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Comparison of Metabolizable Energy Values of Roughages Determined by Regression Equations Using *in vivo* and *in vitro* Parameters

Hulya Ozkul, Yilmaz Sayan, Muazzez Polat and Tulug Capci
Department of Animal Science, Faculty of Agriculture, Ege University, Turkey

Abstract: The aim of the study was to compare the Metabolizable Energy (ME) values of some roughages which were calculated from regression equations using *in vivo* and *in vitro* parameters and to determine the most suitable regression equations using *in vitro* parameters. For this purpose, *in vivo* ME values and *in vitro* Crude Nutrients (CN), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), enzyme soluble/insoluble organic matter (ELOS/EULOS) and 24 h Gas Production (GP) amounts of 40 roughages were used. In the study, *in vitro* ME values of roughages were calculated by regression equations where *in vitro* parameters were used and these results were compared with their *in vivo* ME values. According to the obtained results, *in vitro* ME values were estimated more better with equations where, NDF or especially ADF was used than with equations where, CN or ADL was used. Besides, the prediction was improved in regression equations which were constituted by the combination of crude nutrients and ELOS/EULOS or combination of crude nutrients and GP. However, it can be suggested that, using of ADF instead of Crude Fiber (CF) in equations would be more correct.

Key words: Roughages, metabolizable energy, regression equations

INTRODUCTION

Regression equations based on digestible crude nutrients determined by expensive and time consuming *in vivo* classic digestion trials have been accepted as the most reliable equations and are still in use to predict ME values of roughages[1,2]. However, feed values of roughages and feedstuffs such as industry by-products are very variable. So it is important that parameters of regression equations used for determining ME values of these feeds should be reliable, economical and practical. Therefore, researchers have been intensively studying to generate regression equations by using some *in vitro* parameters and have been suggesting some regression equations[3-6].

The aim of this study was to compare ME values of some roughages which were calculated from regression equations by using *in vivo* and *in vitro* parameters and to determine the most suitable regression equations using some *in vitro* parameters.

MATERIALS AND METHODS

In this study, *in vivo* and *in vitro* parameters of 40 roughages (10 Maize Silage: MS, 10 Alfalfa Hay: AH, 10 Grass Hay: GH, 10 Wheat Straw: WS) determined by Sayan *et al.*[7], were used (Table 1 and 2). This data have been conducted in Ege University, Agriculture Faculty, the chemical and biological analyses units between 2000-2003 years.

Table 3 shows various regression equations used to calculate *in vitro* ME values of experimental roughages. All data were subjected by using SAS package programme. When significant differences occurred, Duncan test was used to compare means[12].

RESULTS AND DISCUSSION

In the first step of this study, the ME values of roughages were determined by using CN, NDF, ADF, ADL, ELOS/EULOS and GP amounts with some regression equations where these parameters were used (Table 4).

As given in Table 6, ME_1, ME_2, ME_3, ME_4, ME_5 and ME_6 results were found 8.08-10.46, 8.81-10.37, 8.42-11.05, 7.70-10.06, 6.78-9.89 and 7.83-9.58 MJ/kg DM for MS; 7.85-8.79, 9.74-11.32, 8.71-10.26, 6.56-8.66, 8.44-9.25 and 8.49-9.41 MJ/kg DM for AH; 6.55-8.42, 8.15-9.46, 7.45-9.34, 6.95-9.22, 7.04-8.94 and 6.92-8.89 MJ/kg DM for GH; 5.56-6.78, 7.21-8.03, 5.83-7.17, 6.59-8.37, 6.48-7.99 and 6.33-7.60 for WS MJ/kg DM for WS, respectively. In the

Corresponding Authors: Dr. Hulya Ozkul, Feeds and Animal Nutrition Unit, Department of Animal Science, Faculty of Agriculture, University of Ege, Bornova 35100, Izmir, Turkey
E-mail: ozkul@ziraat.ege.edu.tr

