Animal Science Papers and Reports vol. 37 (2019) no. 4, 379-392
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Digestible energy value of oat and barley grain for horses*

Piotr Micek[1], Olga Lasek[1], Magdalena Pieszka[2], Agnieszka Waliczek[1]**

[1] Department of Animal Nutrition and Dietetics, University of Agriculture in Cracow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

[2] Department of Horse Breeding, University of Agriculture in Cracow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

(Accepted August 5, 2019)

An experiment was conducted to determine the digestible energy value of oat and barley grain based on their chemical composition and an *in vitro* digestibility trial for horses in relation to the type of grain variety and estimation method used. The study involved 9 oat and 11 barley cultivars. Chemical composition and *in vitro* organic matter digestibility were determined by standard procedures using the Daisy[II] Incubator with cellulase solution. Digestible energy was estimated using five different regression equations. The results of these studies suggest that the chemical analysis of grains and *in vitro* digestibility trials should be a laboratorial routine, since these are relatively simple and allow for establishing basic parameters for evaluation of digestible energy content for horses. The correct nutritive estimation of grains for horses should include, at the very least, protein and starch content, but also fiber content and composition.

KEYWORDS: barley / cultivars / oat grain / digestible energy / in vitro digestibility

Cereal grains have always been a traditional feedstuff fed to horses for a source of energy. Usually, oat grain is fed to race horses, corn and barley to draft horses, and different corn-oat-barley mixes as a treat or as a staple of the diet. However, Lewis [1995] has proven that oat grain is safer and healthier for horses than other cereal grains. Oats provide a moderate amount of starch therefore their energy content is relatively low. Nevertheless, some studies [Frape 1998, Julliand *et al.* 2006] suggest

*This work was supported by grant no. DS-3217/KZiDZ/2017-2018
**Corresponding author: rzmicek@cyf-k.edu.pl

that oats are very efficient as a source of energy due to the digestible starch which creates glucose in the small intestine. In comparison to other grains, oats contain more fiber, are less likely to contain mycotoxins and do not have to be processed in order to be safe for horse consumption [Julliand *et al.* 2006]. In turn, barley has lower fiber and starch content, falling somewhere between oat and corn grains. Barley is also a valuable energy source, but due to its tough hull it has to be processed when put into horse feeds [Bailoni *et al.* 2006].

The chemical composition and physical characteristics of cereal grains included in the tables of composition and nutritional values of feed materials [Abdouli and Ben Attia 2007, NRC 2007] usually comprise only average values, not assuming differences between varieties. Bailoni *et al.* [2006] and Micek [2008] showed that chemical composition and organic matter digestibility of oat or barley grains can vary widely in relation to variety or their physical treatments. Therefore, in most systems of energy evaluation, at least digestible energy content is estimated on a routine basis. For this purpose, *in vivo* methods for determining dry matter (DM) or organic matter (OM) digestibility are used but because they are all laborious and expensive, *in vitro* techniques have been developed for most livestock species, including equines. Many of these methods have provided estimates highly correlated to *in vivo* studies [Micek 2008, Rosenfeld and Austbø 2009a]. Lowman *et al.* [1999] as well as Smolders *et al.* [1990] suggested also that some *in vitro* techniques used for the determination of digestibility of ruminant feedstuffs can be applied to predict the *in vivo* apparent digestibility of equine feeds. Microbial inoculum (rumen fluid) is commonly used in these techniques, which need to have surgically modified (fistulated) animals. Such techniques have been adapted in equines using the gas test method and either inoculum sampled from caecal fluid of fistulated animals [Micek 2008] or faeces as a source of inoculums [Martin-Rosset *et al.* 2012]. In recent years, in equines [Earing *et al.* 2010, Martin-Rosset 2015], as in ruminants [Kowalski *et al.* 2014], these enzymatic methods have been implemented more frequently to predict the digestibility of feeds.

Various methods have been used for estimating the digestible energy content of feeds for horses. Most of them assume that DE values can be predicted directly from chemical composition, without any measures of digestibility [Fehrle 1999, Zeyner and Kienzle 2002]. Unfortunately, in many cases it can lead to significant differences in estimated energy value of feeds and consequently to feeding unbalanced rations to animals. Regarding cereal grains in the scientific literature, there are very few complex *in vivo* studies on their DE content estimated in relation to origin, cultivars or chemical composition of seeds. Therefore, in the current work, based on *in vitro* studies, we hypothesized that inappropriate energy evaluation of cereal grains for horses may be caused not only by the differences in their chemical composition or origin, but also by the use of inadequate regression equations.

The aim of the study was to determine the digestible energy value of oat and barley grain for horses based on its chemical composition and an *in vitro* digestibility trial in relation to the type of grain variety and estimation method used.

Material and methods

Grains of 9 oat cultivars and 11 grains of barley cultivars, varied in type, form, colour of lemma or technological suitability for processing and industrial usefulness, were investigated (Tab. 1). Air-dried samples of oat and barley grain were ground to pass through a 1 mm sieve and analysed for content of dry matter (DM), ash, crude protein, crude fat and crude fiber using standard analytical procedures (procedure nos. 934.01, 942.05, 976.05, 920.39 and 962.09, respectively AOAC [2007]. The content of gross energy (GE) was measured using a Parr adiabatic oxygen bomb calorimeter (KL-10, Precyzja, Bydgoszcz, Poland). The content of neutral detergent fiber (aNDF determined with a heat stable amylase and expressed inclusive of residual ash), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using an ANKOM220 Fiber Analyzer (Ankom Products, NY, USA) according to AOAC [2007]. Total dietary fiber (TDF) was analyzed by an enzymatic method according to Englyst and Cummings [1998]. Beta-glucans were determined according to McCleary and Mugford [1997]. Furthermore, non-starch polysaccharides (NSP = TDF – ADL) and non-cellulosic polysaccharides (NCP = TDF – CEL) were calculated. Starch content was determined by colorimetric method with α-amylases according to the procedure of Faisant *et al.* [1995].

