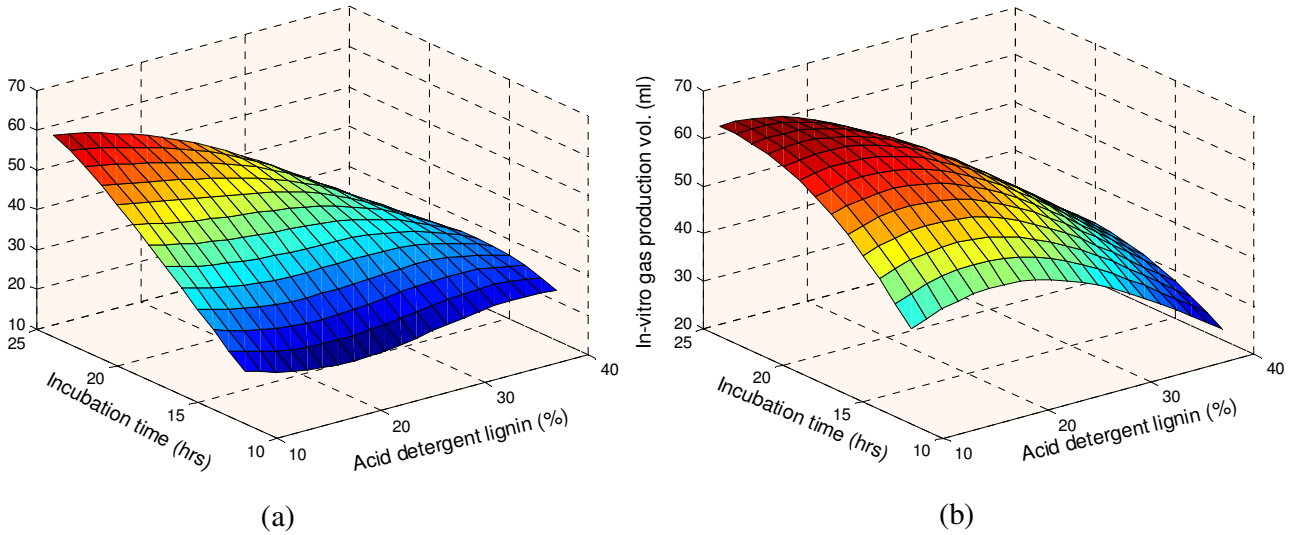


Figure 8. Effect of acid detergent lignin and incubation time on *in vitro* gas production for extracted (a) and unextracted forage at DMC = 30%, OMC = 92%, CP = 15%, ADF = 35% and NDL = 15%.

of NDF. The gas production however, was noticed to increase tremendously at higher values of NDF.

Effect of acid detergent lignin

Figure 8 indicates the influence of acid detergent lignin (ADL) on *in vitro* gas production. Gas production was increasing with incubation time at lower values of ADL and remained fairly constant at higher values for both extracted (Figure 8a) and unextracted forage (Figure 8b). Increase in ADL showed no significant effect on the *in vitro* gas production for extracted forage (Figure 8a). There was a slight increase in gas production with increasing ADL (from 10 to 20%) while a reduction with further increase in ADL above 20% was observed for unextracted forage (Figure 8b).

Conclusion

Prediction of *in vitro* gas productions of the four browse plants showed that the multilayer network with single hidden layer having 45 'tangent sigmoid' neurons trained with Livenberg-Marquard algorithm combined with 'early stopping' technique was found to be the optimum network for the model with R-value: mean = 0.9504; max. = 0.9618; min. = 0.9343; and std. =0.0059. The interrelationships between the chemical composition and the processing parameters investigated were found to be complex and non-linear. Characteristic differences were observed in the gas production for extracted and unextracted forages. The model offers a more cost and time efficient strategy in feed evaluation for ruminant animals.

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