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Chemical Composition and Nutritive Value of Oats (*Avena sativa*) Grown in Mixture with Vetch (*Vicia villosa*) with or Without Phosphorus Fertilization in East shoa Zone, Ethiopia

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Abstract

A study was conducted to determine forage chemical composition as well as *in vitro* and *in sacco* dry matter digestibility of oats-vetch mixture forage. The study was conducted in a 5 × 2 factorial arrangement of treatments in a Randomized Complete Block Design (RCBD) with three replications. The first factor was five different seed proportions, i.e., 0, 25, 50, 75 and 100% of oats and the remains vetch for SP1, SP2, SP3, SP4 and SP5, respectively. The sole oat had a seeding rate of Oats 80 kg/ha or 48 gm/plot, and that of sole vetch had a seeding rate of 25 kg/ha or 15 g/plot. The second factor was either without (P0) or with (P1) phosphorus fertilizer (DAP) application at a rate of 100 kg/ha or 60 g/plot. The size of the plots was 3 × 2 m. Seeds were drilled by hand in rows 30 cm apart, spaced approximately 5 cm between plants and covered with soil to about 3 cm depth. The CP content of forages was affected ($P<0.01$) by seed proportion but not by phosphorous application. The CP content increases with increasing proportion of vetch in the forage mixture and ranged from 12.4 to 23.5%. The contents of NDF, ADF and ADL appeared to increase with increasing proportion of oats in the mixture. *In Vitro* Dry Matter Digestibility (IVDMD) was not affected ($P>0.05$) by both seed proportion and phosphorus application. Conversely, *in sacco* DM degradability was significantly affected ($P<0.01$) by seed proportion across the different incubation hours, but without a consistent trend for a conclusive remark.

Keywords: *In Vitro* Dry Matter Digestibility (IVDMD); *In sacco*; Oats; Vetch

Introduction

Ethiopia has one of the largest livestock populations in Africa, which is supporting and sustaining the livelihoods of an estimated 80% of the rural population [1]. Livestock is an integral part of the farming systems in the country. It is the source of many social and economic values such as food, draught power, fuel, cash income, security and investment in both the highlands and the lowlands/pastoral farming systems. The contribution of livestock to the national economy is estimated to be 30% of the agricultural GDP and 19% of the export earnings [2]. In spite of the immense contribution of the livestock sector to the national economy, animal productivity is extremely low mainly due to poor standard of feeding both in terms of quality and quantity as the production performance of an animal often reflects its nutritional status [3].

In most tropical countries, inadequate supply of feed is the bottleneck to livestock production. This is due to the dependence of livestock on naturally available feed resources and little development of forage crops for feeding to animals. Like in other tropical countries, in Ethiopia, most of the areas in the highlands of the country are nowadays put under cultivation of cash and food crops. This resulted in keeping large number of livestock on limited grazing area leading to overgrazing and poor productivity of livestock. Though, expansion in the cultivation of cereal crops increased the supply of crop residues for animal feeding, crop residues have low nutritive value and could not support reasonable animal productivity. Hence, shortage of nutrients for livestock is increasingly becoming serious. One of the alternatives to improve livestock feeding, and thereby their productivity could be the cultivation of grass-legume mixtures and offer them to animals during critical periods in their production cycle and when other sources of feeds are in short supply [4].

Profitable livestock production could be easily achieved partly through the feeding of quality forage. Mixed cropping of cereals with forage legumes can improve both quality and quantity of fodder over a pure cereal crops. Vetches (*Vacia sativa*) are reputed for their beneficial compatibility with cereal crops when they grow in mixture [5]. It was reported that mixture containing 25-50% legume produces more quality forage and yield per unit area than those of pure sowings [5]. In forage crop production systems, grass-legume mixtures are preferred due to their several advantages over monoculture. Legumes have ability to fix atmospheric free nitrogen into the soil by symbiotic living with bacteria of *Rhizobium* species and sustaining of soil fertility [6]. Legumes are rich in terms of protein concentration, whereas cereals have higher carbohydrate contents, and cereals benefit from the nitrogen fixed by legumes when they are grown together. Forage species such as vetch (*Vacia sativa*) and oat grass (*Avena sativa*) are high potential feed sources to fill the gap of feed shortage. They are also well adapted to drought stress areas. They are promising due to their high quality feeding value. Their production potential and utilization under arid conditions to provide low cost fodder to animal particularly during the dry season, and the potential ease of agronomic practices to produce these forage species make them of higher choice and priority [7]. Their