Table 1. Description of oat and barley cultivars

Species	Form	Cultivar	Symbol	Type or technological group of variety
Oats	spring	Akt	ANN	*Avena nuda*, naked
		Flamingsprofi	WeH	white hull
		Czarnoziarnisty	BkH	black hull
		Gniady	BnH	brown hull
		Cwał	YGH	yellow-green hull
		Szakal	YH	yellow hull
		Rajtar	YH	yellow hull
		Bajka	YH	yellow hull
		Dukat	YH	yellow hull
Barley	spring	Rastik	SFN	fodder, naked
		Stratus	SM2R	malting, 2-row
		Rudzik	SM2R	malting, 2-row
		Rodos	SF2R	fodder, 2-row
		Orthega	SF2R	fodder, 2-row
		Rodion	SF2R	fodder, 2-row
	winter	Gregor	WF6R	fodder, 6-row
		Sigra	WF6R	fodder, 6-row
		Gil	WF6R	fodder, 6-row
		Bombay	WF2R	fodder, 2-row
		Tiffany	WM2R	malting, 2-row

Determination of in vitro organic matter digestibility

The test was carried out using F57 bags (Ankom Technology, USA). Bags were marked, rinsed in acetone for 5 min and weighed. Accurately, 0.500 g of sample

ground for 1.5 mm was weighed directly into the bag and heat-sealed. Eight bags with each feed were incubated in four jars (2 bags with each feed/jar) and this procedure was repeated 3 times (3 runs). One bag per every jar was left empty (blank bag) and one bag per every jar was filled with standard feed. Incubation was carried out in 4 jars of Daisy^{II} Incubator (Ankom Technology, USA) with cellulase solution [Kowalski *et al*. 2014]. Cellulase solution was prepared by dissolution of 1 g cellulase (Onozuka R10, Trichoderma viride) in 1 liter of 0.01 M acetate buffer. Incubation started after the device reached a temperature 39.5°C. All bags were placed at the same time and removed after 48 h of incubation. Bags removed from incubation jars were rinsed with cold tap water and aNDF content in the bags was analyzed.

In vitro organic matter digestibility (IVOMD) was calculated according to formula:

$$IVOMD = 100 - (W_3 - (W_1 \times W_4)) \times 100 / W_2$$

where:

W_1 – bag tare weight;

W_2 – organic matter sample weight;

W_3 – final bag weight after *in vitro* and sequential neutral detergent treatment expressed exclusive of residual ash;

W_4 – blank bag correction (final oven-dried weight/original blank bag weight).

Statistical analysis

Digestible energy (DE) was calculated according to formulas by: NRC [2007], INRA [2004], Zeyner and Kienzle [2002], Fehrle [1999] and DLG [1974]. In this experiment INRA [2004] model based on *in vivo* studies was modified by substituting *in vivo* OMD for in vitro OMD (IVTD). Means for winter and spring form of barley were compared using one-way ANOVA. Pearson's correlation coefficients were calculated as a measure of strength of the association between variables [StatSoft 2011]. The stepwise multiple regression method was used in order to estimate an equation for the prediction of *in vitro* organic matter digestibility in cereals from chemical parameters. Paired t-Student test was used to compare DE values predicted by different mathematical equations. The significance level was set at $P \leq 0.05$.

Results and discussion

Oats and barley are widely used as a feed grain for horses but their nutritive value is often discounted because of the large variation in composition. Variability in the nutritional usefulness of these grains has been the subject of numerous studies which clearly show that there are a number of factors to be considered for proper evaluation [Rowe *et al.* 2011]. In the present study, the nutrient content as well as the carbohydrate structure of oat or barley grains varied considerably between cultivars within each species (Tab. 2-4). The oat cultivars differed mostly in crude fiber and

aNDF content, whereas the maximum differences for barley cultivars was observed in aNDF and starch content. The lowest DE values for both oat and barley grains were found when the equation proposed by DLG [1974] was used. In turn, the highest DE values was estimated by the Zeiner and Kienzle [2002] model for oats and by the INRA [2004] model for barley (Tab. 5). Thus, estimated DE differed highly not only among various mathematical equations used (Tab. 6) but also among cultivars within each species (Tab. 5). The highest *in vitro* organic matter digestibility (IVOMD) values were observed for naked forms of oat (Akt) and barley (Rastik), with important variation between regular cultivars, especially for oats (SD = 6.5 vs 2.3 for barley).

