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Modeling *in vitro* dry matter digestibility of oat (*Avena sativa*) fodder based on proximate and fiber fractions

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Abstract

The present investigation was conducted to evaluate the *in vitro* Dry Matter Digestibility (IVDMD) of oat (*Avena sativa* L.) fodder and to develop predictive models based on proximate and fiber fractions. Eight oat cultivars were studied at two growth stages (60 and 90 days after sowing) under a factorial randomized block design. The results revealed that IVDMD was significantly influenced by cultivar, growth stage, and their interaction ($p < 0.01$). The overall mean IVDMD was 61.47%, with a marked decline from 65.51% at 60 DAS to 57.42% at 90 DAS, indicating reduced digestibility with advancing maturity due to increased structural carbohydrate accumulation.

Among cultivars, RO-2000-19 exhibited the highest digestibility (67.07%), whereas RO-2000-16 recorded the lowest (54.23%), reflecting genotypic variation in fiber composition and degradability. Correlation analysis demonstrated that IVDMD was positively associated with crude protein ($r = 0.700$), ether extract ($r = 0.697$), and ash ($r = 0.653$), while it was negatively correlated with crude fibre ($r = -0.756$) and nitrogen free extract ($r = -0.678$). Furthermore, strong negative correlations were observed with detergent fiber fractions, particularly silica ($r = -0.864$), ADF ($r = -0.801$), and cellulose ($r = -0.774$), indicating their critical role in limiting digestibility.

Regression analysis revealed that the model based on proximate constituents explained 66% of the variation in IVDMD ($R^2 = 0.66$), whereas the model based on detergent fiber fractions showed substantially higher predictive accuracy ($R^2 = 0.85$). These findings clearly indicate that fiber fractions, especially structural carbohydrates and silica, are superior predictors of forage digestibility compared to proximate composition.

The study underscores the importance of early harvesting (60 DAS) for maximizing digestibility and highlights the potential of integrating chemical composition with regression modeling for rapid and reliable prediction of forage quality. The developed models can serve as effective tools for forage evaluation, ration formulation, and selection of nutritionally superior oat cultivars for enhanced livestock productivity.

Keywords: *In vitro* Dry Matter Digestibility (IVDMD), OAT (*Avena sativa*) fodder, fiber fractions, digestibility prediction model, forage quality evaluation

Introduction

Oat (*Avena sativa* L.) is an important temperate forage crop widely utilized in ruminant feeding systems due to its high biomass production, palatability, and relatively balanced nutrient composition. However, the efficiency of forage utilization in ruminants is primarily determined not only by its chemical composition but also by its digestibility, which governs nutrient availability and animal performance. Among the various laboratory methods, *In vitro* Dry Matter Digestibility (IVDMD) has been extensively used as a reliable indicator of forage quality due to its strong association with *in vivo* digestibility and intake (Foster, 2023) [4].

Digestibility of forage is largely influenced by the proportion and composition of cell wall constituents, particularly Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), lignin, and silica. These structural components form the major fraction of plant biomass and act as primary limiting factors for microbial degradation in the rumen. It is well established that increased lignification and cell wall thickening reduce digestibility by restricting microbial access to fermentable substrates (Jung & Allen, 1995; Van Soest, 1994) [7, 13]. Furthermore, silica deposition enhances tissue rigidity and further impedes enzymatic breakdown, thereby exerting a negative influence on forage utilization.

Growth stage is a critical determinant of forage digestibility. As plants mature, there is a progressive increase in structural carbohydrates accompanied by a decline in cell solubles and protein fractions, leading to a reduction in digestibility. Recent studies have confirmed that fiber concentration increases while digestibility decreases with advancing maturity, highlighting a clear inverse relationship between plant age and nutritive value (Tulu, 2025) ^[12]. Similarly, investigations on oat-based feeding systems have demonstrated that IVDMD and nutrient availability decline as forage advances from vegetative to reproductive stages (Gaytán-Aleman, 2025) ^[6].

