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Original Paper

Productive and physiological characteristics of West African dwarf goats fed cassava root sievate-cassava leaf meal based diet

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West African Dwarf (WAD) goat is an important livestock and its production is indispensable in the country's food chain. The WAD goat is a trypanotolerant breed reared mainly for meat. This breed can be bred all year-round, attains sexual maturity early and moderately prolific; thereby satisfying a part of the meat requirement. However, in Nigeria, scarcity, poor utilization of agro-waste and seasonality in feed availability undermines this goat breed in achieving better performance. Hence, the effect with of feeding cassava root sievate – cassava leaf meal (CRSCLM) diets on the productive and physiological characteristics of WAD goats were investigated for 97 days. Thirty six (36) WAD goats of about 8–10 months of age and averaging 7.19kg in weight were selected from the College flock for this experiment. The goats were randomly divided into four groups of nine animals each with three goats constituting a replicate. Feed intake and body weight changes were recorded accordingly. Blood samples were drawn from each goat on the last day of the trial and evaluated for haematological, biochemical and electrolyte profiles. Daily feed intke, daily weight gain and feed conversion ratio were not (P >0.05) influenced by the treatment diets. The haematological parameters indicated no significant difference (P >0.05) among the treatment groups. There was significant (P <0.05) difference for globulin (23.80–31.40), Creatinine (0.085–1.025) Cholesterol (97.125–120.46) and alanine aminotransferase (ALT) (13.96–18.22) across the treatment groups. Cholesterol and ALT were significantly (P <0.05) increased with increasing levels of CRSCLM. Globulin and creatinine however did not follow any specific trend with increasing or decreasing levels of CRSCLM. Sodium, potassium and chloride were significantly (P <0.05) different across the treatment groups with sodium being significantly (P <0.05) higher among the treatment groups than the control. The study revealed that CRSCLM in the diet of WAD goats had no deleterious effect on the growth performance and blood indices of WAD goats and could therefore be included in goat diets up to 60%.

Keywords: WAD goats, feed intake, growth performance, blood indices, serum electrolyte, cassava and agro waste

1 Introduction

Goats (Capra hircus) are unique ruminants owing to their ability to meet their nutritional requirements easily because of their moveable mandibles and ability to stand with their hind limbs which enables them to browse the most nutritious plant parts and even from thorn bushes and high tree branches. They easily adapt to different weather conditions which has helped their distribution and population in the world today. The West African dwarf (WAD) goat is believed to have originated from the south eastern Nigeria. This breed is one of the dominant breeds of goat in West Africa as a meat animal but however known as Nigerian dwarf goat in USA where it ranks fifth as a dairy breed; thus explaining its universal importance. They are trypanotolerant and widely distributed across the rainforest and derived Savannah zones of Nigeria (Ahamefule et al., 2005) where it makes significant contribution to the livelihoods of most families and the country at large. They are characteristically good mothers with high reputation for high meat and milk yield. The WAD goat is the dominant breed of small ruminants found in West Africa where they are raised under small holder management system (Jiwuba et al., 2017). Regardless of these advantages, the production of this indigenous breed of goat with great potentials is hampered due to high cost of feed and poor nutrient intake as a result of seasonal variations. This has necessitated the need to search for cheaper and readily available feed supplements to reduce feed cost for the ruminant farmers. Local and non-competitive

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feed supplement is the only means of reducing the production cost of WAD goats (Jiwuba et al., 2017), and this could be achieved through the use of cassava or cassava by products, since Nigeria is the highest world producer of cassava.

Cassava or its by products (Tijani et al., 2012; Anaeto et al., 2013; Jiwuba et al., 2016a; Jiwuba and Ezenwaka, 2016; Jiwuba et al., 2018) has been used as feeds for sheep and goats. The cassava leaf meal is very nutritious with nutrient compositions higher than that in the fresh leaves. Results on the chemical profile of cassava leaf meal revealed high protein content of 16.6% to 39.9% (Khieu et al., 2005; Jiwuba et al., 2018), high mineral content and also a major source of vitamin B_1, B_2 and C and carotenes (Adewusi and Bradbury, 1993). High amino acid profile and ME value (1,590 kcal/kg to 1,800 kcal/kg) (Ravindran, 1991; Khajarern and Khajarern, 2007) have been reported. Similarly, cassava root sievate proximate composition revealed 87.06% DM, 1.07% CP, 0.084% EE, 3.25% CF, 2.01% ash, 84.71% NFE and of 3330 Kcal/kg energy (Salami et al., 2003). Cassava and its byproducts however, have been implicated with anti-nutritional factors like cyanides (Morgan and Choct, 2016) which has been reported to affect nutrient availability, utilization and blood formation when not properly processed (Jiwuba et al., 2016b). Thus, the processing methods employed in this study would perhaps further reduce the anti-nutrients to tolerable values for WAD goats. To date, there is paucity of information on the effect of Cassava root sievate-cassava leaf meal supplements as feed component for WAD goats on productive and physiological characteristics.

2 Material and methods

2.1 Location of the experiment

The experiment was carried out at the sheep and goat Unit of Animal Production Technology, Federal College of Agriculture, Ishiagu, Ivo L.G.A., Ebonyi state, Nigeria. The College is located at about three kilometers (3 km) away from Ishiagu main town (Jiwuba et al. 2016b). The College is situated at latitude 5.56^0 N and longitude 7.31^0 E, with an average rainfall of 1,653 mm and a prevailing temperature condition of 28.500^c and relative humidity of about 80%.

2.2 Experimental feeds

The cassava root sievate (CRS) and cassava leaf were sourced and harvested within Ishiagu community. The cassava root sievate is a by product of cassava root processing which is acquired after the cassava roots meant for fufu (a popular food in Nigeria) production are peeled or not, washed clean and soaked in clean water for 3–5 days to ferment so as to reduce the hydrogen cyanide level and also to soften the roots to enable sieving (Jiwuba et al., 2018). Thereafter, the soaked cassava roots were sieved, the sievate (waste) collected and sundried for about 5 days to reduce the moisture contents and possible anti-nutrients that were not removed during the fermentation process. After which, the sundried cassava root sievate were coarsely milled and stored in batches. The cassava leaves were harvested from the College cassava farms after root harvesting. They were also coarsely milled using hammer mill to encourage chyme chewing. The cassava root sievate meal (CRSM) and cassava leaf meal (CLM) were mixed in the ratio of 3 : 1 and used in the formulation of the experimental diet. Four diets T_1, T_2, T_3, and T_4, were formulated. The cassava root sievate-cassava leaf meal (CRSCLM) was included at the levels of 0%, 20%, 40% and 60% for T_1, T_2, T_3, and T_4, respectively as presented in Table 1.