Table 2. Chemical composition of oat and barley grains* (g/kg of dry matter)

	Items	Symbol	Ash	Organic matter	Crude protein	Ether extract	NFE[a]	Starch
Oat cultivars	Akt	ANN	26.7	973.3	163.8	76.5	706.9	713.8
	Flaemingsprofi	WeH	25.8	974.2	148.7	51.5	689.3	498.8
	Czarnoziarnisty	BkH	27.4	972.6	126.8	59.5	707.2	459.2
	Gniady	BnH	19.8	980.2	167.8	32.7	672.2	390.7
	Cwał	YGH	24.4	975.6	148.6	37.9	678.5	359.0
	Szakal	YH	21.8	978.2	139.9	42.9	724.9	589.5
	Rajtar	YH	24.7	975.3	149.0	40.6	659.1	328.0
	Bajka	YH	27.7	972.3	116.6	41.9	698.9	654.8
	Dukat	YH	30.5	969.5	125.7	48.1	689.8	571.8
	Regular variety	mean	25.3	974.7	140.4	44.4	690.0	481.5
		SD[b]	3.4	3.4	16.6	8.4	20.7	118.0
		CV[c], %	13.5	0.3	11.8	18.9	3.0	24.5
Barley cultivars	Rastik	SFN	21.6	978.4	141.0	19.4	805.3	740.8
	Stratus	SM2R	24.7	975.3	131.6	18.7	789.8	622.9
	Rudzik	SM2R	26.7	973.3	112.3	22.5	809.3	677.1
	Rodos	SF2R	26.7	973.3	136.1	24.3	770.1	652.9
	Orthega	SF2R	24.0	976.0	134.9	21.5	774.8	534.4
	Rodion	SF2R	21.1	978.9	139.7	15.8	780.5	642.6
	Spring form (regular)	mean	24.6	975.4	130.9	20.6	784.9	626.0
		SD	2.3	2.3	10.8	3.3	15.5	54.8
		CV, %	9.4	0.2	8.3	16.3	2.0	8.8
	Gregor	WF6R	25.3	974.7	96.4	25.5	791.5	672.3
	Sigra	WF6R	22.9	977.1	124.3	23.9	768.6	553.7
	Gil	WF6R	23.7	976.3	95.0	22.9	804.5	606.0
	Bombay	WF2R	22.2	977.8	106.3	18.6	819.4	696.9
	Tiffany	WM2R	23.7	976.3	108.1	16.9	811.5	726.6
	Winter form (regular)	mean	23.6	976.4	106.0	21.6	799.1	651.1
		SD	1.2	1.2	11.8	3.7	19.9	70.3
		CV, %	4.9	0.1	11.1	16.9	2.5	10.8
	Overall (regular)	mean	24.1	975.9	118.5	21.1	792.0	638.5
		SD	1.8	1.8	16.9	3.3	18.4	60.9
		CV, %	7.5	0.2	14.3	15.9	2.3	9.5
		P-value[e]	NS[d]	NS	0.044	0.001	0.003	0.026
		SEM[f]	0.6	0.6	5.4	1.1	5.8	19.3

*Data are the mean of duplicate analyses of each ingredient; NFE[a] – nitrogen free extract; SD[b] – standard deviation; CV[c] – coefficient of variation; NS[d] – not significant ($P>0.05$); *P*-value[e] – winter vs. spring form; SEM[f] – standard error of the mean.

Regardless of species, IVOMD of grain was highly correlated ($P<0.05$) with CF (-0.95), cellulose (-0.85), aNDF (-0.79) and starch (0.74) content (Fig. 1). Lower and not statistically significant ($P>0.05$) correlation indices were found between IVOMD and β-glucans (0.65), TDF (-0.64) or NSP (-0.54). However, in the case of β-glucans, TDF and NSP important variation in correlation values with IVOMD were shown separately for oats and barley. This may indicate different influences of these substances on IVTD depending on the type of grain. According to Jensen *et al.* [2010], the TDF analysis method gave the most appropriate differentiation of the fiber fractions and their digestibility in horses, compared to the traditional CF, ADF and NDF analyses. Furthermore, a major advantage of the TDF analysis is the capacity of recovering soluble fibers. In the present study, determination of β-glucans, as one of the main components of soluble fiber, did not improve the precision of IVTD and DE estimation. This phenomenon could be partially explained by the relatively high proportion of insoluble fiber, which increase intestinal motility and impair the digestibility of nutrients in the small intestine. The quantity and the quality of fiber in the horse diet may therefore modify intestinal transit, leading to changes in digestibility characteristics [Moreira *et al.* 2015]. From a practical standpoint, β-glucans and TDF are expensive, and time- and labor-intensive indicators and their inclusion into the regression equations would probably require the preparation of separate formulas for each cereal species.

Table 3. Content of structural carbohydrates in oat grains* (g/kg of dry matter)

Items		Symbol	CF	aNDF	ADF	ADL	CEL[a]	HCEL[b]	β-glucans	TDF[c]	NSP[d]	NCP[e]
Regular variety		ANN	26.1	137.1	32.1	5.9	26.2	104.9	43.6	114.8	108.8	82.6
		WeH	84.7	236.3	122.1	11.7	110.3	114.2	35.8	363.7	352.0	253.4
		BkH	79.0	266.5	119.5	6.2	113.3	147.0	37.5	450.0	443.8	336.7
		BnH	107.6	378.6	237.7	38.3	199.4	140.9	36.7	365.3	327.0	165.9
		YGH	110.6	377.2	204.7	36.1	168.6	172.5	37.0	261.2	225.1	92.6
		YH	70.5	210.8	82.8	17.4	65.5	127.9	31.2	252.8	235.4	187.3
		YH	126.5	397.8	241.0	45.9	195.1	156.8	31.6	312.1	266.2	117.0
		YH	114.8	313.4	147.7	19.0	128.7	165.6	30.8	338.7	319.7	210.0
		YH	105.9	269.8	121.6	22.2	99.4	148.2	32.6	245.8	223.6	146.4
	mean		100.0	306.3	159.6	24.6	135.0	146.7	34.2	332.6	299.1	188.7
	SD[f]		19.5	71.3	60.1	14.0	48.0	19.2	2.9	70.9	76.8	78.6
	CV[g], %		19.5	23.3	37.7	56.9	35.6	13.1	8.5	21.3	25.7	41.7