In addition to maturity, genotypic variation among oat cultivars plays a significant role in determining digestibility. Differences in cell wall composition, lignin concentration, and anatomical characteristics contribute to variability in IVDMD among cultivars. Previous studies on oat genotypes have reported substantial variation in digestibility values, emphasizing the potential for selection of superior cultivars with improved feeding value (Gadisa, 2023) ^[5].

Given the complexity of factors influencing digestibility, there has been increasing interest in developing predictive models based on chemical composition. Regression approaches utilizing proximate and detergent fiber fractions have been widely applied to estimate IVDMD, enabling rapid and cost-effective assessment of forage quality. It is well recognized that fiber fractions, particularly NDF, ADF, and lignin, are more reliable predictors of digestibility than proximate components, as they directly represent the structural barriers to microbial degradation (Van Soest, 1994) ^[13]. Such predictive relationships are valuable tools for forage evaluation, ration formulation, and breeding programs aimed at improving feed efficiency.

Despite extensive research, there remains a need for integrated evaluation of oat cultivars combining digestibility assessment with predictive modeling under specific agro-climatic conditions. Particularly, limited information is available on the relative efficiency of proximate versus detergent-based models in predicting IVDMD in oat cultivars grown under Indian conditions.

Therefore, the present investigation was undertaken to evaluate the *in vitro* dry matter digestibility of different oat cultivars at varying growth stages and to develop predictive relationships between IVDMD and chemical composition, with the objective of identifying key determinants of digestibility and improving forage quality assessment.

Materials and Methods

1. Experimental material and sampling

The study utilized eight oat (*Avena sativa*) cultivars grown under rabi season at MPKV, Rahuri. Samples were collected at two growth stages (60 and 90 DAS), sun-dried, and ground (2 mm sieve).

2. Chemical composition analysis

Ground samples were analyzed for:

- Proximate constituents (AOAC, 2012) ^[1]
- DM, CP, EE, CF, NFE, Total Ash

Fiber fractions (Van Soest method, 1968) ^[14]

- NDF, ADF, cellulose, hemicellulose, lignin, silica

- Minerals
- Calcium and phosphorus

3. *In vitro* Dry Matter Digestibility (IVDMD)

IVDMD was determined using the Tilley and Terry (1963) ^[11] two-stage technique, modified by Van Soest *et al.* (1968) ^[14].

a) Rumen liquor collection

- Collected from fistulated cattle
- Filtered through muslin cloth
- Maintained at 39 °C
- Flushed with CO₂ to maintain anaerobic conditions

b) Digestion procedure

- 0.25 g sample + artificial saliva + rumen liquor
- Incubated at 39 °C for 48 hours
- Followed by pepsin digestion for 24 hours

c) Calculation

IVDMD (%) was calculated based on dry matter disappearance after incubation.

4. Statistical and regression analysis

- Data analyzed using FRBD
- Regression analysis performed to develop prediction equations of IVDMD using:
 - CP
 - NDF
 - ADF
 - Lignin
 - Other chemical constituents

Results and Discussion

1. *In vitro* Dry Matter Digestibility (IVDMD)

The *In vitro* Dry Matter Digestibility (IVDMD) of oat cultivars was significantly influenced by strain, growth stage, and their interaction ($p < 0.01$) (Table 1 and Table 2). The overall mean IVDMD was 61.47%, with values ranging from 54.23% to 67.07% among different cultivars (Table 1).

A pronounced decline in IVDMD was observed with advancement in maturity, decreasing from 65.51% at Stage I (60 DAS) to 57.42% at Stage II (90 DAS) (Table 1). This reduction in digestibility is primarily associated with increased accumulation of structural carbohydrates and lignification of plant tissues at later growth stages.

Among the cultivars, RO-2000-19 recorded the highest mean IVDMD (67.07%), followed by Kent (65.93%), while the lowest was observed in RO-2000-16 (54.23%) (Table 1). The superior digestibility of RO-2000-19 may be attributed to its lower fiber fractions and lignin content, whereas the poor performance of RO-2000-16 is associated with higher structural components.