Table 1 Composition of the experimental diets for West African Dwarf Goats

	Dietary levels (%)			
Ingredients	T_1	T_2	T_3	T_4
CRSCLM	0.00	20.00	40.00	60.00
Palm kernel meal	48.00	38.00	30.00	20.50
Brewer's dried grain	47.50	37.50	25.50	15.00
Molasses	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Limestone	1.00	1.00	1.00	1.00
Total	100	100	100	100

2.3 Animal management

Thirty six (36) WAD goats of about 8–10 months of age and averaging 7.19 kg in weight were selected from the College flock for this experiment. The goats were randomly divided into four groups of nine animals each with three goats constituting a replicate. The groups were randomly assigned the four experimental diets (T_1, T_2, T_3, and T_4) in a completely randomized design (CRD). The animals were housed individually in a well-ventilated cement floored pens equipped with feeders and drinkers. Each animal received a designated treatment diet in the morning (8 am) for 97 days. Feed offered was based on 3.5% body weight per day; the animals in addition were fed 1 kg wilted chopped *Panicum maximum* later in the day (5 pm). Regular access to fresh drinking water was made available. Initial live weights of the animals were taken at the beginning of the feeding trial and weekly thereafter. Final live weight was obtained by weighing the goats at the end of the experiment. Daily weight gain, daily feed intake and feed conversion ratio were calculated.

2.4 Blood studies

Ten ml of blood samples were drawn from each animal on the last day of the study. The goats were bled through the jugular vein. The samples were separated into two lots and used for haematological and biochemical determinations. An initial 5ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid (EDTA) and used for haematological analysis. Another 5ml was collected over anti-coagulant free bottle and used for the serum biochemical studies. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000$^+$ HbA1c analyzer, respectively. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentrations (MCHC), were calculated. However, the coagulated blood samples were subjected to standard method for evaluation of serum electrolyte levels using ELISA kit technique as described by Nowshari (1995). The standard flame photometry using Gallenkamp analysis were used to determine serum sodium (Na^+) ion, Potassium (K^+) ion. Chloride ion was determined according to the method of Baker and Silverton (1995).

2.5 Proximate analyses

All the sample of feed and test ingredients were analyzed for their proximate composition using the method of AOAC (2000). The following were determined and analyzed ; dry matter content (DM), crude protein (CP), crude fibre (CF), ether extract (EE), nitrogen free extract (NFE), ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF), hemicellulose and metabolizable energy. Gross energy was calculated using the formula $T = 5.72Z_1 + 9.50Z_2 + 4.79Z_3 + 4.03Z_4 \pm 0.9\%$; where T = gross energy, Z_1 = crude protein, Z_2 = crude fat, Z_3 = crude fibre, Z_4 = nitrogen free extract (Nehring and Haelein 1973).

2.6 Data analyses

The results were analyzed using the Statistical Package for Social Sciences Window 17.0. One – way analysis of variance (ANOVA) was employed to determine the means and standard error. Treatment means were compared using Duncan's new multiple range test (Duncan, 1955).

3 Results and discussion

The proximate composition of the experimental diets, CRSM, CLM and *Panicum maximum* are presented in Table 2. The DM, CF and gross energy of the experimental diets tend to increase with increasing levels of CRSCLM while CP, NDF and ADF decreased with increasing levels of CRSCLM. Ash, EE, NFE and hemicellulose failed to follow a specific pattern across the treatment groups. The CLM is comparable with the DM, CP, ash and NFE values reported by Akinfala et al. (2002). The crude protein content of the CRSM is below the acceptable 7% CP for ruminant performance as recommended by ARC (1980) and 8% suggested by Norton (1994) for ruminal function. The fibre fractions (NDF, ADF and hemicellulose) have implication on the digestibility of plants. The NDF is a measure of the plant cell wall contents, used in determining the rate of digestion of feed. The NDF comprises mainly the cell wall fraction of forages and roughages and includes a complex matrix of lignin, small amounts of protein, and various polysaccharides. Odedire and Babayemi (2008) noted that the higher the NDF, the lower the plant's digestible

Table 2 Proximate composition of the experimental diets, cassava root sievate meal, cassava leaf meal and *Panicum maximum*

Nutrients (%)	Treatments				CRSM	CLM	*PM*
	T_1(0)	T_2(20)	T_3(40)	T_4(60)			
Dry matter	89.95	90.40	91.00	91.44	88.60	89.12	30.93
Crude protein	15.36	14.87	13.64	13.00	2.57	17.66	5.34
Crude fibre	18.96	18.96	19.65	20.11	18.96	5.38	12.64
Ash	5.44	5.59	4.91	4.50	1.80	9.87	4.01
Ether Extract	2.15	3.26	3.38	2.90	2.71	3.93	3.17
Nitrogen free extract	48.04	42.72	50.14	50.93	68.14	52.28	26.37
Gross energy (MJ/kg)	3.94	3.89	4.07	4.04	3.79	3.76	2.27
Neutral detergent fibre	62.44	42.08	35.75	33.93	25.34	39.90	58.31
Acid detergent fibre	58.35	38.30	25.11	23.69	6.68	33.25	28.60
Hemicellulose	4.09	3.78	10.64	10.24	8.66	6.65	19.17

CRSM – cassava root sievate meal; CLM – cassava leaf meal; *PM – Panicum maximum* energy. The values obtained for the CRSM may imply moderate cell wall content, moderate digestible energy and DM intake. The ADF consist mainly the lignin and cellulose. Hemicellulose has been reported to be more digestible than cellulose (Gillespie, 1998). The reportedly lower values of the fibre fractions is in agreement with the findings of Boonnop et al. (2009) for the same cassava by product. The high energy value reported for the CRSM is in agreement with Khampa et al. (2009) who noted that cassava roots contain high levels of energy and have been used as a source of readily fermentable energy in ruminant rations. The high dry matter value reported is favourably compared with the values of Boonnop et al. (2009). The proximate composition of the experimental diets revealed that the crude protein and the energy requirements are within the ranges reported for goats (ARC, 1980; NRC 1981; Norton, 1994). The DM, CP, ash NDF, ADF and hemicellulose were higher in the control, but however compared to the treatment groups. The proximate composition of *Panicum maximum* in this study is in comparison with the values reported by Odedire and Babayemi (2008), Onyeonagu and Eze (2013) and Jiwuba et al. (2016c) for the same forage.