*Data are the mean of duplicate analyses of each ingredient. CEL[a] – cellulose; HCEL[b] – hemicellulose; TDF[c] – total dietary fiber; NCP[d] – non-starch polysaccharides; NCP[e] – non-cellulosic polysaccharides; SD[f] – standard deviation; CV[g] – coefficient of variation.

Table 4. Content of structural carbohydrates in barley grains* (g/kg of dry matter)

Items	Symbol	CF	aNDF	ADF	ADL	CEL[a]	HCEL[b]	β-glucans	TDF[c]	NSP[d]	NCP[e]
Rastik	SFN	12.6	185.4	36.2	3.8	32.4	149.2	40.7	135.7	132.0	99.6
Stratus	SM2R	35.1	250.7	40.5	7.8	32.7	210.2	35.6	242.6	234.7	202.0
Rudzik	SM2R	29.2	223.1	43.3	6.1	37.2	179.7	40.0	225.7	219.6	182.4
Rodos	SF2R	42.8	246.1	47.3	5.8	41.5	198.8	37.1	241.5	235.7	194.2
Orthega	SF2R	44.9	247.8	72.4	11.3	61.0	175.5	45.7	258.7	247.4	186.4
Rodion	SF2R	43.0	215.2	78.8	9.0	69.7	136.5	49.9	246.4	237.4	167.6
Spring form (regular)	mean	39.0	236.6	56.5	8.0	48.4	180.1	41.7	243.0	235.0	186.5
	SD[f]	6.6	16.2	17.8	2.3	16.1	28.2	6.0	11.8	10.0	13.0
	CV[g], %	16.9	6.8	31.5	28.8	33.3	15.7	14.4	4.9	4.3	7.0
Gregor	WF6R	61.4	234.2	96.0	8.9	87.2	138.1	35.1	361.8	352.9	265.8
Sigra	WF6R	60.3	271.5	88.2	16.4	71.8	183.3	40.1	329.2	312.8	240.9
Gil	WF6R	53.9	230.5	74.7	14.2	60.5	155.8	35.5	245.5	231.3	170.8
Bombay	WF2R	33.6	217.5	62.1	17.4	44.6	155.5	44.8	279.8	262.4	217.8
Tiffany	WM2R	39.8	220.8	73.2	4.4	68.8	147.6	37.1	202.5	198.0	129.3
Winter form (regular)	mean	49.8	234.9	78.8	12.3	66.6	156.1	38.5	283.8	271.5	204.9
	SD[f]	12.5	21.6	13.3	5.5	15.6	16.9	4.0	63.7	62.1	54.9
	CV[g], %	25.1	9.2	16.9	44.7	23.4	10.8	10.4	22.4	22.9	26.8
Overall (regular)	mean	44.4	235.7	67.7	10.1	57.5	168.1	40.1	263.4	253.2	195.7
	SD[f]	11.0	18.0	18.9	4.6	17.7	25.3	5.1	48.3	46.1	38.8
	CV[g], %	24.8	7.6	27.9	45.5	30.8	15.1	12.7	18.3	18.2	19.8
	P-value (winter *vs.* spring form)	NS[h]	0.036	0.023	NS	0.019	0.000	NS	NS	NS	NS
	SEM[i]	3.5	5.7	6.0	1.4	5.6	8.0	1.6	15.3	14.6	12.3

*Data are the mean of duplicate analyses of each ingredient; CEL[a] – cellulose; HCEL[b] – hemicellulose; TDF[c] – total dietary fiber; NCP[d] – non-starch polysaccharides; NCP[e] – non-cellulosic polysaccharides; SD[f] – standard deviation; CV[g] – coefficient of variation; NS[h] – not significant (P>0.05); SEM[i] – standard error of the mean.

Using the step-wise multiple regression method for chemical components, the first variable entered into the model for IVOMD prediction was CF (Tab. 7). This single parameter, routinely analyzed in laboratories, can explain a large part of the variability in IVOMD between variety (R^2= 0.92). NDF introduced to the model with CF resulted in only a slight increase of R^2 (R^2= 0.93). The best regression equation

Table 5. *In vitro* organic matter digestibility (IVOMD), gross energy (GE) and digestible energy (DE) content estimated using different regression equations in oat and barley grains