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multipurpose role as source of food, fodder and improve soil fertility through legume and oat production as food-feed source under farmers homestead could be promoted. However, there is limited information on the agronomic practices, biomass production and feeding value of annual grass species like oat grass when grown alone or in mixture with legumes like vetch with and without phosphorus fertilization. Therefore, this study was conducted with the following objectives:

- Determine the effect of mixed cropping of vetch and oats at different seed proportion with or without phosphorus fertilization on chemical composition and *in vitro* and *in sacco* digestibility.

Material and Methods

Description of the study area

The experiment was conducted at Debre Zeit Agricultural Research Centre (DZARC) [8]. Debre Zeit is located at 47 km South East of Addis Ababa at 8^{0}44' North latitude and 38^{0}58' East longitude. The altitude is 1900 m above sea level. It receives 1100 mm rainfall per annum. The minimum and maximum mean annual temperature is 8.9-28.3^{o}C, respectively [9]. The soil of the experiment site is black cracking type clay (*Vertisol*) soil [8].

Land preparation and time of sowing

The experiment plot consisted of heavy black clay soil (Vertisol) the major arable soil type around Debre Zeit. The plots used for this study were located within DZARC main campus, flat land protected by fencing for forage research purpose. Land was ploughed in May and harrowed in June. After preparing a fine seedbed free of weeds, planting was done in July when continuous rain was assured for successful germination.

Treatment and experimental design

A grass-legume mixture of hairy vetch (*Vicia villosa*) and oats (*Avena sativa*) were used for the study. The selected varieties were oats variety CI-8237 and hairy vetch variety DZF-00329 from DZARC gene bank collections. The rationale of selecting the aforementioned grass and legumes species for the study was based on their high yield and quality fodder potential and the easy of cultural practice in growing these fodder crops that has close relationship with that of arable crops.

The experiment was a factorial arrangement of treatments in a Randomized Complete Block Design (RCBD) with three replications. Factor 1, was the oats-vetch mixture at different seed proportion including mono-crops of either species; factor 2 was phosphorus fertilizer application. Factor 1 consisted of 5 seed proportion, i.e., mono-crop of either species (Oats CI-8237, and hairy vetch), and three different oats-vetch seed mixtures with the combination 25% vetch+75% oat; 50 vetch%+50% oat; 75% vetch+25% oat (Table 1). The seed proportion is based on 25 kg seed/ha or 15 g/plot for the 100% vetch and 80 kg seed/ha or 48 g/plot for 100% oat treatment [5,10]. Factor 2 consisted of two levels of phosphorus fertilizer application: with and without 100 kg Di-ammonium Phosphate (DAP) on the basis of recommendations for vetch and oats crops, which is 100 kg/ha DAP as an optimum level [5,10,11].

The experiment consisted of three blocks; each block contained 10 plots, which were fully randomly assigned to treatments. The spacing between blocks and plots was 1.5 m and 1 m, respectively. The size of the plots was 3 × 2 m. Seeds were drilled by hand in rows 30 cm apart, spaced approximately 5 cm between plants and covered with soil to about 3 cm depth. Inoculation of the vetch component was not necessary because vetches are known to take up the right strains of *Rhizobium* bacteria from the soil (Solomon Mengistu, personal Communication). Coarse weeds were removed by hand throughout the growing period.

