



**INFLUENCE OF CUTTING AGE ON CHEMICAL COMPOSITION, RUMEN DEGRADATION KINETICS AND *in vitro* DIGESTIBILITY OF GREEN HYDROPONIC FODDER OF *Avena sativa*<sup>†</sup>**

**[INFLUENCIA DE LA EDAD DE CORTE SOBRE LA COMPOSICIÓN QUÍMICA, CINÉTICA DE DEGRADACIÓN RUMINAL Y DIGESTIBILIDAD *in vitro* DE FORRAJE VERDE HIDROPÓNICO DE *Avena sativa*]**

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#### SUMMARY

The aim of this research was to evaluate the influence of cutting age on chemical chemistry, rumen degradation kinetics and *in vitro* digestibility of hydroponic green forage of *Avena sativa*. The treatments were: T1: 8 days, T2: 11 days, T3: 14 days and T4: 17 days after germination. The DM showed a linear increase ( $P = 0.0034$ ) at the harvest days. The NDF was higher in treatments T3 and T4 (457.2 and 479.1 g/kg DM respectively). The OM, AFD, GE and the digestibility of the DM and OM showed no differences ( $P > 0.05$ ) between treatments. The ruminal degradation of DM fractions A, B and A + B showed no differences ( $P > 0.05$ ). The degradation rate (c% h1) was higher in T2. The effective degradation showed a linear reduction ( $P < 0.05$ ) as the days to cut increased. The degradation of the OM fraction A was reduced linearly ( $P < 0.05$ ) within days at the cut. Fraction B showed no differences ( $P > 0.05$ ) between treatments and the degradation rate (c% h1) was higher ( $P = 0.0009$ ) in T2. The effective degradation decreased linearly ( $P = 0.0001$ ) as the days to cut increased. The CP fraction A was higher ( $P = 0.0001$ ) in T3 (888.0 g / kg DM). Fraction B and degradation rate (c% h1) was higher in T1 and T4. The effective degradation (0.02% k) showed no differences ( $P > 0.05$ ), at 0.05% k it was higher in T1, T2 and T3. And at 0.08% k was higher in T2 and T3. The degradation of the FDN fraction A was higher in T1, T3 and T4. Fraction B and A + B showed no differences ( $P > 0.05$ ) between treatments. The degradation rate (c% h1) was higher in T1 and T2. And the effective degradation showed no differences ( $P > 0.05$ ) between treatments. It's concluded that the optimum harvest time to achieve a better chemical composition and greater ruminal digestion is between the 11 days of harvest of the hydroponic green forage of *A. sativa*.

**Keywords:** hydroponic green fodder; degradation; *Avena sativa*; digestibility.

#### RESUMEN

El objetivo de la investigación fue evaluar la influencia de la edad de corte sobre la composición química, cinética de degradación ruminal y digestibilidad *in vitro* de forraje verde hidropónico de *Avena sativa*. Los tratamientos fueron: T1: 8 días, T2: 11 días, T3: 14 días y T4: 17 días de corte posterior a la germinación. La MS mostró un incremento lineal ( $P=0.0034$ ) a los días de cosecha. La FDN fue mayor en los tratamientos T3 y T4 (457.2 y 479.1 g/kg MS respectivamente). La MO, FDA, EB y la digestibilidad de la MS y MO no mostraron diferencias ( $P>0.05$ ) entre tratamientos. La degradación ruminal de MS fracción A, B y A+B no mostraron diferencias ( $P>0.05$ ). La tasa de

<sup>†</sup> Submitted May 26, 2017 – Accepted July 30, 2018. This work is licensed under a CC-BY 4.0 International License.  
ISSN: 1870-0462