	Items	Symbol	GE MJ/kg of dry matter	DE (MJ/kg of dry matter)					IVOMD[a] %
				NRC [2007]	INRA* [2004]	DLG [1974]	Zeiner and Kienzle [2002]	Fehrle [1999]	
Oat cultivars	Akt	ANN	20.2	16.3	18.6	12.7	16.5	15.8	97.6
	Flaemingsprofi	WeH	19.9	14.2	14.5	12.1	14.9	14.5	77.3
	Czarnoziarnisty	BkH	18.8	14.3	14.1	12.0	15.1	14.5	75.1
	Gniady	BnH	19.0	11.6	14.6	12.3	14.2	14.1	78.0
	Cwał	YGH	18.9	12.3	14.2	12.0	14.1	13.9	76.0
	Szakal	YH	20.9	15.1	14.9	12.8	15.0	14.4	80.3
	Rajtar	YH	19.2	11.5	11.4	11.6	13.9	13.8	60.6
	Bajka	YH	19.5	13.6	13.1	11.8	14.0	13.6	69.4
	Dukat	YH	19.3	14.2	13.8	11.7	14.3	13.9	74.1
	Regular variety	mean	19.4	13.4	13.8	12.0	14.4	14.1	74.5
		SD[b]	0.7	1.4	1.1	0.4	0.5	0.3	6.5
Barley cultivars	Rastik	SFN	18.2	16.2	17.1	14.6	15.4	14.4	97.0
	Stratus	SM2R	18.4	16.1	16.0	14.1	14.9	14.0	90.6
	Rudzik	SM2R	18.0	16.0	15.8	14.1	15.1	13.9	91.5
	Rodos	SF2R	18.5	15.9	16.1	13.8	14.9	14.1	90.8
	Orthega	SF2R	18.1	15.4	15.8	13.8	14.9	14.0	90.7
	Rodion	SF2R	18.0	15.2	15.8	14.0	14.8	14.0	89.4
	Spring form (regular)	mean	18.2	15.7	15.9	14.0	14.9	14.0	90.6
		SD	0.2	0.4	0.1	0.1	0.1	0.1	0.8
	Gregor	WF6R	17.9	14.8	15.4	13.4	14.6	13.6	87.1
	Sigra	WF6R	18.5	15.0	15.0	13.4	14.6	13.8	83.9
	Gil	WF6R	18.3	15.3	15.4	13.6	14.7	13.6	87.4
	Bombay	WF2R	17.7	15.6	15.1	14.2	15.0	13.8	88.5
	Tiffany	WM2R	18.6	15.3	15.8	14.0	14.8	13.7	88.2
	Winter form (regular)	mean	18.2	15.2	15.3	13.7	14.7	13.7	87.0
		SD	0.3	0.3	0.3	0.3	0.1	0.1	1.6
	Overall (regular)	mean	18.2	15.5	15.6	13.8	14.8	13.6	88.8
		SD	0.3	0.4	0.4	0.3	0.2	0.2	2.3
		P-value[e]	NS[c]	0.049	0.007	NS	NS	0.001	0.004
		SEM[f]	0.10	0.14	0.12	0.09	0.05	0.06	0.73

*INRA [2004] model based on *in vivo* studies modified in this study by substituting *in vivo* OMD for *in vitro* OMD (IVOMD); IVOMD[a] – *in vitro* organic matter digestibility; SD[b] – standard deviation; NS[c] – not significant ($P>0.05$); SEM[d] – standard error of the mean; *P*-value[e] – winter vs. spring form.

Table 6. Differences (*P*-value) between digestible energy values estimated by different regression equations

Source	NRC [2007]	INRA* [2004]	DLG [1974]	Zeiner and Kienzle [2002]
INRA [2004]	0.039			
DLG [1974]	<0.001	<0.001		
Zeiner and Kienzle [2002]	0.772	0.433	<0.001	
Fehrle [1999]	0.058	0.060	0.001	<0.001

*INRA [2004] model based on *in vivo* studies modified in this study by substituting *in vivo* OMD for *in vitro* OMD (IVOMD).

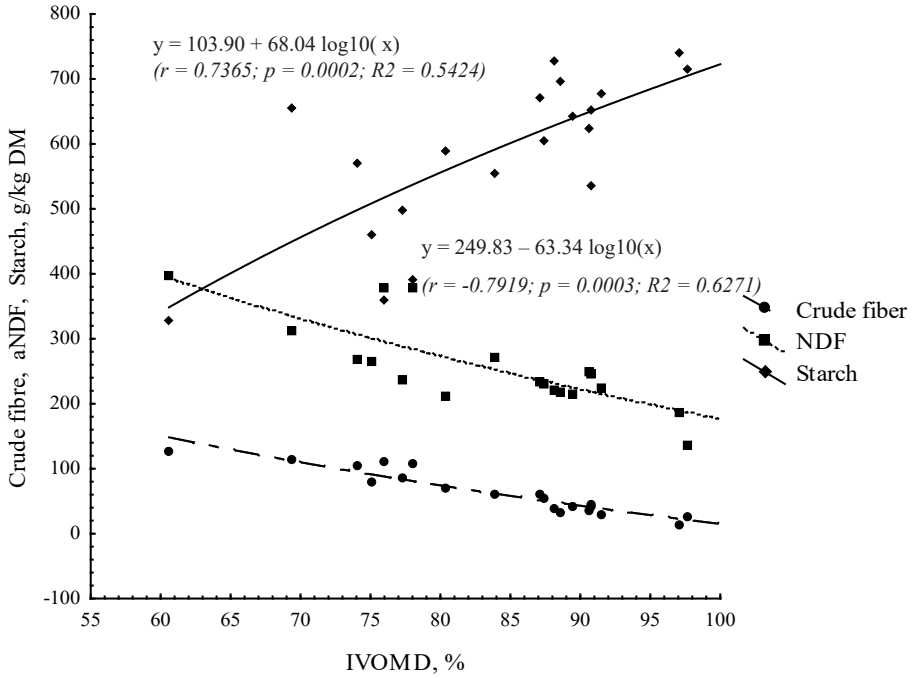

Fig. 1. Relationship between in vitro organic matter digestibility (IVOMD, %) and crude fiber, aNDF and starch content in oat and barley grains.