The productive performance of West African Dwarf goats fed cassava root sievate-cassava leaf meal is presented in Table 3. There was no significant (P >0.05) difference for the parameters evaluated. There was (P >0.05) improvement on the feed intake among the treatment groups (T_2, T_3 and T_4) in comparison with the control diet. This may be attributed to high crude protein value of cassava leaf meal. Cassava leaves have been reported (Khieu et al., 2005) to have high crude protein, ranging from 16.6% to 39.9%; hence amino acid profiles of cassava leaves have been compared to be similar with alfalfa. Similarly, the improved intake observed among the treatment groups may also be attributed to high mineral, vitamin B_1, B_2, C and carotene levels, reported for CLM which tend to boost the appetite, vitality and general wellbeing of the goats. The quantity of feed consumed is largely dependent on the palatability of the diet and cassava or it by products has been generally observed to be highly palatable to goats.

There is also improvement (P >0.05) in the body weight changes from T_1 to T_4 with treatment groups having better performance than the control. The lower body weight gain of T_1 animals compared to other treatments maybe attributed to the lower feed intake recorded for the respective treatment. Jiwuba et al. (2016c) reported that dry matter intake is an important factor in the utilization of feed by goats. Feed conversion ratio was also better (P >0.05) for the treatment group.

Table 3 Growth performance of West African Dwarf Goats fed cassava root sievate-cassava leaf meal diets

Parameters	T_1	T_2	T_3	T_4	SEM
Total Feed intake (kg)	39.535	42.015	39.710	43.215	2.17
Daily Feed intake (kg/d)	0.408	0.423	0.409	0.446	0.07
Initial Body Weight (kg)	5.850	6.955	6.900	7.150	0.26
Final Body Weight (kg)	11.605	14.620	14.940	15.415	0.70
Total Weight Gain (kg)	5.755	7.665	8.040	8.265	0.32
Average Weight Gain (kg/d)	0.059	0.079	0.083	0.085	0.01
Feed Conversion Ratio	6.912	5.354	4.807	5.247	0.20

means in the row with no superscript are similar (P >0.05)

Table 4 Haematology of West African Dwarf Goats fed cassava root sievate-cassava leaf meal diets

Parameters	T_1	T_2	T_3	T_4	SEM
Packed cell volume (%)	31.23	31.66	29.90	30.36	3.2
Haemoglobin (g/dl)	9.97	9.59	9.04	10.00	0.18
Red blood cell (x106/mm^3)	13.89	14.70	14.56	14.99	3.96
White blood cell (mm^3)	10.96	11.80	11.15	11.71	2.19
Mean corpuscular volume (fl)	20.27	18.18	17.49	18.24	0.51
Mean corpuscular haemoglobin (pg)	5.57	7.11	6.83	7.00	0.69
mean corpuscular haemoglobin conc. (g/dl)	35.78	31.86	33.28	32.61	3.58

means in the row with no superscript are significantly similar (P >0.05)

Table 5 Serum Biochemistry of West African Dwarf Goat fed cassava sievate-cassava leaf meal diets

Parameters	T_1	T_2	T_3	T_4	SEM
Total Protein (g/dl)	6.035	6.515	6.27	6.340	0.28
Globulin (g/dl)	2.845^{ab}	3.140^{a}	2.385^{b}	2.820^{ab}	0.12
Urea (mg/dl)	11.290	13.970	14.275	15.090	1.14
Creatinine (mg/dl)	0.085^{b}	0.900^{ab}	1.025^{a}	0.485^{ab}	0.16
Cholesterol (mg/dl)	99.270^{b}	97.125^{b}	110.735^{ab}	120.460^{a}	3.92
Bilirubin (mg/dl)	0.370	0.030	0.035	0.140	0.09
Aspartate aminotransferase (U/l)	178.5	195.505	179.05	194.765	7.29
Alanine aminotransferase (U/l)	13.960c	16.265^{b}	18.215^{a}	15.640^{b}	0.58
Alkaline phosphatase (U/l)	68.29	67.55	69.66	67.6	1.2

$^{a, b}$ – means in the row with different superscripts are significantly different (p <0.05)

Table 6 The serum electrolyte of West African dwarf (WAD) goats fed cassava root sievate-cassava leaf meal based diet

Parameters	Treatment				SEM
	T_1	T_2	T_3	T_4	
Sodium (mmol/L)	146.11^{d}	152.36^{b}	152.17^{c}	153.44^{a}	2.18
Potassium (mmol/L)	3.95^{c}	4.26^{b}	4.02^{b}	4.64^{a}	0.08
Chloride (mmol/L)	100.75^{c}	108.42^{a}	100.14^{c}	102.72^{b}	1.85

$^{a–d}$ – means value different superscript in a row differ significantly (P <0.05)

The haematology of West African dwarf (WAD) goat fed cassava root sievate-cassava leaf meal based diets is presented in Table 4. All the haematological indices examined in this study showed no significant difference (P >0.05) across the treatment groups. This indicated that the treatment diets were non toxic but nourishing and supported the haematological indices since all the parameters were within the normal physiological range for apparently healthy goats.