degradación ( $c \%h^{-1}$ ) fue mayor en T2. La degradación efectiva presentó una reducción lineal ( $P < 0.05$ ) a medida que incrementa los días al corte. La degradación de la MO fracción A se redujo de forma lineal ( $P < 0.05$ ) a los días al corte. La fracción B no mostró diferencias ( $P > 0.05$ ) entre tratamientos y la tasa de degradación ( $c \%h^{-1}$ ) fue mayor ( $P = 0.0009$ ) en T2. La degradación efectiva se redujo linealmente ( $P = 0.0001$ ) a medida que se incrementaba los días al corte. La PC fracción A fue mayor ( $P = 0.0001$ ) en T3 (888.0 g/kg MS). La fracción B y tasa de degradación ( $c \%h^{-1}$ ) fue mayor en T1 y T4. La degradación efectiva (0.02 % k) no mostró diferencias ( $P > 0.05$ ), al 0.05 % k fue mayor en T1, T2 y T3. Y al 0.08 % k fue mayor en T2 y T3. La degradación de la FDN fracción A fue mayor en T1, T3 y T4. La fracción B y A+B no mostraron diferencias ( $P > 0.05$ ) entre tratamientos. La tasa de degradación ( $c \%h^{-1}$ ) fue mayor en T1 y T2. Y la degradación efectiva no mostró diferencias ( $P > 0.05$ ) entre tratamientos. Se concluye que el tiempo óptimo de cosecha para lograr una mejor composición química y una mayor digestión ruminal es entre los 11 días de cosecha del forraje verde hidropónico de *A. sativa*.

**Palabras clave:** forraje verde hidropónico; degradación; *Avena sativa*; digestibilidad.

## INTRODUCTION

The feeding of ruminants in the tropics is based mainly on grazing of monocultures of grasses that contain high levels of fiber and low protein, which leads to a decrease in animal productivity (Barros-Rodríguez *et al.*, 2015). The association of limiting factors such as small areas for forage production, prolonged droughts, poor quality seeds and inappropriate management of pastures, contribute to the fluctuating availability of quality forage throughout the year (Naik *et al.*, 2015).

In this context, the production of hydroponic green fodder (HGF) is considered as an alternative in the production of feed for ruminants with the purpose of covering the nutritional needs and improving the productive behavior of the animals, as well as optimizing the use of natural resources (earth, water). Hydroponics is an agricultural technique that guarantees the permanent obtaining of green fodder throughout the year (Ata, 2016). The HGF is obtained as response to the germination of grains in a period of 9 to 15 days with great nutritional qualities (Maldonado Torres *et al.*, 2013), and requires small areas for its production (Emam, 2016). Promising results on dry matter consumption, feed conversion efficiency, nutrient digestibility, growth rate and weight gain have been reported with a feed based on FVH in goats, calves and lambs (Sánchez Del Castillo *et al.*, 2013; Gebremedhin, 2015; Verma *et al.* 2015; Ata, 2016; Acosta Lozano *et al.*, 2016). Effects attributed to the high content of soluble carbohydrates and their high digestion (Farghaly *et al.*, 2019).

However, it is essential to determine the appropriate germination age to take full advantage of the qualities of hydroponic forage, since this is related to the nutrient content, as forage development progresses (Fouad and Rehab, 2015). Fazaeli *et al.*, (2012) indicate that as the period of germination of barley grains extended (6 to 8 days), the NDF, ADF, CP and ashes increased. Peer and Leeson, (1985) found decreasing values in the digestibility of DM, as the

development of hydroponic barley fodder continued (7 to 8 days). Therefore, studies are needed to establish the effects in terms of germination time of HGF, directed towards the development of strategic foods for tropical areas. Based on the above, the objective of the present work was to determine the effect of four harvest times of hydroponic green fodder of *Avena sativa* on rumen degradation and *in vitro* digestion.

## MATERIALS AND METHODS

### Location

The trial was carried out at the Faculty of Agricultural Sciences of the Technical University of Ambato, Cevallos, Tungurahua – Ecuador.

### Experiment Management

Seeds of *Avena sativa* L, variety INAIIP 82 with 95% germination were used, which were pre-germinated in a bowl with water for 24 hours. The disinfection was performed with a 5% sodium hypochlorite solution for 10 minutes, then the seed was washed with running water.

Germination was carried out in polyethylene trays inside a greenhouse, with a sowing density of 6.4 kg per m<sup>2</sup> maintaining a temperature of approximately 20 °C. Watering were made every 6 hours at a dose of 1 liter of water per m<sup>2</sup>; From the first day of germination (4 days after sowing) up to two days before the forage harvest, nutritive applications were made with a formulation: 192.84 mg L<sup>-1</sup> of N, 24.9 mg L<sup>-1</sup> of P, 190 mg L<sup>-1</sup> of K, 100 mg L<sup>-1</sup> of Ca and 67 mg L<sup>-1</sup> of Mg, as established by Rodríguez (2003), cited by (Fuentes-Carmona *et al.*, 2011).