Table 7. Equation for predicting *in vitro* organic matter digestibility (IVOMD) of grains from chemical composition

Intercept	CF	NDF	Starch	R^2	RSD	*P*-value
102.99	-0.31			0.92	2.26	<0.001
108.40	-0.28	-0.03		0.93	2.11	<0.001
75.83	-0.20		0.03	0.96	1.60	<0.001

for estimating IVOMD was found when CF and starch content were entered into the model. These two variables explained 96% of the dependent variable variation, and the discrepancy between empirical values and those predicted by the model were 1.6.

The substantial differences in chemical composition between oat and barley cultivars shown in the present study are comparable to results presented by Holtekjølen *et al.* [2006] and Givens *et al.* [2004]. Also the fibrous fraction content of analysed cereals are in agreement with those reported in literature [Bailoni *et al.* 2006]. Naked oat and barley cultivars were characterised by lower content of CF, NDF and ADF and higher starch content, in comparison to regular ones. It is worth noting the very low proportion of cellulose and high proportion of β-glucans in DM demonstrated in the naked form of oat (Akt variety). Hussein *et al*. [2004] suggest that naked oats was developed as a variety with 20 to 27% greater DE (i.e., 15.57 MJ/kg of DM) than

conventional oats and, as a result, its use for feeding horses has gradually increased. In the case of naked barley (Rastik variety), the concentration of cellulose in grain was similar to some of the regular varieties. In turn, the differences in crude protein content between spring and winter forms of barley were higher than averages between oat and barley species.

The above-described differences in the chemical composition of grains in most cases explain the variability in their *in vitro* organic matter digestibility as well as digestible energy content. A similar conclusion has been drawn by Sarkijarvi and Saastamoinen [2006] from studies carried out on various processed oat grains in equine diets. According to Rowe *et al.* [2001], differences in lignin content in high- and low-lignin oat cultivars had a particularly significant effect on their digestibility and the prediction of DE based on feed intake. Additionally, lignin incorporation into the model could be further improved by considering ADF content. Hussein et al. [2004] pointed out higher fiber and lower energy content in oats compared with other grains making them a safer energy source for horses, especially when fed in excess. Oats are highly palatable and can be fed whole or processed (e.g., rolled or crimped). In comparison to oats, barley is considered a "heavy" feed due to its greater energy value and, at the same time, harder physical quality than most grains, requiring processing before feeding. The DE values reported in NRC [2007] for oats and barley are 13.39 and 15.40 MJ/kg of DM, respectively.

Routine prediction of equine feed values has been reported using the chemical composition of feedstuffs, a pepsin-cellulase method, and near infrared reflectance spectrophotometry as predictor variables [Lowman *et al.* 1999, Micek 2008]. Nowadays, the main general purpose of nutritional research is to rapidly evaluate the nutritive value of feeds based either on the knowledge of their chemical composition [Martin-Rosset *et al.* 2006] or using enzymatic and NIRS methods [Martin-Rosset *et al.* 2012]. In this context, the estimation of feed digestible energy values using regression equations, without experiments carried out on animals, seems justified on both economical and ethical grounds. For cereal grains such procedures are commonly applied in the formulation of equine diets [Zeyner and Kienzle 2002, Abdouli and Ben Attia 2007, Martin-Rosset 2015]. However, in order to increase the precision of such estimations, the equations to predict energy value of cereal grains should be based not only on chemical composition but also on *in vitro* digestibility trials. On the other hand, it should be noted that *in vitro* techniques are not always designed to accurately measure absolute digestibility coefficients but rather to predict it or to compare the relative digestibility of different feeds.

According to Martin-Rosset *et al.* [2012] and Krizsan *et al.* [2012], *in vitro* organic matter digestibility is highly correlated with *in vivo* OMD, therefore this parameter is increasingly proposed in the majority of regression equations for predicting energy value of feeds for livestock [Martin-Rosset 2015, Tagliapietra *et al.* 2011]. Abdouli and Ben Attia [2007] suggest that *in vitro* OM digestibility determination for low-fiber, high-quality feeds represented by barley grains or soybean meal needs to be carried

out in two stages: a predigestion stage with pepsin and α-amylase for at least 2 h each, and a fermentation stage using horse faeces as the source of microbial inoculum. In their opinion, for high-fiber, low-quality feeds such as oat hay, only the fermentation stage is needed. In turn, Lowman *et al.* [1999] have confirmed the suitability of simple cumulative gas volumes or modelled gas production parameters as good predictors of *in vivo* nutritive feed values for equines and demonstrated the widespread applicability of a *in vitro* batch culture technique for routine feed evaluation.