The blood biochemistry of the West African dwarf (WAD) goat fed cassava root sievate-cassava leaf meal based diet is presented in Table 5. Globulin, Creatinine, Cholesterol and ALT were significantly (P <0.05) influenced by the treatment diets and tended to increase with an increasing levels of the test ingredients. Total protein, urea, bilirubin, AST and ALP were not significantly (P >0.05) affected and failed to follow a specific pattern. The globulin values in this study compared with the reported values by Oni et al. (2012) for WAD goat fed dried cassava leaf based concentrate diets and fell within the reference range of 2.7–4.1 (g/dl) for healthy goats. This is an indication of proper functioning of the liver and high immunity response of the experimental animals; a view corroborated with Jiwuba et al. (2016a). This further suggested that the processing methods of cassava root sievate-cassava leaf meal reduced the antinutritional factor like tannin and HCN to tolerable values for goats. HCN and tannin have been reported (Mitjavila et al., 1977) to diminish nutrient permeability and increase excretion of endogenous protein. The creatinine value was highest (1.02 mg/dl) in T_3 goats and lowest (0.09 mg/dl) among the T_1 goats, but perhaps fell within the range 0.7–1.5 (mg/dl) for apparently healthy goats as reported by Fraser and Mays (1986); thus suggesting that the animal did not survive at the expense of body reserve. Privuloric et al. (2012) noted that creatinine level is directly correlated with muscle mass and kidney function in animals. This further indicated that CRSCLM did not hamper the physiological functioning of the organs (liver and kidney).

Zilva and Pannall (1984) stated that normal enzyme level in serum is a reflection of a balance between synthesis and their release, as a result of the different physiological process in the body. The activities of the enzyme alanine transminase (ALT) studied were influenced (P <0.05) significantly among the treatment groups but however are within the reported reference range of 15.3–52.3 (μ/l) for goats. ALT is an enzyme found in the highest amount in liver and typically used to detect liver injury (Pratt, 2010). The within normal physiological range reported in this study gave a clear indication of absence of liver malfunctioning or injury.

The serum electrolyte of West African dwarf (WAD) goats fed cassava root sievate-cassava leaf meal based diet is presented in Table 6. The serum electrolytes showed significant (*P* <0.05) difference but however fell within the normal physiological range of 142–155 mmol/L, 3.5–6.7 mmol/L and 99–110.3 mmol/L respectively for sodium, potassium and chloride for apparently healthy goats. Serum electrolytes regulate plasma volume and acid-base balance, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function and involve in the maintenance of body water. The within normal physiological reported for these electrolytes gave a clear indication that the diets supported the normal physiological functions of the goats since there was no liver or kidney damage. Similarly, nervousness, cardiac weakness, failure of the respiratory muscles, increased PCV above the normal range, and impairment of adrenal function which are common signs of chloride, potassium and sodium deficiency were not observed during the study.

Conclusions

It could be concluded that cassava root sievate-cassava leaf meal could be included in the diets of goats up to 60% in compounded ration without deleterious effect on the growth performance and blood characteristics of West African dwarf goats. The use of cassava root sievate-cassava leaf meal based diets is therefore recommended for enhanced goat production.

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Original Paper

Effects of low protein diets with amino acids supplementation on biochemical and faeces parameters in weaned piglets

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The goal of this study was to determine the effects of a low-protein diet supplemented with crystalline amino acids on the biochemical parameters in the blood serum, and the indicators of fermentation in the faeces in 12 crossbred piglets. The weaned piglets (at 28 days of age) were divided into two groups with 6 piglets each. The control diet contained 195 g/kg crude protein and the experimental diet contained 167 g/kg. The experimental diet was supplemented with lysine, methionine and threonine to achieve a more ideal amino acid pattern. The blood collections from the *sinus ophthalmicus* for the determination of the biochemical parameters were performed 2 times at 2 weekly intervals in both groups. The faeces were taken from the rectum at the end of the study period. The decrease in the dietary crude protein content of the experimental group was manifested by a significant decrease of the blood urea level (3.77 mmol/l average concentration) compared to the control group (4.97 mmol/l average concentration) (P <0.001). The serum concentrations of other components showed no significant statistical changes between the control and experimental groups. The results of the fermentation process analysis indicated that the acetate and the butyrate concentration decreased in the experimental group compared to the control group (P <0.05; 0.01, respectively). The decrease crude protein intake in the experimental group revealed significant lover levels of ammonia (P <0.001) and crude protein (P <0.01) compared to the control group.

Keywords: pigs, amino acids, proteins, metabolism; fermentation

1 Introduction

Dietary protein is the fundamental source of amino acids for pigs. The high inclusion of dietary protein and the imbalance of amino acid (AA) composition in animal husbandry result in inefficient utilization of protein resources and increased nitrogen excretion. Therefore, an efficient approach to alleviate the nitrogen excretion and increase the utilization of protein resources is to formulate the AA-balance protein-restricted diet with crystalline AA supplementation (Kim, Chen and Parnsen, 2019). Because of the increased availability of crystalline AA (lysine, methionine, and threonine, including the 'new' amino acids isoleucine and valine), and the continual need to improve the utilization of nutrients to reduce the impact of livestock production on the environment, there is always a need to more fully understand amino acid nutrition of non-ruminants (Kerr, 2006). Lysine, the first-limiting AA in typical swine diets, plays very important roles in exerting many metabolic and physiological functions in pigs (Liao, Wang, and Regmi, 2015). Threonine is considered an essential amino acid and is commonly the second or third limiting AA in pig diets based on corn and soybean meal; however, it may be the first limiting AA when diets are supplemented with synthetic lysine (Saldana et al., 1994).Threonine is critical for maintenance because it is used for the synthesis of muscle protein, mucin in the gastrointestinal system, and immunoglobulins (Nichols and Bertolo, 2008). A reduction of dietary crude protein (CP) could limit the growth performance of growing pigs, but a low-protein diet, supplemented with deficient amino acids, could reduce the excretion of nitrogen into the environment without affecting weight gain (Ball et al., 2013; He et al., 2016; Wang et al., 2018). It was also concluded that the supplementation of limited amounts of synthetic amino acids to diets for swine could spare 2 to 3 percentage units of dietary protein and substantially

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reduce the nutrient excretion, especially of nitrogen (Han and Lee, 2000). More studies indicated that the reduction of dietary CP and the supplementation of limiting AA could effectively decrease the nitrogen emission (Heo et al., 2008; Toledo et al., 2014). This fact motivated us to determine the effects of reducing the dietary CP content from 19.5 to 16.7% on serum biochemical parameters and indicators of fermentation processes in category of weaned piglets.