Table 1: *In vivo* ME values and crude nutrient amounts of roughages

Feeds	ME_{DCN}, (MJ kg^{-1} DM)	DM (g kg^{-1})	CA (g kg^{-1} DM)	CP	EE	CF	Feeds	ME_{DCN}, (MJ kg^{-1} DM)	DM (g kg^{-1})	CA (g kg^{-1} DM)	CP	EE	CF
MS 1	9.07	349.2	103.1	59.0	34.9	184.4	GH 1	8.79	890.0	99.9	93.7	15.7	318.4
MS 2	10.37	355.4	56.8	69.2	30.7	176.7	GH 2	7.99	899.6	119.9	102.5	17.3	315.0
MS 3	9.48	290.3	76.5	87.2	28.6	219.8	GH 3	8.88	902.6	99.3	91.1	20.4	275.3
MS 4	9.44	297.7	84.6	80.6	27.5	208.6	GH 4	9.06	915.9	96.0	91.6	23.1	262.3
MS 5	10.40	192.9	81.4	96.4	36.8	267.0	GH 5	7.26	922.7	93.1	66.3	11.7	362.1
MS 6	10.13	309.7	73.3	61.3	27.4	217.0	GH 6	9.78	917.6	77.6	91.3	17.3	318.1
MS 7	10.38	327.5	64.7	81.8	28.4	206.7	GH 7	7.56	918.0	164.5	73.1	12.1	302.2
MS 8	9.26	292.9	73.4	68.6	21.9	230.1	GH 8	8.00	910.9	60.3	89.4	12.6	330.0
MS 9	10.06	286.1	71.0	71.7	24.5	220.6	GH 9	7.40	910.2	90.5	108.3	12.0	366.0
MS 10	8.55	197.2	95.8	93.3	20.3	279.9	GH 10	8.60	919.7	92.4	68.1	12.0	351.2
AH 1	8.58	883.5	105.8	183.9	16.2	267.3	WS 1	7.26	935.4	80.3	34.6	11.4	360.8
AH 2	9.52	871.6	107.8	219.4	12.4	235.4	WS 2	6.78	903.0	130.8	54.7	9.6	367.4
AH 3	8.14	895.4	118.3	196.0	14.4	281.7	WS 3	7.17	921.9	111.1	54.8	11.2	365.4
AH 4	8.81	890.7	134.7	154.6	17.9	232.7	WS 4	6.43	929.0	86.0	29.3	8.6	359.7
AH 5	9.08	871.1	163.6	193.5	18.7	201.5	WS 5	7.65	909.3	59.9	28.7	11.4	406.4
AH 6	8.62	887.8	62.4	163.7	11.0	292.6	WS 6	7.14	920.2	63.8	26.2	10.1	425.5
AH 7	9.68	891.1	129.3	207.4	17.7	246.4	WS 7	6.92	928.4	75.0	28.0	7.0	416.1
AH 8	8.96	867.9	86.1	165.8	13.6	302.6	WS 8	6.56	926.9	57.0	34.2	13.5	447.9
AH 9	9.13	919.1	87.4	191.8	20.8	263.4	WS 9	7.61	915.2	70.7	30.4	16.3	377.0
AH 10	8.76	897.1	107.6	188.3	13.5	282.6	WS 10	7.22	919.5	82.3	59.3	11.9	356.8

It was reported that *in vivo* ME values were calculated with the regression equation of ME, MJ kg^{-1} DM= 0.0152xDCP+0.0342xDEE+0.0128xDCF+0.0159x DNFE in which Digestible Crude Nutrients (DCN), Digestible Crude Protein (DCP), Digestible Ether Extract (DEE), Digestible Crude Fiber (DCF), Digestible Nitrogen Free Extract (DNFE)- obtained by Classic Digestion Trials and Dry Matter (DM), Crude Ash (CA), Crude Protein (CP), Ether Extract (EE) and Crude Fiber (CF) amounts of crude nutrients were obtained by Weende analysis method[7]