### Harvest and sample collection

The harvest was carried out according to the days assigned for each treatment in the following order: T1: 8 days, T2: 11 days, T3: 14 days and T4: 17 days

after germination. About 3 kg of forage harvested on days 8, 11, 14, 17 were taken for each repetition (n=5) and dried in an oven at 60 °C until constant weight to determine dry matter. The dried material was crushed in a hammer mill, subsequently screened until a homogeneous particle of 1 mm was obtained to perform the different analyzes.

### Response variables

*Chemical analysis of nutrients:* It was estimated following the methods of the AOAC (1990) for dry matter (DM) and ashes. The content of ADF, NDF was determined according to method 12 and 13 of fiber digestion ANKOM<sup>2000</sup>, the energy content by means of a calorimetric pump and the protein by means of elementary analysis of (N) using the LECO 268 (LECO Corporation) equipment.

*In situ degradation of nutrients:* It was evaluated using the nylon bag technique in the rumen described by Ørskov *et al.*, (1980). Six bulls provided with a fistula in the rumen were used, two bags were incubated with 5g of DM of each treatment at intervals of 0, 3, 9, 12, 48 and 72 h. After 72 h the bags were removed, washed with running water, dried at 60 °C. The bags used to measure the loss by washing (0 h), were not incubated in the rumen and only washed with running water. The residues were stored in polyethylene bags at -4 °C until further analysis in the laboratory. The disappearance of nutrients was calculated as a proportion of the incubated and residual material. The data were adjusted to the equation:  $Y = a + b(1 - e^{-ct})$  and the effective degradation was adjusted using the equation  $ED = a + [(b * c) / (c + k)]$  considering a rate of 2, 5

and 8% passage (Ørskov and McDonald, 1979; Prism 4, GraphPad Software, Inc. of San Diego, CA, USA.).

### *In vitro* Apparent digestibility of DM and OM

The ruminal content (liquid and solid fractions) was obtained separately from 7 cannulated bulls before feeding and kept at 39 °C in a sealed plastic container; He was immediately transferred to the laboratory for processing, in a time no longer than 1 hour after collection. A medium rich in nitrogen (artificial saliva) was prepared according to Menke and Steingass (1988). 0.5 g of DM was taken from each treatment and repetition and placed in glass bottles (100 mL nominal capacity). Then 60 mL of ruminal inoculum (70:30 medium/ruminal inoculum) was added under a constant CO<sub>2</sub> flow. The jars were sealed and incubated at 39-40 °C. For each treatment, 7 glass bottles (repetitions) and 3 additional bottles as white were used. At the end of the incubation, *in vitro* digestibility (DM and OM) was estimated by filtering the residues and correcting with the residual DM and OM in the blanks.

### Experimental design

A completely randomized design was used, with four treatments (harvest times), seven repetitions for *in situ* degradation and *in vitro* digestibility. All data were analyzed according to the GLM PROC model. The comparison of means of the variables was performed using the Tukey test and finally, an orthogonal polynomial transformation of the data was performed to see if the response is linear or quadratic (SAS 2009).

Table 1. Chemical composition and *in vitro* digestibility of GHF (g/kg DM except where otherwise mentioned)

	Treatments				SEM	P	Contrasts	
	T1	T2	T3	T4			linear	quadratic
DM	921.6b	945.6ab	952.9a	952.8a	9.21	0.0012	0.0034	0.0578
OM	963.0a	962.1a	955.4a	950.9a	5.98	0.0634	0.0745	0.0598
NDF	434.2b	414.5c	457.2ab	479.1a	2.01	0.0001	0.0510	0.0345
ADF	206.3a	200.4a	212.1a	218.6a	2.14	0.4561	0.0647	0.0589
GE (kcal)	45.9a	46.1a	45.8a	45.9a	0.122	0.0765	0.0854	0.0679
<i>In vitro</i> digestibility								
IVDDM	614.1a	623.8a	603.6a	599.1a	12.37	0.5068	0.2500	0.5703
IVDOM	640.4a	667.8a	643.0a	636.8a	12.20	0.2881	0.5198	0.1825

<sup>a,b,c</sup> Means with different letters between rows differ significantly (P <0.05). SEM: standard error of the mean. DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, GE: Gross energy, IVDDM: *In vitro* digestibility of dry matter, IVDOM: *In vitro* digestibility of organic matter, T1: 8, T2: 11, T3: 14, T4: 17 days of harvest.