2 Material and methods

2.1 Animals and diets

Twelve crossbred piglets (Slovakian White × Landrace) were divided in two groups (6 animals in the control group and 6 animals in the experimental group; 50% male: 50% female in both of groups); with an initial average body weight (BW) of 8.70 ±0.53 in the control and 8.65 ±0.60 kg in the experimental group with weaning at 28 days of age in both groups. The experimental period lasted 4 weeks.

The same basic ingredients for the control and experimental groups were used in the study. The diets were formulated based on corn, wheat, barley, soybean meal, vitamin + mineral premix, and salt. The animals were divided into two groups according to the two different CP levels of diet (19.5% and 16.7%) with different soybean meal concentration in diets (25 vs. 18.5%). The addition of limiting amino acids (AA) – lysine Lys, methionine Met, and threonine Thr was used in the experimental diet according to the National Research Council amino acids recommendations (NRC, 2012) for limiting amino acids in category of growing pigs with body weight up to 25 kg. The concentrations of dietary metabolizable energy were calculated according to Šimeček, Zeman and Heger (1994). Feed and water were allowed on an *ad libitum* basis. The feed composition of the diets used in the study and their nutrient content are shown in Table 1.

Table 1 Composition and chemical composition (g/kg; as fed basis) of diets containing different levels of crude protein for piglets

Ingredients (%)	Control diet	Experimental diet
Corn	28.5	29.0
Wheat	33.4	38.4
Barley	10.0	10.0
Soybean meal	25.0	18.5
Premix Vitamin Mineral	3.0	3.0
Salt	0.1	0.1
L-Lysine HCl 78%	–	0.49
DL-Methionine	–	0.25
L-Threonine 98%	–	0.26
Parameters (g/kg)		
Dry mater	888.4	887.5
Crude protein	195.0	167.0
Etheric extract	21.5	20.4
Crude fiber	33.1	32.9
Neutral detergent fiber	198.7	162.8
Acid detergent fiber	54.1	54.6
Ash	57	54.6
Nitrogen free extract	581.8	612.6
Lysine	12.8	14
Threonine	7.6	8.7
Methionine+Cysteine	6.6	7.9
Metabolizable energy	13.2	13.1

The investigation was carried out in the animal quarters of the Institute of Animal Nutrition and Dietetics at the University of Veterinary Medicine in Košice in compliance with the EU regulations concerning the welfare of experimental animals

2.2 Analysis

The diets were analysed for their dry mater, crude protein (CP), crude fiber, acid detergent fiber, neutral detergent fiber, ether extract and ash by the AOAC (2001). The nitrogen free extract was mathematically calculated from previous parameters. The amino acids content in both diets were calculated according to the program for formulation of diets for pigs from AA composition of feeds and the addition of crystalline limiting amino acids. The blood collection from the *sinus ophthalmicus* for the determination of the biochemical parameters was performed 2 times at 2 weekly intervals in the control and experimental group, 4–5 hours after feeding. The biochemical parameters of the blood serum (total proteins, albumin, urea, glucose, triglycerides, cholesterol, AST aspartate aminotransferase, and AP alkaline phosphatase) were determined using a fully automatic random access benchtop analyser Ellipse (Italy).

The faeces were taken directly from the rectum at the end of the investigation. The quantitative determination of the short chain fatty acids (SCFAs) was done by the method of isotachophoresis employing a two-capillary analyser EA100 (Villa Labeco, Slovakia). The content CP and ammonia in the faeces was determined according to the AOAC (2001).

2.3 Statistical methods

All data were reported as the mean ± S.D. (standard deviation). The differences between means were determined according to the unpaired *t*-test using GraphPad Prism statistical program (Graph Prism software, USA). By conventional criteria, differences ($P < 0.05$; $P < 0.01$; $P < 0.001$) were considered to be statistically significant.

3 Results and discussion

The first goal of our study was performed to investigate the effects on the biochemical parameters in the blood serum following the feeding of a low crude protein diet to piglets. The metabolic variables in the blood serum are shown in Table 2.

No significant differences were seen between the control and experimental groups in the serum total protein and albumin. The average concentrations of total protein from two collections of the study period were slightly higher in the experimental group compared to the control group. Opposite tendency was observed in the albumin concentration. The average total protein concentrations determined in both groups were lower than the reference values reported by Doubek et al. (2010) (65–90 g/l).These differences could be due to the very young category of the animals used in our investigations. The reference concentration of albumin in serum ranges between 19 and 39 g/l according to Doubek et al. (2010). The average albumin concentrations determined in both groups were consistent with those reference values. The blood urea level as an important indicator of protein nutrition showed marked changes. Throughout the study, the serum urea concentration was significantly lower (P <0.001) in pigs fed the experimental diet, with the lower CP content supplemented with essential limiting amino acids (Lys, Met, Thr), compared to the control diet which contained higher CP. Significantly higher levels of blood urea in our work were recorded in the control group compared to the experimental group at both intervals (week 2. and 4.). The mean values in the control group corresponded to the reference values of Doubek et al. (2010) (3.6–10.7 mmol/l). In the experimental group, the mean values of the urea parameter in the monitored intervals were just above the lower reference range. No significant differences between groups in other serum parameters were found. The concentrations of glucose, cholesterol, triglycerides, AST, and AP in the blood serum in weaning pigs were within the physiological values for pigs (Kraft and Dürr, 2001; Doubek et al., 2010).

Protein synthesis requires a complete set of AAs presented at the synthesis site simultaneously, and when the first-limiting AA is used up, protein synthesis stops and the remaining free or unbound AAs will be catabolized via deamination (NRC, 2012; Liao et al., 2015). The increasing availability of synthetic amino acids allows for the reduction of the crude protein level in piglet diets in association with adequate AA supplementation, which maintains sufficient essential AA supply (Figueroa et al., 2002). Lysine is the first-limiting amino acid in typical swine diets and plays very important roles in promoting growth performance of pigs. Limiting dietary lysine supply to late-stage finishing pigs can increase the blood plasma concentrations of urea nitrogen and total cholesterol (Regmi et al., 2018).