Abdouli and Ben Attia [2007] reported OMD values for barley grains ranged from 71.3 to 80.1% depending on the *in vitro* method used. In the present study, estimation of DE according to INRA [2004] was performed with a new IVOMD method instead of OMD determined by *in vivo* studies. De Marco *et al.* [2014] suggest that among the various *in vitro* methods, the pepsin-cellulase technique is one of the most tested, in particular by INRA researchers, highlighting its suitability both in ruminants and horses. These authors reported that OM digestibility with the pepsin-cellulase method was highly correlated (R = 0.978 and 0.943, respectively for ruminants and horses) with *in vivo* digestibility values for both forages and concentrates. In our study, for regular forms of barley, the OMD values ranged from 83.9 to 88.5% and from 89.4 to 91.5% for winter and spring cultivars, respectively. These values, although generally slightly higher, indicate a very important role not only of the method used for OMD estimation but also of the origin of the tested cultivar. This last remark can be explained by high correlation indices between IVOMD and CF (-0.95) or starch (0.74) content in grains, which was already observed by Bailoni *et al.* [2006] in a trial on differently processed barley, oat and corn grain. In their studies, *in vitro* OMD values for barley were in agreement with our findings, which may suggest that the physical treatment of grains could have effects on digestibility parameters similar in importance to the effects of cultivar diversity. Rosenfeld and Austbø [2009b] reported that maize had less pre-cecal starch digestibility in horses than oats and barley. These authors suggest that the increased amount of starch supplied to the microbiota in the time-dependent mixing compartment allows the grain particles to remain in that compartment for an extended period. Indeed, it is well recognised that the proportion of different fractions of starch resistant to enzyme attacks vary according to either the cereal grain species and/or the effects of industrial processing. In addition, it has been established by Martin-Rosset [2015] that *in vivo* OMD of cereal grains is well predicted mostly from CF content (R^2 = 0.987; RSD = 1.3) but that CP and cytoplasmic content (starch + soluble sugars) improve the prediction slightly (R^2 = 0.969; RSD = 0.2). So far, the *in vivo* OMD models (Tab. 6) using CF or CF and starch are relevant.

In summary, numerous regression equations are known to predict the DE value of cereal grains for horses, resulting in considerable variation in results obtained for the same cultivar. DE predicted using INRA or NRC models are close and the variations are consistent for all cereal grains and cultivars. The effects of cultivars on DE values are much higher for oat than for barley (31% vs 5%, respectively) and even higher for their naked forms. Thus, being able to use the prediction of OMD using an IVOMD

model based on a cellulase technique dedicated to cereal grains with high cell wall content may be useful. In order to help predict cereal grain variation, this model, with CF as the major variable and starch as an additional variable, would be most useful when cereal grains are processed or naked thereby improving adequacy of nutrients for horses fed with a high proportion of cereal grains. But regardless of this finding, it seems that more research is needed to better validate known equations, not only with a greater number of cereal varieties but above all, involving *in vivo* studies.

REFERENCES

1. ABDOULI H., BEN ATTIA S., 2007 – Evaluation of a two-stage in vitro technique for estimating digestibility of equine feeds using horse faeces as the source of microbial inoculum. ***Animal Feed Science and Technology*** 132,155-162.
2. AOAC., 2007 – Association of Official Analytical Chemists, Official Methods of Analysis. 18th ed. Rev. 2. Washington, DC, US.
3. BAILONI L., MANTOVANI R., PAGNIN G., SCHIAVON S., 2006 – Effect of physical treatments on the resistant starch content and in vitro organic matter digestibility of different cereals in horses. ***Livestock Science*** 100, 14-17.
4. DE MARCO M., PEIRETTI P.G., MIRAGLIA N., BERGERO D., 2014 – Apparent digestibility of broken rice in horses using in vivo and in vitro methods. ***Animal*** 8, 245-249.
5. DLG, 1974 – Futterwerttabellen für Pferde. Verlag Frankfurt am Main, Germany. In German.
6. EARING J.E., CASSIL B.D., HAYES S.H., VANZANT E.S., LAWRENCE L.M., 2010 – Comparison of in vitro digestibility estimates using the Daisy^{II} Incubator with in vivo digestibility estimates in horses. ***Journal of Animal Science*** 88, 3954-3963.
7. ENGLYST H.N., CUMMING J.H., 1988 – Improved method for measurement of dietary fiber as non-starch polysaccharides in plant food. ***Journal - Association of Official Analytical Chemists*** 71, 8083-814.
8. FAISANT N., PLANCHOT V., KOZLOWSKI F., PACOURET M.P., COLONNA P., CHAMP M., 1995 – Resistant starch determination adapted to products containing high level of resistant starch. ***Science Aliment*** 15, 83-89.
9. FEHRLE S., 1999 – Untersuchungen zur Verdaulichkeit von Mischfutterbeim Pferd in. Abhangigkeit von der Rauhfutteraufnahme. Thesis, Ludwig-Maximilians-University, Munich, Germany. In German.
10. FRAPE D., 1998 – Equine Nutrition and Feeding, 2nd Ed., Blackwell Science, Malden, MA.
11. GIVENS D.I., DAVIES T.W., LAVERICK R.M., 2004 – Effect of variety, nitrogen fertilizer and various agronomic factors on the nutritive value of husked and naked oats grain. ***Animal Feed Science and Technology*** 113, 169-181.
12. HOLTEKJØLEN A.K., UHLEN A.K., BRÅTHEN E., SAHLSTRØM S., KNUTSEN S.H., 2006 – Contents of starch and non-starch polysaccharides in barley varieties of different origin. ***Food Chemistry*** 94, 348-358.
13. HUSSEIN H.S., VOGEDES L.A., FERNANDEZ G.C.J., FRANKENY R.L., 2004 – Effects of cereal grain supplementation on apparent digestibility of nutrients and concentrations of fermentation end-products in the feces and serum of horses consuming alfalfa cubes. ***Journal of Animal Science*** 82,1986-1996.
14. INRA, 2004 – Tables of composition and nutritional value of feed materials. Pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. 2nd revised edition, Editors: Sauvant D, Perez J-M, Tran G; INRA Wageningen Academic Publishers.