## RESULTS

### Chemical composition and *in vitro* digestibility

The DM showed a linear effect ( $P=0.0034$ ) with respect to the treatments with longer germination days ranging between 92 and 95% (T1 and T4 respectively). In the FDN a quadratic response was obtained ( $P=0.0345$ ). with respect to the OM, ADF, GE, IVDDM and IVDOM showed no difference ( $P>0.05$ ) between the evaluated treatments (Table 1).

### *In situ* rumen degradation of DM and OM

The parameters of ruminal degradation of the DM in fraction A and B, as well as, the degradation potential A+B showed no differences between treatments ( $P>0.05$ ). However, the percentage degradation rate per hour ( $c$ ) and the effective degradation was higher ( $P<0.05$ ) in T1 and T2 compared to the other treatments. The ruminal degradation of the OM in fraction (A) was higher ( $P=0.0412$ ) in treatments T1, T2 and T3 (61, 58 and 59% respectively). The soluble but potentially degradable fraction (B) and the degradation potential A+B showed no differences ( $P>0.05$ ) between the evaluated treatments. However,

the percentage degradation rate per hour ( $c$ ) showed a quadratic effect ( $P=0.0133$ ), and the effective degradation at the different passage rates showed a downward linear response ( $P<0.0001$ ) to treatments (Table 2).

### Rumen degradation of CP and NDF

The parameters of rumen degradation of the crude protein in the soluble fraction (A) were higher ( $P=0.0001$ ) in the T3 treatment (88%). The insoluble but potentially degradable fraction (B), degradation rate in % per hour ( $c$ ) and degradation potential (A+B) showed linear and quadratic effect to the treatments ( $P<0.05$ ). The degradation of the NDF in the soluble fraction (A) was higher ( $P=0.0043$ ) in treatments T1, T3 and T4 (29, 27 and 30% respectively), and the degradation rate in % per hour ( $c$ ) was higher ( $P=0.0028$ ) in T2 (0.051%) compared to the other treatments. Regarding the insoluble but potentially degradable fraction (B), degradation potential (A+B) and effective degradation did not show differences ( $P>0.05$ ) between treatments evaluated (Table 3).

Table 2. Degradation kinetics of DM and OM (g/kg DM) of hydroponic green fodder of *Avena sativa*

	Treatments				ESM	P	Contrasts	
	T1	T2	T3	T4			linear	quadratic
Degradation DM								
A	613.4a	592.2a	593.5a	584.1a	8.70	0.1386	0.0371	0.5109
B	242.3a	242.7a	246.0a	248.0a	8.51	0.9519	0.5876	0.9676
$c$	0.028b	0.059a	0.028b	0.028b	0.0059	0.0031	0.2841	0.0193
A+B	855.8a	835.0a	840.4a	832.0a	11.72	0.5010	0.2228	0.6027
Effective degradation *								
0.02	750.8ab	768.1a	729.3bc	727.8c	5.61	0.0001	0.0004	0.1093
0.05	698.0ab	719.0a	677.1bc	673.1c	6.10	0.0001	0.0004	0.0540
0.08	674.8ab	691.8a	654.3bc	648.8c	5.92	0.0002	0.0003	0.0719
Degradation MO								
A	617.0a	588.6ab	594.1ab	580.6b	8.58	0.0412	0.0139	0.3937
B	249.6a	250.1a	257.9a	258.2a	8.90	0.8350	0.4068	0.9926
$c$	0.028b	0.060a	0.026b	0.028b	0.0057	0.0009	0.2111	0.0133
A+B	866.6a	838.7a	852.1a	838.9a	12.13	0.3374	0.2134	0.5529
Effective degradation *								
0.02	757.8a	772.3a	732.0b	728.3b	5.50	0.0001	0.0001	0.1148
0.05	703.6a	722.0a	678.0b	671.6b	5.97	0.0001	0.0001	0.0522
0.08	679.8a	693.6a	654.8b	646.8b	5.87	0.0001	0.0001	0.0779

<sup>a,b,c</sup> Means with different letters between rows differ significantly ( $P < 0.05$ ). ESM: standard error of the mean. A: soluble fraction, B: soluble but potentially degradable fraction,  $c$ : degradation rate in percentage per hour, A+B: ruminal degradation potential. L: linear, C: quadratic. T1: 8, T2: 11, T3: 14, T4: 17 days of harvest. \*: Passage rate (k) per hour.

