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RESEARCH ARTICLE

Comparison of *İn-Vitro* Digestibility of Commonly Used Forage and Concentrate Feeds in Dairy Buffalo, Cow and Sheep by Using Daisy Incubator

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ABSTRACT

In this study was evaluated in-vitro true dry matter (DM) digestibility of 8 different feedstuffs by using in vitro system. Eight different feeds were grouped as roughage and concentrate and tested by using Buffalo, cow and sheep inoculums.. The experiment was replicated on three different timelines for all feeds and the three inoculum sources. The incubation time for the digestibility was 48 hours for each species inoculum. In the study, rumen fluids from different animals did not have a significant effect on the in vitro true? dry matter (DM) digestibility of different feedstuffs during the 48-hour incubation period (P>0.05). The pellet wheat bran showed the lowest mean value in sheep among all feedstuffs. It was concluded that the daisy incubator method could be used to predict the true digestibility of different feedstuffs in different species animals.

Keywords: Concentrate, daisy, dry matter, digestibility, feed, ruminant

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Sütçü Manda, İnek ve Koyunlarda Yaygın Olarak Kullanılan Kaba ve Konsantre Yemlerin *İn-Vitro* Sindirilebilirliğinin Daisy İnkübatör Kullanılarak Karşılaştırılması

ÖZ

Bu araştırmada, manda, inek ve koyunlarda in vitro sistem kullanılarak 8 farklı yem maddesinin in vitro gerçek (NDF) sindirilebilirliği değerlendirilmiştir. Yemler, kaba ve konsantre olarak gruplandırıldı ve manda, sığır ve koyun rumen sıvıları kullanılarak test edildi. Araştırmada, tüm yemler için, üç tekerrür, üç farklı inokulum kaynağı (rumen sıvısı) kullanıldı. Sindirilebilirlik için inkubasyon süresi her tür rumen sıvısı için 48 saatti. Araştırmada, farklı hayvanlara ait rumen sıvılarının, 48 saatlik inkubasyon süresi boyunca yem maddelerinin in vitro gerçek? kuru madde sindirilebilirliği üzerinde önemli bir etkisi olmamıştır (P>0.05). Pelet buğday kepeği, sindirilebilirlik değeri açısından, diğer yem maddelerine göre koyunlarda en düşük değeri vermiştir. Sonuç olarak, in vitro sindirilebilirlik deneme yönteminin, farklı hayvan türlerinde yemlerin gerçek sindirilebilirliğini tahmin etmek için kullanılabileceği kanısına varılmıştır.

Anahtar kelimeler: Daisy, kuru madde, sindirilebilirlik, ruminant, yem

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INTRODUCTION

The rumination and peristalsis of the gastrointestinal tract are triggered by the fibers included in the animal's diet. Chewing lessens the particle size of the engulfed feed, enhances the microbial attachment by improving the surface area, and maintains the rumen pH by affecting saliva production (Van Soest, 1994). Better peristalsis ensures the polite rumen movements for effective digestibility of the feedstuffs. It provides a homogenous environment for good bioavailability by particle retention and efficient gut motility by the outflow from the rumen (Van Soest, 1994). The particle size, density, palatability, freshness, and digestibility of fibers are physical parameters that control the rumen fill and dry matter intake (DMI) (Conrad et al., 1964). The DMI and gut filing are a more conscious determinant when *ad-libitum* feed is offered and during the first lactation phase (Allen & Piantoni, 2014). Rumen fill promotes rumination by stimulating the pressure and stretching receptors in the reticulum and rumen wall (Allen & Piantoni, 2014). Moreover, the effect of amylase and sodium-treated with ash correction ($aNDF_{om}$) digestibility on dry matter intake was studied by Kendall et al. (2009). The main fibrous components in feedstuffs are cellulose, hemicellulose, and pectin. Even after a long time, the protein that remains indigested by ruminal microorganisms is subtracted from the NDF to measure the potentially digestible NDF (Nousiainen et al., 2004). It is very important to measure the total tract digestibility (Huhtanen et al., 2006), rumen fill (Krizsan & Huhtanen, 2013), and DMI (Cotanch et al., 2014). For some dynamic rumen models such as Cornell Net Carbohydrate and Protein System (CNCPS), indigestible NDF is a helping tool (Fox et al., 2004; Tylutki et al., 2008; Van Amburgh et al., 2015). Lignin is understood as a major fraction of indigestible fiber (Besle et al., 1994).

With the advancement in human population, climatic circumstances, and scarcity of water resources, animal feed is being sold at high prices in many countries (Ajila et al., 2012). The agricultural by-products found after processing fruits, vegetables, crops, and nuts are valuable resources that overwhelm this threat (Rojas-Downing et al., 2017). By-product feedstuffs are more abundant and economical energy and fibrous sources for livestock (Devendra & Sevilla, 2002). Subsequently, the animals could be fed effectively without disturbing the human need for food (Odum et al., 2018). The in vitro methods are efficiently used to evaluate the quality and digestibility of the different feedstuffs offered to the ruminants (Getachew et al., 1998). The most precise and applied research technique available for accessing ruminant digestibility (Goldman et al., 1987). The strategy has been modified and adjusted for starch feedstuff examination (Holden, 1999). A few analysts have value-added its calculation precision (Mabjeesh et al., 2000). Distinctive dilution buffers for the rumen alcohol have been created to alter the pH of the inoculum (Tylutki et al., 2008).