Treatment			Phosphorus level	Seed required for sole and mixture planting/plot	
Oats (%)	Vetch (%)	SP	(P1: with P; P0: without P)	Oats (*Avena sativa*) Amount (g/plot)	Vetch (*Vacia villosa*) Amount (g/plot)
0	100	SP1	P0	0	15
25	75	SP2	P0	12	11.25
50	50	SP3	P0	24	7.5
75	25	SP4	P0	36	3.5
100	0	SP5	P0	48	0
0	100	SP1	P1	0	15
25	75	SP2	P1	12	11.25
50	50	SP3	P1	24	7.5
75	25	SP4	P1	36	3.5
100	0	SP5	P1	48	0

SP: Seed Proportion; P: Phosphorus

Table 1: Treatments for the study and amount of seed used for sole and grass-legume mixture treatments.

Data collection

Chemical analysis: The partially dried forage sample was ground in a cyclone mill to pass 2 mm screen for *in sacco* and 1 mm screen for other analysis at Holleta Agricultural Research Canter Animal Nutrition Laboratory. The Dry Matter (DM), Crude Protein (CP) and ash contents was analyzed following the procedure described by AOAC [12]. The Nitrogen (N) content was analyzed using the Kjeldhal [12] and converted to CP as N × 6.25. Analysis for Acid Detergent Lignin (ADL), Neutral Detergent Fiber (NDF), and Acid Detergent Fiber (ADF) was carried out using the methods of Van Soest and Robertson [13]. Hemicellulose was determined by difference of NDF and ADF.

***In vitro* dry matter digestibility:** The two stage rumen inoculums-pepsin method of Tilley and Terry [14] were used to determine IVDMD. Rumen liquor was collected from three ruminally festulated steers and transported to the laboratory using thermos flasks that had been pre-warmed to 39^{o}C. Rumen liquor was taken in the morning before animals are offered feed. A duplicate sample of 0.5 g of each were incubated with 30 ml of rumen liquor and a buffer in 100 ml test tube in water bath at 39^{o}C for a period of 48 h for microbial digestion followed by another 48 h for enzyme digestion with acid pepsin solution. Blank samples containing buffered rumen fluid were incubated in duplicates for adjustment.

***In sacco* dry matter degradability:** The ruminal *in sacco* DM degradability was determined by incubating 3 g of dried forage sample in nylon bags (41 μm pore size and 6.5 × 14 cm dimension) in three rumen fistulated steers for 6, 12, 24, 48, 72, and 96 h. Upon the removal of nylon bags at the end of each incubation hours, all bags including zero hour were washed manually under a running tap water until the water is clean, gently squeezed to remove excess water, and dried at 60^{o}C for 48 h in a forced draft oven. DM content was determined in the original samples as well as in the residues according to standard procedure [12]. The degradability of DM (DMD) was determined for each incubation time using the following formula; DMD (%)=100 × (DM in forage sample–DM in residue)/DM in forage.

Statistical analyses

The data of the study was subjected to ANOVA using the General Linear Model Procedure of SAS. Least significant difference at 5% level of significance was used for comparison of means when treatment effect is significant. The model for data analysis is shown below. For *in sacco* data analysis, block effect was removed from the model as the samples are bulked per treatment and the three festulated animals serve as a replication.

Yijk=μ+Fi+Pj+FPij+BK+eijk

Where:

Yijk=Individual observation;

μ=Overall mean;

Fi=Effect of forage species mixture;

Pj=Effect of P fertilization;

BK=Block effect;

FPij=Interaction effect of forage species mixture and P fertilization;

Eijk=Random error term.

Results and Discussion

Chemical composition of mixed and pure stands of oats and vetch

Dry mater content was not significantly affected (P>0.05) by seed proportion, phosphorous application and their interaction (Table 2). The obtained results of chemical composition of oats and vetch mixture were comparable to those reported by Alemu et al. [15]. The differences observed between various research findings, can be attributed to the differences in soil related factors, climate and probably the physiological stage of the plant at harvest [16].

The total ash content was not significantly affected (P>0.05) by seed proportion, phosphorous fertilization and their interaction (Table 2). The ash content in all treatments is about 13%. Assefa [17] reported higher ash value for two vetch verities and lower ash content for three oat verities used in his oat/vetch trail, which is not in line with the results of the present study. The higher concentration of ash in vetches than oats has been also reported by Bediye [18]. According to Jennings [19], herbaceous forage legumes have higher content of some minerals like calcium, sulfur and possibly phosphorus than grasses, and well nodulated legumes contain large amount of calcium, magnesium and other essential elements. Concentration of minerals in forage varies due to factors like plant developmental stage, morphological fractions, climatic conditions, soil characteristics and fertilization regime [20].