Table 2: NDF, ADF, ADL, ELOS and GP amounts of roughages

Feeds	NDF (g kg^{-1} DM)	ADF	ADL	ELOS (g kg^{-1} DM)	GP (mL/ 200 mg DM)	Feeds	NDF (g kg^{-1} DM)	ADF	ADL	ELOS (g kg^{-1} DM)	GP (mL/ 200mgDM)
MS 1	459.1	311.3	35.1	603.90	48.15	GH 1	652.0	441.2	107.5	443.09	34.10
MS 2	452.7	243.4	47.6	690.68	51.73	GH 2	649.0	436.5	90.3	406.07	27.64
MS 3	486.2	293.0	85.4	634.30	54.59	GH 3	562.0	381.6	85.0	501.23	35.10
MS 4	560.4	295.6	53.6	631.04	47.85	GH 4	561.0	357.6	73.1	560.29	40.07
MS 5	585.2	352.7	78.4	598.84	47.41	GH 5	664.8	457.2	94.5	372.45	31.35
MS 6	533.8	306.3	79.6	626.75	50.95	GH 6	627.0	426.7	70.8	478.94	37.29
MS 7	545.6	283.5	45.5	638.79	49.41	GH 7	671.3	447.3	54.6	386.87	31.64
MS 8	569.7	313.9	44.2	607.62	48.25	GH 8	718.0	415.8	102.8	419.33	33.10
MS 9	536.1	283.7	52.0	623.27	52.77	GH 9	643.4	483.4	91.4	456.36	32.08
MS 10	638.9	418.7	90.0	475.11	40.40	GH 10	667.0	448.1	58.7	409.95	33.25
AH 1	519.7	365.9	85.0	550.50	37.70	WS 1	778.0	510.1	93.0	360.63	34.43
AH 2	470.0	352.0	95.1	590.87	34.56	WS 2	756.4	571.7	98.8	288.08	29.57
AH 3	442.1	371.9	104.3	566.07	34.41	WS 3	731.9	501.7	105.6	296.67	27.00
AH 4	423.8	340.2	86.8	583.54	38.05	WS 4	807.7	549.9	97.6	260.46	29.00
AH 5	338.6	296.1	82.4	599.09	37.24	WS 5	798.4	538.4	87.5	322.26	33.30
AH 6	527.7	399.6	116.4	534.12	35.38	WS 6	830.6	584.8	115.7	267.08	27.87
AH 7	428.7	330.6	99.8	609.99	35.08	WS 7	825.7	591.5	95.6	230.29	26.73
AH 8	497.1	388.3	94.7	567.53	37.48	WS 8	805.0	529.8	90.0	316.04	31.23
AH 9	443.5	339.5	67.7	595.08	36.53	WS 9	781.9	525.5	92.4	395.43	38.97
AH 10	489.1	354.4	110.8	550.43	35.57	WS 10	777.5	502.5	74.3	355.99	34.60

It was reported that Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) were obtained by Van Soest analysis method, enzyme soluble/insoluble organic matter (ELOS/EULOS, EULOS= 1-ELOS) was obtained by Enzymatic (cellulase) method, GP (mL/200 mg DM) was obtained by *in vitro* Hohenheim Feed Test[7]

Table 3: Regression equations used in calculation of *in vitro* ME values of roughages

Regression equations			References
1)	ME_{CN}, kcal/kg OM	= 3260+(0.455xCP+3.517xEE)-4.037xCF	[8]
2)	ME_{NDF}, kcal/kg DM	= 3381.9-19.98xNDF and 3) ME_{ADL}, kcal/kg DM= 2764.4-12.73xADL	[9]
4)	ME_{ADF}, MJ/kg DM	= 14.70-0.150xADF	[10]
5)	$ME_{ELOS+CN}$, MJ/kg DM	= - 1.04+(0.00001611xELOSxELOS)-(0.0003674xELOSxEE)+(0.3724xEE)-(0.0004919xEExCF)+(0.01548xCF)	[11]
6)	$ME_{EULOS+CN}$, MJ/kg DM	= +14.27-(0.0120xEULOS)+(0.00234xCP)-(0.0147xCA)	[11]
7)	ME_{GP+CN}, MJ/kg DM	= +4.99+(0.1695xGP)-(0.001134xGPxEE)-(0.0003105xGPxCF)+ (0.0002373xEExCF)-(0.00006067xCAxCA)+(0.00006168xCAxCP)	[11]
8)	ME_{GP+CN}, MJ/kg DM	= - 2.60+(0.2353xGP)-(0.003768xGPxEE)+(0.1438xEE)+(0.0002798xEExCP)+(0.00002146xCPxCF)	[11]