The display considers assessed the in-vitro genuine $aNDF_{om}1$ digestibility of diverse feedstuffs for buffalo, cattle, and sheep after 48 hours of incubation (% of NDF/dry matter). This study was carried out to measure their in vitro (real) digestibility of different by-product feeds by using different ruminant inoculants.

MATERIAL and METHODS

The study was conducted at the dep. of Animal Nutrition, Faculty of Veterinary Medicine, University of Afyon Kocatepe. All procedures were approved by the local ethics committee (No: 495337002-07, Date: 14/01/2019).

Feedstuff Collection and Sample Preparation

Eight different feedstuffs were selected for the study, including dried tomato leaves (coarse), dried tomato leaves (fine), hazelnut, pellet wheat bran, grape pulp silage, biscuits, bulgur bran pellet, and poppy seed meal. The feedstuffs were obtained from the local livestock farms in Afyonkarahisar, Turkey. The rumen fluid for the in vitro incubation was obtained from the cannulated dry dairy cattle and dry dairy buffalo in the Education Research and Practice Farm, Faculty of Veterinary Medicine, Afyon Kocatepe University. Rumen fluid of sheep was obtained from the local slaughterhouse after immediately slaughtering.

The small part of the feeds was grounded separately by using Variable Speed Rotor Mill Pulverisette 14 Premium. All of the samples were dried in a hot air oven. Grape pulp silage was put in the oven at 65-70 °C 48 h, and for other concentrate samples, 100-105 °C for overnight was provided. The dry matter (DM) values of the feedstuffs are determined by gravimetric analysis according to the Affiliation of Official Expository Chemists, 1997 (Official Strategies of Examination, 16th ed. (AOAC Universal: Washington, D.C.)

Daisy Incubator Filter Bags Preparation

After calculating the dry matter, F57 filter bags (ANKOM, Macedon, Unused York; U.S) were pre-rinsed in filtered acetone for three to five minutes and air-dried to maintain a strategic distance from underestimation of NDF assimilation of scrounge tests in ANKOM F57 packs (Adesogan 2005). Moreover, the acetone wash evacuates a surfactant that represses microbial assimilation. Each pack was labeled with a dissolvable solvent-resistant marker. The empty weight of each F57 bag was recorded. Each samples was weighed and recorded (0.25g~0.5 g) and heat-sealed employing a 200 mm Parker IS/7300H motivation sealer. One purge fixed clear sack was utilized for the redress calculation.

Buffer arrangement was based on that of Tilly and Terry (1963). (Table 1) with McDougall-manufactured spit modifications (last pH 6.8 at 39°C). In partitioned holders, 266 ml of arrangement B and 1330 ml of arrangement A were arranged agreeing to the equation concentration of the reagents. A rise to the sum of Buffer arrangements A and B was included in all of the four assimilation jugs with tests. Eight samples were picked for the … (IVNDF) digestibility, and two tests were included for each jolt.

Table 1. Solutions

Buffer Solution A reagents	Quantity, g/ liter
KH2PO4	10
MgSO4•7H2O	0.5
NaCl	0.5
CaCl2•2H2O	0.1
Urea (reagent grade)	0.5
Buffer Solution B reagents	
NA2CO3	15.0
NA2S•9H20	1.0
Neutral Detergent Solution	Quantity for 2 liter
In	120 gm
Triethyl Glycerol	20 gm
Sodium Sulfite	20 gm

After obtaining, the rumen fluid for each animal species (~2 L) was transported to the lab by storing in a tightly closed thermos that pre-warmed (38°C) with distilled water. Each of the jars was continuously gassed with CO_2 before and during the placement of samples. The incubator was operated at 39°C temperature. After 48 hours of incubation, the samples were removed from the jars and were left to dry in a room overnight. After the samples were dried, $aNDF_{om}$ values of samples with heat-stable α-amylase and sodium sulfite were determined according to Van Soest et al. (1991) using the FibreTherm apparatus (Fibretherm®, C. Gerhardt GmbH & Co. KG, C., Konigswinter, Germany). The fiber values were expressed without residual ash (Mertens 2002). The in vitro 48h NDF digestibility ($IVNDFD_{48}$) was calculated with the following formula:

$IVNDFD_{48}$ (DM basis) = 100 – (W3 - (W1 x C1)) x 100/ (W2 x DM, %)

Where:

W1 = Dried bag tare weight

W2 = Sample weight

W3 = Dried final bag weight after in vitro and sequential NDF treatment

C1 = Blank bag correction (final oven-dried weight/original blank bag weight)

Statistical Analysis

In vitro digestibility data of each feedstuff were analyzed using the Kruskal-Wallis nonparametric test with MedCalc statistical software (v 19.0.3; MedCalc Software bvba, Ostend, Belgium). Data were expressed in tables as $\pm SEM$. Statistical significance was declared at P<0.05.