15. JENSEN R.B., BROKNER C., KNUDSEN K.E., TAUSON A.H., 2010 – A comparative study of the apparent total tract digestibility of carbohydrates in Icelandic and Danish warmblood horses fed two different haylages and a concentrate consisting of sugar beet pulp and black oats. ***Archives of Animal Nutrition*** 64, 343-56.
16. JULLIAND V., DE FOMBELLE A., VARLOUD V., 2006 – Starch digestion in horses: The impact of feed processing. ***Livestock Science*** 100, 44-52.
17. KOWALSKI Z.M., LUDWIN J., GÓRKA P., RINNE M., WEISBJERG M.R., JAGUSIAK W., 2014 – The use of cellulase and filter bag technique to predict digestibility of forages. ***Animal Feed Science and Technology*** 198, 49-56.
18. KRIZSAN S.J., NYHOLM L., NOUSIAINEN J., SÜDEKUM K-H., HUHTANEN P., 2012 – Comparison of in vitro and in situ methods in evaluation of forage digestibility in ruminants. ***Journal of Animal Science*** 90, 3162-3173.
19. LEWIS L.D., 1995 – Equine clinical nutrition: Feeding and care. Williams and Wilkens, Baltimore, MA.
20. LOWMAN R.S., THEODOROU M.K., HYSLOP J.J., DHONOA M.S., CUDDEFORD D., 1999 – Evaluation of an in vitro batch culture technique for estimating the in vivo digestibility and digestible energy content of equine feeds using equine faeces as the source of microbial inoculum. ***Animal Feed Science and Technology*** 80, 11-27.
21. MARTIN-ROSSET W., 2015 – Nutritive value of feeds. Chapter 12 In: Equine nutrition, INRA nutrient requirements, recommended allowances and feed tables. Martin-Rosset W (Ed.), Wageningen Academic publishers, Wageningen, The Netherlands.
22. MARTIN-ROSSET W., ANDRIEU J., JESTIN M., ANDUEZA D., 2012 – Prediction of organic matter digestibility using different chemical, biological and physical methods. In: SAASTAMOINEN M, FRADINHO MJ, SANTOS SA, MIRAGLIA N. (eds.). Forages and grazing in horse nutrition. EAAP Publications no. 132. Wageningen Academic Publishers, Wageningen, the Netherlands 83-96.
23. MARTIN-ROSSET W., ANDRIEU J., VERMOREL M., JESTIN M., 2006 – Routine methods for predicting the net energy and protein values of concentrates for horses in the UFC and MADC systems. ***Livestock Science*** 100, 53-69.
24. MCCLEARY B.V., MUGFORD D.C., 1997 – Determination of ß-glucan in barley and oats by streamlined enzymic method: Summary of collaborative study. ***Journal of AOAC International*** 80, 580-583.
25. MICEK P., 2008 – Nutritional usefulness to ruminants of grain of Polish cereal species and cultivars. ***Scientific Papers of University of Agriculture in Krakow***. Dissertations 449:1-127. In Polish, with English summary.
26. MOREIRA C.G., BUENO I.C.S., MENEZES M.L., MOTA T.P., SOUZA A.D., TAVARES A.F., AUGUSTO L.S., BRANDI R.A., 2015 – Palatability and digestibility of horse diets containing increasing levels of citrus pulp. ***Revista MVZ Córdoba*** 20, 4544-4555.
27. NRC., 2007 – Nutrient requirement of horses, 6th ed. The National Academies Press, Washington, DC, US.
28. ROSENFELD I., AUSTBØ D., 2009a – Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. ***Animal Feed Science and Technology*** 150, 249-258.
29. ROSENFELD I., AUSTBØ D., 2009b – Effect of type of grain and feed processing on gastrointestinal retention times in horses. ***Journal of Animal Science*** 87, 3991-3996.
30. ROWE J.B., BROWN W., BIRD S., 2011 – Safe and effective grain feeding for horses. ***Rural Industries Research and Development Corporation, Kingston***, ACT, Pub. Vol. 01, no. 148.
31. ROWE J.B., MAY P.J., CROSBIE G., 2001 – Knowing your oats. ***Recent Advances in Animal Nutrition in Australia*** 13, 211-221.

32. SARKIJARVI S., SAASTAMOINEN M., 2006 – Feeding value of various processed oat grains in equine diets. ***Livestock Science*** 100, 3-9.
33. SMOLDERS E.A.A., STEG A., HINDLE V.A., 1990 – Organic matter digestibility in horses and its prediction. ***Netherlands Journal of Agricultural Science*** 38, 435-447.
34. STATSOFT., 2011 – STATISTICA (data analysis software system), version 10. www.statsoft.com.
35. TAGLIAPIETRA F., CATTANI M., HANSEN H.H., HINDRICHSEN I.K., BAILONI L., SCHIAVON S., 2011 – Metabolizable energy content of feeds based on 24 or 48 h in situ NDF digestibility and on in vitro 24 h gas production methods. ***Animal Feed Science and Technology*** 170, 182-191.
36. ZEYNER A., KIENZLE E., 2002 – A method to estimate digestible energy in horse feed. ***Journal of Nutrition*** 132, 1771-1773.