animals

MDPI

Review

Supplementary File: In Vitro Techniques Using the Daisy^{II} Incubator for the Assessment of Digestibility: A Review

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Table S1. Practical recommendations on the use of the Ankom Daisy^{II} incubator (AD^{II}).

		Step	Further Researches
1	**Sample preparation**		
1a	Pre-rinse F57 filter bags in acetone for 3–5 ′ and completely air-dry		
2	Weight samples; 0.250 g (or 0.500) ±0.05, in triplicate	1. Number the bags with a solvent resistant marker 2. Homogenized sample 3. Weight directly into filter bag 4. Clean the top edge of the bag sealing area to avoid sample particles	Test F58 filter bags for AD^{II} [28]
2a	Seal the bags with heat sealer; set 4–5 for 2–3 ″	5. Seale no more than 4 mm from the open end of F57	
2b	Place bags in the jar with a blank bag (up to 25 samples)	6. Distribute bags on both sides of the perforated agitator baffle 7. Place the same sample in three different jars to reduce influence of position of the jar in the incubator 8. Use a standard in each jar	Study the effects of jar position in AD^{II}
3	Prepare and pour the buffer solutions in the digestion jars	9. Do not store the buffer for further analysis 10. Adjuste pH at 6.8 (at 39 °C)	Define a buffer solution to compare experiments
4	Turn on heat and agitation switches	11. Allow temperature of digestion jars to equilibrate for at least 20–30 ′	Modify AD^{II} to allow the same speed of rotation of all jars
5	Rumen fluid collection	12. Use donor animals with feed-controlled diets 13. Maintain anaerobic conditions and temperature (39 °C) 14. Filter rumen fluid with cheese clothes	Define a standard protocol to collect, transport and store the inoculum.
6	Add 400 mL rumen fluid to each jar	15. Purge the jar with CO_2 for 30 ″	
7	Incubate 48 h	16. Check the rotation of jars and the temperature (39 °C)	
8	Remove jars and drain fluid	17. Rinse bags with cold tap water until water is clear	Standardize the washing of bags (type and time)
9	Oven dry and weight samples	18. Store the bags in a freezer for postponed analysis	

		Formulae
10a	$TDMD_{AD^{II}}$/FM)	%: $100 - (W_4 - (W_1 \times C_1)) \times 100 / W_2$
10b	$TDMD_{AD^{II}}$/DM	%: $100 - (W_4 - (W_1 \times C_1)) \times 100 / (W_2 \times$ DM g/g) or $100 - ((100 - NDFD) \times (NDF / 100))$
		g/g: $1 - (W_4 - (W_1 \times C_1)) \times 1000 / (W_2 \times$ DM g/Kg)
		g/kg: $1000 - (W_4 - (W_1 \times C_1)) \times 1000 / (W_2 \times$ DM g/g)
10c	$ADMD_{AD^{II}}$/FM	%: $100 - (W_3 - (W_1 \times C_1)) \times 100 / W_2$
11d	NDFD/NDF	%: (NDF_{OM} *ai.* − NDF_{OM} *pi.*) × 100 / NDF *ai.*
		%: $100 - (W_3 - (W_1 \times C_1)) \times 100 / (W_2 \times$ (NDF *ai.* g/g FM))
		g/g: $1 - (W_3 - (W_1 \times C_1)) \times 1000 / (W_2 \times$ NDF *ai.* g/kg FM)
		g/kg: $1000 - (W_3 - (W_1 \times C_1)) \times 1000 / (W_2 \times$ NDF *ai.* g/g FM)
		$1 - ((100 - TDMD) / NDF\%DM\ ai.)) \times 100$
11e	dNDF/DM	%: % NDF%DM *ai.* × (NDFD / 100)

AD^{II} = Ankom Daisy^{II} Incubator; $ADMD_{AD^{II}}$ = apparent dry matter digestibility measured with AD^{II}; dNDF = digestible neutral detergent fiber; FM= fresh matter; NDFD = neutral detergent fiber digestibility; $TDMD_{AD^{II}}$ = true dry matter digestibility measured with AD^{II}; TDMD = true dry matter digestibility. W_1: F57 weight; W_2: sample weight (without the bag) *ante*-incubation; W_3: sample weight (with the bag) *post*-incubation; W_4: sample weight (with the bag) NDF treatment; C1: F57 *ante/post* incubation (final oven-dried weight/original blank bag weight); *ai.*: *ante-* incubation; *pi.*: *post*-incubation.