RESULTS

The results showed that the digestibility of all the feedstuffs under trial had a non-significant effect. Moreover, it could be seen that in sheep, the mean value of the pellet wheat bran was the lowest among all species. In differentiation, the penetrability of the sacks and the test weight per sack surface range may disturb the (IVTD) values. The starch degradability from distinctive feedstuffs was higher when the benefactor dairy animals were encouraged a proportion containing 1:1 feed: concentrate (on a DM premise) than when eat less was based only on roughage. In-vitro true digestibility of feedstuffs for water buffalo, cattle and sheep after 48 hours of incubation were shown in Table 2.

Table 2. In-vitro true $aNDF_{om}$[1] digestibility of different feedstuffs for water buffalo, cattle and sheep after 48 hours of incubation, % (DM basis)

Item	Feedstuffs	Cattle		Buffalo		Sheep		P-Value
		Mean	SEM	Mean	SEM	Mean	SEM	
No.1	**Coarse Tomato leaf meal**	36.70	0.53	36.43	1.34	35.43	2.33	0.999
No.2	**Fine Tomato leaf meal**	29.24	2.03	23.64	2.93	17.61	0.25	0.102
No.3	**Hazelnut Meal**	24.97	0.13	27.59	7.45	20.52	0.49	0.368
No.4	**Pellet Wheat Bran**	35.39	2.33	39.47	1.99	12.62	3.16	0.156
No.5	**Grape Silage**	68.44	0.01	65.53	0.53	65.74	0.58	0.151
No.6	**Biscuit Bran**	46.68	0.56	44.24	1.41	37.48	1.19	0.101
No.7	**Bulgur Bran Pellet (cracked Wheat)**	29.49	2.35	25.59	0.45	21.35	1.29	0.102
No.8	**Poppy Seed Meal**	35.78	3.56	35.70	1.58	33.59	4.01	0.867

[1] NDF with heat-stable amylase treated and without residual

DISCUSSION

The in vitro method of surveying the digestibility of ruminant feedstuffs is utilized universally. The strategy is less demanding than in vivo studies and avoids the prerequisite of surgically planning creatures in totally different positions within the gastrointestinal tract. The IVTD decided by the Daisy strategy can be influenced by a few flows related to the packs utilized, nourish characteristics, and the device itself. One apparent advantage of the Daisy instrument over the in vivo strategy is the nonstop revolution of the maturation vessels, which efficiently mixes the assimilation inoculum amid the hatching period and eradicates the prerequisite for a time-consuming centrifugation step after brooding (Hogan & Flinn, 1999). These perspectives have been broadly surveyed for the in-situ strategy /DESI Hatchery (Nocek, 1988; Vanzant et al., 1998). Cone et al. (1989) found that the assortment of diets encouraged to the benefactor creature influences the values of in vitro degradability. In any case, the adaptation of the concentrated blend had as it were an immaterial impact on degradability values (Devendra & Sevilla, 2002; Richards et al., 1995). (Cone et al., 1989) appeared that the activity of rumen microflora, measured at diverse times after feeding, was higher in alcohol taken from dairy animals bolstered the next level of DM when bolsters were hatched for 6 hours. The pore measure of the sacks (50±15 µm) was inside the extent summarized by (Vanzant et al., 1998) for numerous considerations detailed within the writing. Test handling, especially crush measure, interatomic with pore estimate of the pack and influences the degree of nourish vanishing (Michalet-Doreau & Ould-Bah, 1992).

CONCLUSION

The daisy system is a more straightforward, less time-consuming method of measuring IVTD of ruminant feed. However, in the current study, the results for the IVTD % of eight feedstuffs were non-significant. Moreover, it is advised to conduct more trials to develop appropriate sample sizes and estimate the appropriate feedstuffs for the livestock according to their percentage of digestibility so that the economics for feed of livestock is improved.

Conflict of Interest: The authors declare no conflict of interest.

Authors' Contributions: Conceptualization, A.I. and I.B.; Methodology, Ü.Ö and E.E.G.; Investigation, S.R.A.S.; Resources, I.S.C. and I.B.; Data curation, A.Q Writing—original draft, A.I. and Ü.Ö.; Writing—review & editing, İ.B; Visualization, E.E.G.; Supervision, İ.S.Ç. and I.B. All authors have read and agreed to the published version of the manuscript.

Ethical Approval: This study was carried out at Afyon Kocatepe University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University (AKUHADYEK, Ref No: 49533702/07, Tarih: 01/2019). This study involves protecting the welfare of animal subjects.

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