## DISCUSSION

### Chemical composition

The results obtained in the DM and NDF content (Table 1) were probably due to the increase in structural carbohydrates as germination days increased (Fazaeli *et al.*, 2012; Acosta Lozano *et al.*, 2016). These results are consistent to those reported by Herrera-Torres *et al.*, (2010).

### Rumen Digestion

Regarding the results obtained in both *in vitro* digestibility of DM and MO, and rumen degradation of nutrients (Table 1 and 2), it was possibly due to an increase in structural carbohydrates as the days of harvest increase, this influences the nutritional characteristics of the fodder and with it, the action of the microorganisms of the rumen to degrade the nutrients (Herrera-Torres *et al.*, 2010; Garcez *et al.*, 2016; Acosta *et al.*, 2016). However, the result observed in the effective degradation of the NDF was possibly due to the high protein content that the GHF possesses, which by action of the degradation at the ruminal level releases peptides, amino acids, NH<sub>3</sub> and branched short chain fatty acids. These compounds

can improve the function of the rumen and with it, the degradation of the fiber. Due to the stimulation on microbial growth of the rumen in general, but specifically of cellulolytic and proteolytic bacteria (Hoover and Stokes 1991; Barros-Rodríguez *et al.*, 2017). These results are consistent with those respected by Pérez *et al.* (2012) and Barros-Rodríguez *et al.* (2015).

## CONCLUSIONS

Under the conditions of this study, it is concluded that the optimum harvest time to achieve a better chemical composition and greater ruminal digestion is between the 11 days of harvest of the hydroponic green forage of *Avena sativa*.

### Acknowledgments

The authors thank the financial support from Research and Development Direction (DIDE, acronym in Spanish) of the Technical University of Ambato, Ecuador, project (PFCAGP19).

### Conflict of interest

The authors declare that they have no conflict of interest

Table 3. Degradation of neutral detergent fiber (NDF) and effective degradation of hydroponic green fodder of *Avena sativa*

	Treatments				SEM	P	Contrasts	
	T1	T2	T3	T4			linear	quadratic
Degradation CP								
A	835.1c	862.5b	888.0a	831.7c	3.327	0.0001	0.0001	0.8102
B	128.0a	79.0b	67.9b	128.4a	3.562	0.0001	0.0001	0.0003
c	0.085b	0.119a	0.050c	0.090b	0.005	0.0001	0.0003	0.0001
A+B	963.2a	941.6c	955.9b	960.3ab	1.678	0.0001	0.0057	0.0001
Effective degradation *								
0.02	938.7a	929.9b	936.6a	936.8a	1.608	0.0052	0.3632	0.0008
0.05	915.8ab	918.3ab	922.1a	914.4b	1.653	0.0180	0.0135	0.7515
0.08	901.2b	909.6a	914.3a	899.8b	1.712	0.0001	0.0001	0.3799
Degradation NDF								
A	298.7a	225.9b	276.1ab	301.3a	14.25	0.0043	0.3731	0.0026
B	511.3a	475.6a	505.6a	429.4a	26.97	0.1571	0.0889	0.4612
c	0.019b	0.051a	0.022b	0.025b	0.0056	0.0028	0.6491	0.0196
A+B	810.7a	701.6a	781.7a	730.8a	34.27	0.1383	0.3103	0.4064
Effective degradation *								
0.02	533.1a	546.8a	517.8a	534.8a	9.67	0.2417	0.5851	0.8649
0.05	432.6a	449.8a	417.3a	442.0a	10.95	0.2145	0.9277	0.7356
0.08	393.0a	399.1a	376.5a	402.0a	10.47	0.3401	0.9272	0.3673

<sup>a,b,c</sup> Means with different letters between rows differ significantly ( $P < 0.05$ ). ESM: standard error of the mean. A: soluble fraction, B: soluble but potentially degradable fraction, c: degradation rate in percentage per hour, A + B: rumen degradation potential. L: linear, C: quadratic. T1: 8, T2: 11, T3: 14, T4: 17 days of harvest. \*: Passage rate (k) per hour.

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