Regression Eq. 1-4 were used in calculation of ME values of MS, AH, GH and WS, 5 and 7 were used in AH, GH and WS, 6 and 8 were used in MS. Afterwards, ME values calculated from regression Eq. 1, 2 and 3 were converted to MJ/kg DM

Table 4: *In vitro* Metabolizable Energy (ME) values (MJ/kg DM) of roughages

Feeds	ME_1	ME_2	ME_3	ME_4	ME_5	ME_6	Feeds	ME_1	ME_2	ME_3	ME_4	ME_5	ME_6
MS 1	9.75	10.31	10.03	10.06	8.14	8.23	GH 1	7.31	8.70	8.08	6.95	7.88	7.95
MS 2	10.46	10.37	11.05	9.52	9.89	8.86	GH 2	7.13	8.72	8.15	7.68	7.67	7.61
MS 3	9.47	10.09	10.31	7.90	8.96	9.58	GH 3	8.11	9.45	8.98	7.91	8.35	8.42
MS 4	9.52	9.47	10.27	9.26	8.79	8.64	GH 4	8.42	9.46	9.34	8.42	8.94	8.89
MS 5	8.75	9.26	9.41	8.20	8.49	8.82	GH 5	6.55	8.59	7.84	7.50	7.47	7.22
MS 6	9.50	9.69	10.11	8.15	8.86	8.82	GH 6	7.64	8.91	8.30	8.52	8.27	8.27
MS 7	9.84	9.59	10.45	9.61	9.18	8.84	GH 7	6.61	8.54	7.99	9.22	7.04	6.92
MS 8	9.20	9.39	9.99	9.67	8.64	8.68	GH 8	7.60	8.15	8.46	7.15	7.61	7.83
MS 9	9.44	9.67	10.44	9.33	8.87	9.30	GH 9	6.61	8.77	7.45	7.64	8.28	7.50
MS 10	8.08	8.81	8.42	7.70	6.78	7.83	GH 10	6.75	8.57	7.98	9.04	7.69	7.42
AH 1	8.27	9.81	9.21	7.91	8.61	9.11	WS 1	6.68	7.65	7.05	7.57	7.35	7.28
AH 2	8.79	10.23	9.42	7.48	8.72	9.28	WS 2	5.90	7.82	6.12	7.32	6.81	6.55
AH 3	7.85	10.45	9.12	7.08	8.86	8.79	WS 3	6.22	8.03	7.17	7.03	6.97	6.76
AH 4	8.43	10.61	9.60	7.84	8.83	9.09	WS 4	6.57	7.40	6.45	7.37	6.48	6.82
AH 5	8.65	11.32	10.26	8.02	8.86	9.41	WS 5	6.18	7.48	6.62	7.81	7.54	6.99
AH 6	8.32	9.74	8.71	6.56	8.44	8.49	WS 6	5.78	7.21	5.93	6.59	7.35	6.59
AH 7	8.37	10.57	9.74	7.28	9.25	9.22	WS 7	5.75	7.25	5.83	7.46	6.84	6.33
AH 8	7.87	9.99	8.88	7.50	9.04	8.65	WS 8	5.56	7.42	6.75	7.70	7.99	6.82
AH 9	8.67	10.44	9.61	8.66	9.25	9.20	WS 9	6.61	7.61	6.82	7.59	7.99	7.60
AH 10	7.96	10.06	9.38	6.80	8.64	8.81	WS 10	6.78	7.65	7.16	8.37	7.31	7.45

ME_{CN}: ME_1, ME_{NDF} : ME_2, ME_{ADF} : ME_3, ME_{ADL}: ME_4, $ME_{ELOS/EULOS+CN}$: ME_5, ME_{GP+CN}: ME_6

Table 5: The comparison of ME (MJ/kg DM) values of roughages using *in vivo* and *in vitro* parameters