The CP content of forages was highly significantly affected (P<0.01) by seed proportion but not with phosphorous fertilizer application (Table 2). The CP content increases with increasing proportion of vetch in the forage mixture, as legumes are rich in protein than cereals. This result is in line with the result of numerous authors. The main advantages of legume-cereal mixtures have been increased CP yield, relative to sole cereal crops. The CP content of all seed proportion in this study was above the minimum level of 7.5% required for optimum rumen function [21]. A review by Adugna and Said [22] indicated that CP value less than 7.5% inhibits intake, digestibility and proper utilization of feeds. In addition, the CP content of the forage species under most of the treatments in this study could also satisfy the requirement for lactation and growth. Norton [23] reported that a minimum of 15% CP is required for lactation and growth. Therefore, oats-vetch mixtures grown at different seed proportion contained high CP, which is well above the critical level for milk production and fattening and body growth. Oats/vetch associated forage crops could be categorized under medium to high quality forage groups and it could be potentially useful as a supplement to crop residue and natural pasture in mixed farming system of Ethiopia [24].

The NDF, ADF and ADL contents of the sole and mixed forages of oats and vetch was significantly affected (P<0.01) by seed proportion, but not by phosphorous fertilizer application and by the interaction of the two factors. The contents of NDF, ADF and ADL appeared to increase with increasing proportion of oats in the mixture. This result was supported by the report of Karachi [25] who reported lower NDF contents of legumes than grass at the same stage of growth. Increasing dietary NDF concentration most often has a negative impact on the amount of DM consumed by animals [26]. However, legume fibers ferment more rapidly in the rumen which is a reason for ruminants to consume larger amounts of legumes than grasses [27]. According to Sing and Oosting [28] roughage diets with NDF of 45-75% and below 45% are generally considered to be medium to high quality feeds, respectively. The current results in NDF content are within the mentioned range signifying the good nutritional value of the forages of the current study.

Acid detergent fiber is the percentage of highly indigestible and slowly digestible material in a feed or forage. Acid detergent fibre was significantly affected (P<0.01) by seed proportion, but not by phosphorous fertilizer application and by the interaction of the two factors (Table 2). Lower ADF indicates more digestible forage and is more desirable. Decrease in ADF levels with increasing vetch proportion observed in this study is in agreement with the finding of Aesen et al. [29], who reported that increasing the legume proportion resulted in decreased ADF and NDF concentrations for the legume-grass mixtures. Acid detergent fiber is a highly determinant factor for the digestibility of forage and intake of the animals. The acid detergent fiber and digestibility of forages have negative relationship with digestibility [30]. Acid detergent fiber has a positive relationship with the ages of the plant [31]. The hemicelluloses content took almost a similar trend like that of the NDF and ADFF content in the current study. The study showed the highest hemicellulose content in grass as compared to grass/legumes as well as to sole legumes. Such trends in hemicellulose content of forage have also been reported by Van Soest [21] who noted that the content of hemicelluloses in grass was higher than the quantity of legumes.

Lignin is a component that gives strength and resistance to plant tissue thereby limiting the ability of rumen microorganisms to digest the cell wall polysaccharides, cellulose and hemicelluloses [32]. As the seed rate of vetch increased the acid detergent lignin decreased in this study. This is in line with the report of Maynard and Loosli [33]. Generally, the presence of insoluble fiber, particularly lignin, lowers the overall digestibility of the feed and limits nutrient availability [34].