Table S2. Variability (CV, %) of the Ankom Daisy^{II} incubator (AD^{II}) after 48 h of incubation.

Variability	ADMD	TDMD	NDFD	AOMD	TOMD	Ref.
Instrument	3.6	4.1	14.6	6.2	4.5	[23]
			2.8			[89]
			9.1			[90]
Within laboratory			6.8			[91]
Between laboratory			10.5			[91]
Within run		14.8	23.6			[87]
Between run		4.7	12.4			[87]
Between jars		2.6				[72]
Within sample		<1–3	2–4.5			[21]
		3.5				[72]

ADMD = apparent dry matter digestibility; TDMD = true dry matter digestibility; NDFD = neutral detergent fiber digestibility; AOMD = apparent organic matter digestibility; TOMD = true organic matter digestibility.

Table S3. Linear equation between Ankom Daisy^{II} incubator (AD^{II}) at 48 h and other digestibility systems.

Instrument	In vivo (vv)	In situ (is)	Tilley and Terry (TT)	Samples	Ref.
AD^{II}	$ADMD_{vv} = 0.335 + 0.402 \times TDMD_{AD^{II}}$ ($r^2 = 0.85\%$, n = 24, $p < 0.001$)	$TDMD_{is} = 0.27 + 0.904 \times TDMD_{AD^{II}}$ ($r^2 = 0.81\%$, n = 115, $p < 0.001$)	$ADMD_{TT} = 0.101 + 0.641 \times TDMD_{AD^{II}}$ ($r^2 = 0.63\%$, n = 115, $p < 0.001$)	Meadow hay, fine fescue straw	[87]
			$ADMD_{TT} = 8.88 + 0.84 \times ADMD_{AD^{II}}$ ($r_2 = 0.81$, n = 17 SD = ±6.24)	Forages, concentrates, protein supplements	[73]
		$NDFD_{is} = 6.39 + 0.74 \times NDFD_{AD^{II}}$ ($r_2 = 0.94$, n = 18, SD = ±1.96)		Hays	[93]
		$NDFD_{is} = 7.31 + 0.95 \times NDFD_{AD^{II}}$ ($r_2 = 0.98$)		Hays	[89]

ADMD = apparent dry matter digestibility; TDMD = true dry matter digestibility; NDFD = neutral detergent fiber digestibility.

Table S4. Acronyms for digestibility trials.

Parameter	Acronyms		Measured
Apparent dry matter digestibility	ADMD	$ADMD_{vv}$	In vivo
		$ADMD_{vt}$	In vitro
		$ADMD_{AD^{II}}$	AD^{II} Incubator procedure
		$ADMD_{TT}$	TT procedure
True dry matter digestibility	TDMD	$TDMD_{is}$	In situ
		$TDMD_{vt}$	In vitro
		$TDMD_{AD^{II}}$	AD^{II} Incubator procedure
Neutral detergent fiber digestibility (% NDF)	NDFD	$NDFD_{vv}$	In vivo
		$NDFD_{is}$	In situ
		$NDFD_{vt}$	In vitro
		$NDFD_{AD^{II}}$	AD^{II} Incubator procedure
		$NDFD_{TT}$	TT procedure
Digestible NDF (% DM)	dNDF	$dNDF_{is}$	In situ
		$dNDF_{vt}$	In vitro
		$dNDF_{AD^{II}}$	AD^{II} Incubator procedure
Apparent organic matter digestibility	AOMD	$AOMD_{vv}$	In vivo
		$AOMD_{is}$	In situ
		$AOMD_{vt}$	In vitro
		$AOMD_{AD^{II}}$	AD^{II} Incubator procedure
True organic matter digestibility	TOMD	$TOMD_{vv}$	In vivo
		$TOMD_{is}$	In situ
		$TOMD_{vt}$	In vitro
		$TOMD_{AD^{II}}$	AD^{II} Incubator procedure