The interaction effect (Table 2) further revealed that RO-2000-19 at Stage I exhibited the highest IVDMD (70.53%), while RO-2000-16 at Stage II recorded the lowest value (49.43%), indicating genotype-specific responses to maturity (Khorasani, 1997 & Cherney, 1982) ^[8, 3].

These findings are consistent with earlier reports that digestibility declines with advancing maturity due to increased cell wall constituents (Coblentz *et al.*, 2010; Van Soest, 1994) ^[15, 13].

Table 1: *In vitro* dry matter digestibility (%) of oat cultivars

Cultivar	Stage I	Stage II	Mean
RO-2000-2	68.13	61.80	64.97
RO-2000-7	65.43	54.40	59.92
RO-2000-19	70.53	63.60	67.07
RO-2000-1	61.87	51.67	56.77
RO-2000-11	66.07	58.20	62.13
RO-2000-16	59.03	49.43	54.23
RO-2000-12	63.53	57.87	60.70
Kent	69.47	62.40	65.93
Mean	65.51	57.42	61.47

Table 2: ANOVA summary for IVDMD

Source	Significance
Strain	**
Stage	**
Strain × Stage	*

2. Relationship between proximate constituents and IVDMD

Correlation analysis revealed significant associations between IVDMD and proximate composition (Table 3). IVDMD showed a strong positive correlation with:

- Crude protein ($r = 0.700$)
- Ether extract ($r = 0.697$)
- Total ash ($r = 0.653$)

Conversely, significant negative correlations were observed with:

- Crude fibre ($r = -0.756$)
- Nitrogen free extract ($r = -0.678$)

These relationships indicate that nutrient-rich fractions (protein and lipids) enhance digestibility, whereas increased fibre content reduces microbial accessibility and digestion efficiency. The strong negative association with crude fibre confirms its role as a major limiting factor in forage utilization (Buxton, 1996 & Mertens, 1997) [2, 9].

Table 3: Correlation coefficients (Proximate vs IVDMD)

Parameter	r-value
CP	+0.700**
CF	-0.756**
Ash	+0.653**
NFE	-0.678**
EE	+0.697**

3. Relationship between detergent fiber fractions and IVDMD

IVDMD exhibited significant negative correlations with all detergent fiber fractions (Table 4), including:

- NDF ($r = -0.728$)
- ADF ($r = -0.801$)
- Cellulose ($r = -0.774$)
- Hemicellulose ($r = -0.528$)
- Lignin ($r = -0.615$)
- Silica ($r = -0.864$)

Among these, silica showed the strongest negative correlation ($r = -0.864$), indicating its critical role in limiting digestibility. Silica deposition increases rigidity of plant

tissues and restricts enzymatic and microbial degradation, thereby reducing feed utilization (Moore, 2001 & Wilson, 1993) [10, 16].

Similarly, lignin acts as a physical barrier by forming complexes with cellulose and hemicellulose, limiting their accessibility to rumen microorganisms (Jung & Allen, 1995) [7]. The strong negative correlations observed in the present study are in agreement with established principles of forage quality evaluation (Van Soest, 1994) [13].

Table 4: Correlation coefficients (Fiber fractions vs IVDMD)

Parameter	r-value
NDF	-0.728**
ADF	-0.801**
Hemicellulose	-0.528*
Cellulose	-0.774**
Silica	-0.864**
Lignin	-0.615*

4. Regression analysis for prediction of IVDMD

4.1 Prediction using proximate constituents

The regression equation developed using proximate constituents is presented in Table 5:

$$Y = 44.94 + 1.31(CP) - 0.20(CF) + 0.96(Ash) + 4.81(EE) - 0.31(NFE)$$

The model explained 66% of the variation in IVDMD ($R^2 = 0.66$) with a multiple correlation coefficient of $R = 0.81$ (Table 5). The positive coefficients for CP, EE, and ash indicate their favorable contribution to digestibility, whereas negative coefficients for CF and NFE indicate their inhibitory effects.

Although this model provides moderate predictive accuracy, it suggests that proximate constituents alone are insufficient to fully explain variability in digestibility.