Feeds	ME_{DCN}	ME_{CN}	ME_{NDF}	ME_{ADF}	ME_{ADL}	$ME_{ELOS/EULOS+CN}$	ME_{GP+CN}
n=10							
MS	9.71±0.21^{a}	9.40±0.21^{ab}	9.67±0.21^{a}	10.05±0.21^{a}	8.94±0.21^{bc}	8.66±0.21^{c}	8.76±0.21^{c}
AH	8.93±0.14^{c}	8.32±0.14^{d}	10.32±0.14^{a}	9.39±0.14^{b}	7.51±0.14^{e}	8.85±0.14^{c}	9.01±0.14^{c}
GH	8.33±0.20^{ab}	7.27±0.20^{c}	8.79±0.20^{a}	8.26±0.20^{ab}	8.00±0.20^{b}	7.92±0.20^{b}	7.80±0.20^{bc}
WS	7.07±0.14^{b}	6.20±0.14^{d}	7.55±0.14^{a}	6.59±0.14^{cd}	7.48±0.14^{a}	7.26±0.14^{ab}	6.92±0.14^{bc}
n=40	8.51±0.11^{b}	7.80±0.11^{d}	9.08±0.11^{a}	8.57±0.11^{b}	7.99±0.11^{cd}	8.11±0.11^{cd}	8.18±0.11^{c}

Different letter(s) in the same row are statistically different ($p<0.01$)

second step of the study, first stage results were compared with their *in vivo* ME values of the roughages (Table 5).

As seen in Table 5, average *in vivo* ME values of MS, AH, GH and WS were 9.71, 8.93, 8.33, 7.07 MJ/kg DM, respectively[7]. But, in this study, average *in vitro* ME values of the same roughages were found between 8.66-10.05 MJ/kg DM for MS, 8.32-10.32 MJ/kg DM for AH, 7.27-8.79 MJ/kg DM for GH and 6.20-7.55 MJ/kg DM for WS. Results were agreed with the ME values of same feeds of literature[13-15]. But, the results of ME values of every feed showed great variation depending on regression equation calculations. In another word, *in vitro* ME values of roughages were estimated at different levels depending on different regression equations. Comparison of the results of *in vitro* ME values with *in vivo* ME values were given in the Table 5. Similar results of *in vivo* ME values were obtained from equations where NDF, ADF, CN were used in MS, where ELOS/EULOS+CN, GP+CN were used in AH, where NDF, ADF, ADL, ELOS/EULOS+CN, GP+CN were used in GH, ELOS/EULOS+CN, GP+CN were used in WS ($p<0.01$). In other words, similar results of *in vivo* ME values were obtained in equations where NDF and ADF are used separately for MS and GH, where ADL is used only for GH where CN is used only for MS. But estimation was improved significantly in combination of CN with ELOS/EULOS and GP separately and similar results of *in vivo* ME values were obtained for AH, GH and WS ($p<0.01$). According to this, it seemed that equations where NDF and ADF were used estimated more better than equations where CN and ADL were used. These results were agreed with the results informing that CN[16,17] and ADL[3,18] were not well predictor alone in the estimation of ME values. But they did not agree with the results of Schwarz *et al.*[17] where *in vivo* ME value and ADL were compared in MS. Furthermore, these results agreed with the declarations where ME values were estimated well in combination of CN with ELOS/EULOS and GP[3,6,19] and where multiple variables were used instead of simple variable in regression equations for getting better results[16,20]. On the other hand, when the number of roughages were increased dealing with the whole roughages average *in vivo* ME value was found 8.51 MJ/kg DM, but ME values using *in vitro* parameters were found 7.80-9.08 MJ/kg DM ($p<0.01$). Similar *in vivo* ME value was obtained from the regression equation as 8.57 MJ/kg DM where only ADF is used alone ($p<0.01$). This result showed consistency with the studies which suggested that ADF parameter is highly related with

in vivo digestibilities[7] and ME values[6,18,21] of roughages. On the other hand in CN analysis of roughages it should be taken into consideration that CF amount was erroneous and determined lower than its actual amount[1,3,15,22]. For this reason, it was suggested that NDF and especially ADF amounts should be taken into consideration instead of CF in meeting the fiber requirements of ruminants[22-24]. So, in combinations of CN with ELOS/EULOS or GP, it was understood that using of NDF and especially ADF instead of CF would be more accurate.

As a result, ME values of roughages were estimated at different levels with different regressions where, *in vitro* parameters were used. On the other hand, it seemed that with the equations where, NDF or especially ADF was used the ME value was estimated more better than with the equations where, CN or ADL was used. Besides, it can be suggested that estimation will be improved in developed equations where, CN are combined with ELOS/EULOS or GP and using of ADF instead of CF will be more accurate.

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