In our study the reduction of CP (19.5% vs.16.7%) only slightly influenced the concentrations of the serum parameters in comparison with the control group, except for the blood urea level. The urea excreted in urine is the main nitrogenous end-product from amino acids catabolism in pigs and plasma or serum urea concentrations may be indicative of excreted nitrogen in urine (Roth and Raczek, 2003). A lower blood urea nitrogen indicated higher availability of dietary nitrogen and a better use for amino acids with the CP reduction (Toledo et al., 2014). Fang et al. (2019) observed significant decrease of the blood urea nitrogen concentration as CP dietary level decreased in the weaning period.

The supplementary part of our study was to investigate the effects of feeding a low CP diet to piglets on short chain fatty acids profile and nitrogen excretion in faeces are shown in Table 3.

Table 2 Effects of different dietary CP content on biochemical parameters of piglets

Parameters	Control diet (19.5% CP)			Experimental diet (16.7% CP)		
Week	2.	4.	2.–4.	2.	4.	2.–4.
Total protein (g/l)	51.04±2.91	53.53±2.06	52.29±2.48	52.70±2.78	53.95±2.44	53.33±2.61
Urea (mmol/l)	4.48±0.31[a]	5.45±0.41[a]	4.97±0.36 [a]	3.44±0.26[b]	4.10±0.30[b]	3.77±0.28[b]
Albumin (g/l)	34.90±1.86	33.35±1.52	34.13±1.69	33.85±2.76	31.50±2.38	32.68±2.57
Glucose (mmol/l)	5.79±0.29	5.54±0.48	5.66±0.38	5.66±0.20	5.29±0.30	5.47±0.25
Triglycerides (mmol/l)	0.37±0.05	0.43±0.04	0.40±0.04	0.33±0.03	0.43±0.22	0.38±0.12
Cholesterol (mmol/l)	2.14±0.13	1.93±0.13	2.03±0.13	2.01±0.11	2.02±0.15	2.01±0.13
AST (µkat/l)	0.38±0.03	0.36±0.01	0.37±0.02	0.35±0.03	0.34±0.03	0.34±0.03
AP (µkat/l)	7.66±0.61	7.83±0.49	7.75±0.55	7.91±0.28	7.77±0.23	7.84±0.26

[a, b] – significant differences (P <0.001); AST – aspartate aminotransferase; AP – alkaline phosphatase

Table 3 Effects of different dietary CP content on faeces indicators of piglets (g/kg dry matter)

Parameters	Control group (19.5% CP)	Experimental group (16.7% CP)
Crude protein	244.50 ±10.80a	203.00 ±11.60c
NH3	1,464.00 ±52.30a	1,176.00 ±55.50b
Acetate	21.70 ±2.21a	18.17 ±2.27d
Propionate	15.12 ±2.24	14.53 ±1.83
Butyrate	7.36 ±0.49a	5.83 ±0.77c
The total SCFAs	44.18 ±4.96	38.53 ±4.89

[a, b] – significant differences (P <0.001); [a, c] – significant differences (P <0.01); [a, d] – significant differences (P <0.05); NH_3 – ammonia; SCFAs – short chain fatty acids

The evaluation of the fermentation processes through the determination of the SCFAs in the faeces showed decreasing tendency in individual acids (acetic, propionic, butyric) as well as in the total short chain fatty acids concentration in the group that received the lower level of dietary CP (experimental group). Short-chain fatty acids (SCFAs), also referred to as volatile fatty acids (VFAs), the end products of fermentation by the anaerobic intestinal microbiota, have been shown to exert multiple beneficial effects on mammalian energy metabolism. In our work, we recorded the decrease of short chain fatty acids contents (significantly for acetic acid, P <0.05 and butyric acid, P <0.01) in the experimental group than in the control group. Decreased concentration of propionic acid and the total SCFAs in the faeces of the experimental group was also observed in our study, but was not significantly affected. The ammonia concentration and the CP content in the faeces revealed an increasing tendency with the higher dietary crude protein concentration. Concentration of crude protein in dry matter of faeces of piglets in the experimental group was significantly lower than in the control group (P <0.01). According to our results the reduction of crude protein in faeces may lead to decreased production of volatile ammonia through microbial fermentation in faeces. The ammonia level in dry matter of faeces of piglets in the experimental group was significantly lower compared to the control group (P <0.001). Our results support the results of Wang et al. (2018) who reported that every 1% reduction of dietary CP can decrease ammonia emission from faeces and urine by 8% to 10%. The high dietary CP concentration, as is common in diets for early-weaned pigs, may increase microbial fermentation of undigested protein. In agreement with our findings also Htoo et al. (2007) detected that the reduction in CP content from 24 to 20% in weaned pigs leads to decreased faecal ammonia nitrogen (P <0.05), acetic acid and volatile fatty acids (VFAs) concentrations. In the study by Heo et al. (2008) feeding low-protein treatments had no effect on the total VFAs level for 14 days after weaning.

Diets with high crude protein (CP) content are commonly used for early-weaned pigs. With the development of industrial synthetic AA technology, supplementary feed grade AA, such as L-valine and L-isoleucine have become available for use in livestock diets, resulting in the potential for further reduction in dietary CP (Wang et al., 2018). According to the results of the Jiao et al. (2016), crystalline amino acids supplementation allows the reduction of dietary CP levels by 3 to 4 percentage units with no effects on carcass traits for finishing gilts. The results of Peng et al. (2016) indicate that reducing dietary CP level from 20% to 15.30%, supplemented with indispensable AA, had no significant effect on growth performance and had a limited effect on immunological parameters. However, a further reduction of dietary CP level up to 13.9% would lead to poor growth performance and organ development, associated with the modifications of intestinal morphology and immune function. Indeed, feeding weaned pigs a lower level of crude protein caused lower ammonia concentrations in the small intestine (Bikker et al., 2006) and decreased plasma urea nitrogen, ammonia nitrogen and volatile fatty acids in the ileal digesta (Nyachoti et al., 2006).