In vitro dry matter digestibility

Analysis of variance data showed that *in vitro* dry matter digestibility (IVDMD) was not significantly affected (P>0.05) by both seed proportion and phosphorus fertilizer application, and their interaction (Table 2). IVDMD of any forage crop varied with harvesting stage, fiber

Factors	Chemical composition (% for DM and%DM for others)							
	DM	Ash	NDF	ADF	CP	ADL	HC	IVDMD
Seed proportion								
SP1	18.05	12.94	56.95^{d}	40.83^{d}	23.54^{a}	9.37^{c}	16.13^{ab}	62.79
SP2	19.29	12.88	61.57^{c}	42.51^{d}	17.73^{b}	9.43^{c}	11.58^{c}	62.84
SP3	22.21	12.85	66.80^{b}	49.13^{c}	17.30^{b}	10.01^{bc}	17.67^{ab}	62.18
SP4	19.43	12.77	70.67^{a}	55.92^{b}	15.56^{c}	10.49^{b}	15.14^{b}	61.14
SP5	20.5	12.41	71.06^{a}	59.09^{a}	12.35^{d}	11.20^{a}	19.06^{a}	58.17
SEM	1.26	0.2	0.89	0.67	0.37	0.24	1.18	1.35
Phosphorus level								
P0	20.05	12.92	65.77	49.86	16.98	10.28	15.19	60.81
P1	19.75	12.62	65.05	49.13	17.61	9.91	15.92	62.04
SEM	0.8	0.13	0.57	0.43	0.23	0.15	0.75	0.85
Interaction of seed proportion and phosphorus level								
P0SP1	18.28	12.81	56.89	41.4	22.81	11.7	15.49	57.01
P0SP2	19.91	12.15	61.94	43.06	24.28	10.7	18.87	60.22
P0SP3	21.7	12.59	65.2	49.95	17.5	9.99	15.25	62.51
P0SP4	19.05	12.54	72.46	55.75	17.95	9.16	16.71	61.8
P0SP5	21.28	13.02	72.39	59.14	17.52	9.85	13.25	60.52
P1SP1	17.82	12.95	57.01	40.25	17.08	10.7	16.76	59.34
P1SP2	18.67	12.68	61.2	41.96	14.99	10.27	19.25	60.05
P1SP3	22.73	12.28	68.41	48.32	16.11	10.17	20.09	63.17
P1SP4	19.5	13.16	69.67	56.1	12.05	9.7	13.57	60.52
P1SP5	19.72	12.52	68.95	59.05	12.64	8.89	9.91	63.84
SEM	1.79	0.28	1.72	0.95	0.52	0.34	1.68	0.52

$^{a-d}$Means with the different superscripts in column within a category differ significantly ($P<0.05$); NDF: Neutral Detergent Fiber; ADF: Acidic Detergent Fiber; ADL: Acidic Detergent Lignin; CP: Crude Protein; HC: Hemicellulose; DM: Dry Matter; IVDMD: *In Vitro* Dry Matter Digestibility; P: Phosphorus; SP: Seed Proportion; SEM: Standard Error Means; P0: No Phosphorus Fertilizer; P1: Phosphorus Fertilizer (100 kg/ha or 60 g/plot); SP1: Oats 0%+Vetch 100% (vetch 25 kg/ha or 15 g/plot); SP2: Oats 25%+Vetch 75%, Oats; SP3: Oats 50%+Vetch 50%; SP4: Oats 75%+Vetch 25%; SP5: Oats 100% (Oats 80 kg/ha or 48 g/plot)+Vetch

Table 2: Chemical composition of pure and mixed stands of oats and vetch.