Table 5: Regression model (Proximate constituents)

Parameter	Coefficient
Intercept	44.94
CP	+1.31
CF	-0.20
Ash	+0.96
EE	+4.81
NFE	-0.31

4.2 Prediction using detergent fiber fractions

A more robust regression model was obtained using detergent fiber fractions (Table 6):

$$Y=124.63-317.94(NDF)+317.69(ADF)+317.71(HC)-0.49(Cellulose)-26.25(Silica)+11.46(Lignin)$$

This model exhibited a significantly higher predictive accuracy, explaining 85% of the variation in IVDMD ($R^2 = 0.85$) with a multiple correlation coefficient of $R = 0.92$ (Table 6).

The higher coefficient of determination indicates that fiber fractions are superior predictors of digestibility compared to proximate components. The strong negative contribution of silica and cellulose further emphasizes their role in limiting digestibility.

Table 6: Regression model (Detergent constituents)

Parameter	Coefficient
Intercept	124.63
NDF	-317.94
ADF	+317.69
Hemicellulose	+317.71
Cellulose	-0.49
Silica	-26.25
Lignin	+11.46

5. Comparative evaluation of prediction models

A comparison of the two regression models (Table 7) clearly indicates that the detergent fiber-based model ($R^2 = 0.85$) is substantially more accurate than the proximate-based model ($R^2 = 0.66$).

This finding highlights that structural carbohydrate fractions, particularly NDF, ADF, and silica, play a dominant role in determining forage digestibility, and should be prioritized in predictive modeling and forage evaluation systems.

Table 7: Model of comparison for detergent

Model	R^2	Accuracy
Proximate	0.66	Moderate
Detergent	0.85	High

6. Biological and nutritional implications

The decline in IVDMD with maturity can be attributed to:

- Increased cell wall thickness and lignification
- Enhanced cellulose crystallinity
- Increased silica deposition
- Reduced availability of cell soluble

These changes collectively reduce microbial colonization and enzymatic breakdown in the rumen, thereby lowering digestibility. Similar mechanisms have been described in forage evaluation studies (Van Soest, 1994; Jung & Allen, 1995) [13, 7].

Conclusion

The present study conclusively demonstrated that *In vitro* Dry Matter Digestibility (IVDMD) of oat (*Avena sativa*) fodder is significantly influenced by both growth stage and genotypic variation, with maturity-induced changes in chemical composition playing a decisive role in determining digestibility. A marked decline in IVDMD from 60 to 90 days after sowing was observed, primarily attributable to increased accumulation of structural carbohydrates, particularly NDF, ADF, cellulose, lignin, and silica, which collectively restrict microbial degradation in the rumen.

Correlation analysis established that IVDMD is positively

associated with nutrient-rich fractions such as crude protein and ether extract, while it is strongly and negatively correlated with fiber components, especially crude fibre and silica. Among all parameters, silica and lignin emerged as critical limiting factors, underscoring their significant role in reducing forage digestibility through structural reinforcement of plant tissues.

Regression modeling further revealed that detergent fiber fractions provide superior predictive accuracy ($R^2 = 0.85$) compared to proximate constituents ($R^2 = 0.66$), highlighting their greater relevance in explaining variability in digestibility. This clearly indicates that structural carbohydrate composition is a more reliable determinant of forage digestibility than conventional proximate analysis alone.

From a practical standpoint, the findings emphasize that early harvesting (around 60 DAS) is essential for maximizing digestibility, while delayed harvesting leads to a substantial decline in nutritive value. Additionally, cultivars such as RO-2000-19 demonstrated superior digestibility characteristics, whereas RO-2000-16 exhibited lower feeding value due to higher fiber and silica content, indicating the importance of genotype selection.

Overall, the study establishes that integrating chemical composition with predictive modeling provides a robust framework for rapid and accurate estimation of forage digestibility, which can be effectively utilized in forage evaluation, ration formulation, and breeding programs aimed at improving feed efficiency and livestock productivity.

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