4 Conclusions

Our study demonstrated that feeding lower CP content in the diet with the addition of limiting amino acids (lysine, methionine, and threonine) for recommendation of ideal amino acids pattern for piglets after weaning, significantly reduces the blood urea concentration (average concentrations from two weekly collection 3.77 vs. 4.97 mmol/l). The statistically significant differences among the groups were found in the acetate and butyric acid concentration in the faeces. Also, lower concentrations of ammonia and CP in the faeces of the experimental group were observed (-288 mg/kg DM and -41.5 g/kg DM, respectively) compared to the control group. The use of crystalline limiting amino acids improved the use of nitrogen from the diet in metabolism, with lower nitrogen excretion into the environment.

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Original Paper

Green fallow soil vs. intensive soil cultivation – a study of soil structure along the slope gradient affected by erosion process

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The type of slope and its interaction with soil management practices are one of the most important factors affecting soil structure along the slope gradient. In this study, the effects of fallow in greening and intensive soil cultivation both located on slopes on changes soil properties especially soil structure were evaluated. Soil samples were collected from two fields (neighbouring fields) between Trakovice and Bučany villages (Slovakia). The terrain of both fields was sloping with a WN – ES orientation and a slope of <8°. Field 1 is used as arable land with intensive cultivation of crops (*IC*). In field 2, the fallow in greening (*G*) was established in 2012 and in 2018 soil samples were taken in five zones of both slopes as follows: on the summit slope, shoulder, back slope, toe slope and flat. Results showed that structure coefficient (*K*) was strongly affected by both land use ($p = 0.0000$) and slope position ($p = 0.0206$) as well as by the interaction of land use and slope position ($p = 0.0010$). The statistically significantly highest structure coefficient of water-stable aggregates (*Kwsa*) and opposite the lowest macro-aggregate destruction (*PAD*) were found for *G* compared to *IC*. In *G*, the index of crusting (Ic) increased by 9% compared to *IC*. The critical level of soil organic matter (S_t) was strongly affected by both land use ($p = 0.0114$) and slope position ($p = 0.0000$). The values of S_t were statistically significantly influenced by interaction of land use and slope position. When land use and slope position were assessed together, positive significant correlations were observed between silt and carbonate contents and Ic. On the other hand, the S_t values were strong effected soil organic matter (SOM) quantity and quality. In *IC*, positive correlations between C_L ($r = 0.773$, $P < 0.01$) and *K* were observed. Ic correlated with silt ($r = 0.650$, $P < 0.05$), carbonates ($r = 0.704$, $P < 0.05$) and lower humus stability. A higher silt and carbonate contents as well as higher content of SOM and better humus quality resulted in higher S_t values. In *G*, the *K* values positive correlated with silt and carbonate contents. Higher humus quality and stability improved soil structure evaluated on the base of *Kwsa*.

Keywords: intensive cultvation, greening, fallow, slope gradient, soil structure

1 Introduction

Soil degradation includes all of the processes leading to aggravation of soil quality and its productivity (Novák and Valla, 2002). One of the most important degradation processes in agricultural areas is soil erosion. It defined as a gradual process (occurring mostly in undulating terrain) consisting of removing soil particles under the impact of different external factors (water, wind, glacier etc.) causing the deterioration of soil. Water erosion constitutes a major global environmental problem threatening agricultural productivity, water quality, infrastructure etc. (Efthimiou, 2018). Moreover water soil erosion is considered as the process responsible for the biggest share of soil loss in Central European agricultural ecosystems (Panagos et al., 2015). Also in Slovakia the attention is paid rather to water than wind erosion when looking into the problem of potential soil loss. According to the outputs of last soil monitoring cycle (Kobza et al., 2017) in Slovakia about 39% of agricultural soils are potentially affected by water erosion (10% in medium, 15% in high and 14% in extremely high level). Regarding individual soil types, categories of extremely high to medium erosion predominates on soil types occurring at the mountainous and submountainous regions (Cambisols and Rendzic Leptosols) where about 75% of

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total area covered with these soil types can be affected by potential water erosion.

The degree of soil erodibility depends on both, soil properties and external factors such as: climate, topography, bedrock, land use, soil management practices etc. and their interrelationships (Morgan, 2005). Incorrect soil management associated with intensive crop production can significantly contribute to soil loss (Moreno-Ramón et al., 2014; Borrelli et al., 2015). The erosion rates in conventionally tilled cropland are on average one- to two-fold greater than the rates of soil production (Montgomery, 2007). It is a reason why implementing of green fallow management is important to be applied in Slovakia. Greening is a set of practices that are beneficial to the environment and climate protection. It consists mainly of crop rotation on arable land (at least three different crops). Very important element of this management type is the omission of the part of land which cannot be used for the crop production. This part of the field (or even the entire field) can be used for the green fallow.

One of the major causes of erosion in cropland is disrupting of soil structure, thus accelerating surface runoff and soil loss. Soil structure is one of the most important indicator of soil quality (Foth, 1990; Pires et al., 2017). The aggregation processes and aggregate stability are very important as the soil aggregate is a fundamental unit of soil structure (Foth, 1990). Aggregate stability depends on the content of base minerals and the type of clay minerals, soil organic matter, electrolyte concentration, texture, soil management practices etc. (Bronick and Lal, 2005; Šimanský et al., 2019). The soil structure have been studied previously in different soil types, climate conditions and under varying soil management practices (Šimanský et al., 2014; Bartlová et al. 2015; Šimanský and Jonczak, 2016; Šimanský et al., 2019). However, the soil structure parameters and their interactions with other soil properties under intensive cultivation and greening system in commercial setting and in field conditions in Slovakia has not been explored yet with few exceptions (Šimanský et al., 2019a). Thus the main assumptions of this study are that selected soil structure parameters depend on the slope position (up slope to down slope direction) and the management type (intensive cultivation and green fallow) what should be connected with changes in soil properties (higher soil organic matter content, higher content of clay, carbonates etc.). Soil structure is a complex system and one of the reasons for the complexity of soil structure is the range of scales it expresses. Structural processes occur at a scale ranging from a few Å (angström) to several centimeters (cm). Another cause of complexity is the dynamic nature of soil structure. Structural attributes vary in time and space, and the attributes observed at any given time reflect the net effect of numerous interacting factors that may change at any moment (Lal and Shukla, 2004), and is difficult to characterize (Coughlan et al., 1991). For comprehensive assessment of the soil is not suitable to indicate only one parameter. Except of soil structure, in order of responsible assessing the quality of the soil there also several soil properties (chemical, physical and SOM) have to be quantified. While previous research carried out by authors in this area (Šimanský et al., 2019a) concerned the influence of slope position and management type on selected soil properties including some of the soil structure parameters and soil organic matter properties this study is focused primarily on the interactions of land use and slope position and their impact on the soil structure indexes connected with vulnerability for the soil erosion. This paper can provide a good basis on topic of different soil management practices impact on soil structure properties and their association with soil loss, as well as the identification of the most erosion vulnerable areas in the study region.