Factors	Incubation period (hours)					
	6	12	24	48	72	96
Seed proportion						
SP1	38.40^{a}	46.92^{c}	58.61^{b}	59.52^{b}	61.63^{b}	64.09^{b}
SP2	33.18^{c}	41.02^{d}	52.63^{c}	65.71^{a}	62.12^{b}	70.29^{a}
SP3	35.83^{b}	50.92^{a}	59.98^{a}	64.87^{ab}	68.73^{a}	70.57^{a}
SP4	37.11^{ab}	49.61^{ab}	53.89^{c}	63.36^{b}	67.83^{a}	70.55^{a}
SP5	36.97^{ab}	48.19^{bc}	57.32^{b}	65.27^{ab}	67.27^{a}	70.76^{a}
SEM	0.68	0.57	0.57	0.67	1.4	0.34
Phosphorus level						
P0	37.07^{a}	45.43^{a}	56.17	63.02^{b}	65.99	69.31
P1	35.53^{b}	46.25^{b}	56	64.48^{a}	65.05	69.2
SEM	0.43	0.36	0.36	0.42	0.88	0.21
Interaction of seed proportion and phosphorus level						
P0Sp1	38.37	46.64^{c}	57.05^{b}	59.48^{f}	61.28	63.36
P0SP2	33.95	43.72^{d}	50.75^{d}	63.09^{cd}	65.64	70.87
P0SP3	37.62	50.61^{a}	57.85^{b}	65.85^{abc}	69.02	70.77
P0SP4	36.63	49.70^{a}	56.99^{b}	62.34^{de}	66.37	70.63
P0SP5	38.76	51.48^{a}	58.20^{b}	64.30b^{cd}	67.63	70.92
P1SP1	38.42	47.28^{bc}	56.17^{bc}	59.56^{ef}	61.98	64.83
P1SP2	32.4	38.32^{e}	54.51^{c}	68.34^{a}	58.59	69.73
P1SP3	34.05	51.24^{a}	62.11^{a}	63.89^{bcd}	68.44	70.36
P1SP4	37.59	49.51^{ab}	50.78^{d}	64.38^{bcd}	69.29	70.47
P1SP5	35.18	44.91^{cd}	56.43^{bc}	66.23^{ab}	66.91	70.59
SEM	0.96	0.8	0.81	0.95	1.98	0.48

$^{a-f}$Means with the different superscripts in column within a category differ significantly ($P<0.05$); P: Phosphorus; SP: Seed Proportion; SEM: Standard Error Means; P0: No Phosphorus Fertilizer; P1: Phosphorus Fertilizer (100 kg/ha or 60 g/plot); SP1: Oats 0%+Vetch 100% (vetch 25 kg/ha or 15 g/plot); SP2: Oats 25%+Vetch 75%, Oats; SP3: Oats 50%+Vetch 50%; SP4: Oats 75%+Vetch 25%; SP5: Oats 100% (Oats 80 kg/ha or 48 g/plot)+Vetch 0%

Table 3: *In sacco* dry matter degradability of mixed and pure stands of oats and vetch.

and cell wall constituents, proportions of morphological fractions, soil, plant species and climate [35]. The composition and content of cell walls are the key factors affecting herbage digestibility. Cell walls are predominately composed of cellulose, hemicellulose, and lignin. The higher structural carbohydrate content in the feed limits digestibility [36]. Generally, with addition of legume fodder to roughages (straw or poor quality hay) both digestibility and intake of the total diet increases, because of desirable nutritional attributes of tropical legumes [37]. However, significant improvement in IVDMD with inclusion of vetch to oats was not observed in this study. This was in contrast to the findings of Getnet [7] who reported average IVDMD result of 57.80% for oats vetch mixtures with an increasing value from pure stands of oats to oats/vetch mixtures and higher at pure vetch stand. The IVDMD values greater than 55% indicates good feeding value (McDowell, 2003) and values below this threshold level results to reduced intake due to lowered digestibility. The IVDMD values observed in this study were above this threshold level which results in higher voluntary intake and digestibility, which is supported by Getnet and Ledin [38].

In sacco dry matter degradability

Analysis of variance data revealed that seed proportion showed significant differences (P<0.01) across all incubation periods (Table 3), although the trend in variation of means across the incubation hours is not consistent. Interaction effect at 12, 24, and 48 h of incubation was significant (P<0.05), but with no consistent trend to make a conclusive remark. On the other hand phosphorus fertilizer application decreased *in sacco* DMD at 6 and 12 h but increased degradability of DM at 48 h. Generally the highest *in sacco* DMD was recorded at 96 h incubation period and the lowest value was obtained at 6 h incubation period in all seed proportions in the present study. This is similar to the result reported by Klopfenstein et al. [39] that indicated that as the period of incubation period increases from 0 to 96 h in the rumen, the *in sacco* DM degradability also increases.

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