2 Material and methods

Study site consists of two adjoining fields located are between villages Trakovice (48° 25′ 46.045″ N; 17° 42′ 20.87″ E) and Bučany (48°25′37.74″ N17° 42′ 16.24″ E) in the north-west part of Danube lowland. The sites have a temperate climate. The average monthly precipitation and temperatures is 634 mm and 9.7°C, respectively (http://www.climate-data.org). Geological substrates of the mentioned region are neogene clays, sands and gravels, mostly covered with loess (silts and silt loams). Several soil types dominates in the study area (Fluvisols, Chernozems, Luvisols, Kastanozems etc.; Fulajtár and Saksa, 2018).

The sampling was carried out within the two adjacent fields located on the slope in complex of Regosols and Chernozems. The terrain of both fields was sloping with a WN – ES orientation with inclination of <8°. Field 1 was used as arable land with intensive cultivation of crops (*IC*). In 2018 (sampling time) maize was planted in field 1. The maize lines were oriented in the slope direction (which is incorrect way) and the spacing was 70 cm apart. The field 2 was abandoned from 2012 and use as a part of greening (G) system (green follow). Weeds were mulched by cutting or disking twice a year. Both fields were divided into five zones for sampling: 1. summit (S), 2. shoulder (SH), 3. backslope (BS), 4. toe slope (TS), and 5. flat (F). In each zone, the soil pits (totally 10) were prepared and a soil samples (disturbed and undisturbed) from cultivated horizon (to the depth of 20 cm) were taken.

Several soil properties were determined in collected disturbed soil samples and described in the previous, above mentioned paper (Šimanský et al., 2019a). These properties were as follows: pH of the soil-to-solution ratio of 1 : 2.5 using H_2O as the suspension medium; content of soil organic carbon (SOC) by sample oxidation in the mixture of $K_2Cr_2O_7$ and H_2SO_4 (Dziadowiec and Gonet 1999); and content of carbonates by the volumetric method using a Jankov calcimeter. Particle-size distribution was determined by pipette method (Hrivňaková et al., 2011), texture classes were described according to USDA (Soil Survey Division Staff 1993). The labile carbon content (C_L) was determined using 0.005 mol/dm^3 $KMnO_4$ (Loginow et al. 1987) and hot water extracted carbon (C_{HWE}) was determined according to the method of Kőrschner et al. (1990). The group and fraction composition of humic substances (HS) was determined by the Belchikova and Kononova method (Dziadowiec and Gonet 1999). The irradiation absorbance of humic substances (HS) and humic acids (HA) was measured at 465 and 650 nm using a Jenway 6400 Spectrophotometer to calculate the colour quotients Q_{HS} and Q_{HA}. Undisturbed soil samples were sieved by dry sieving as dry sieved aggregates as well as by wet sieving as water-stable aggregates – Baksheev method (Vadjunina and Korchagina, 1986). Individual size fractions of both dry and wet sieved aggregates were used for calculation of structure coefficient for dry sieving (K) as well as for wet sieving (*Kwsa*) (Fulajtár 2006). We also calculated the percentage of aggregate destruction (*PAD*) (Zhang and Horn, 2001), index of crusting (I_c) and critical level of soil organic matter (S_t) (Lal and Shukla, 2004).

The experimental results were compared and analysed in terms of its variability following both land use types and slope positions. Multifactor analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests were used to compare above-mentioned factors. All results were reported at α = 0.05 level of significance. Regression analyses were used to establish the relationships between parameters of soil structure and texture, carbonate contents and soil organic matter parameters. The coefficient of determination (R^2) was used to evaluate the performance of the applied regression equations. All statistical analyses were performed using the Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA).

3 Results and discussion

On the intensively cultivated slope (*IC*) content of silt and clay increased along the slope gradient. Different pattern was observed on the slope used as the green fallow (G), where the sand content increased and silt content decreased from the summit to the lower parts of the slope. In both different used slopes the particle size distribution was very similar. Soil texture was silt loam, with the clay content ranging from 14% to 22% (Šimanský et al., 2019a). As reported Bronick and Lal (2005) soil texture has a significant effect on aggregation as well as other soil properties such as soil organic matter, carbonate content and etc. (Šimanský et al., 2014; Paradelo et al., 2013). In previous paper (Šimanský et al., 2019a) dependencies between soil organic matter

Table 1 Dependencies between the soil structure parameters, land use type and slope position

	K		*Kwsa*		*PAD*		I_c		S_t	
Land use										
Intensive cultivation	1.27		1.20		27.3		1.22		3.02	
Green fallow	2.29		2.36		16.4		1.11		3.10	
P-value	0.0000		0.0056		0.0009		0.0000		0.0114	
Slope position										
	intensive cultivation	green fallow	intensive cultivation	green fallow	intensive cultivation	green fallow	intensive cultivation	green fallow	intensive cultivation	green fallow
Summit	1.21	2.57	0.81	1.34	40.5	23.0	1.24	1.06	3.07	3.05
Shoulder	1.06	2.74	1.14	1.26	26.6	28.5	1.50	1.43	2.57	1.68
Backslope	1.78	1.99	0.93	1.25	42.0	22.8	1.11	1.13	3.32	2.72
Toe slope	1.21	2.29	1.84	4.28	11.0	5.50	1.16	1.04	3.36	3.70
Flat	1.08	1.85	1.25	3.69	16.5	2.44	1.09	0.91	3.17	3.94
P-value	0.0206		0.0089		0.0001		0.0000		0.0000	
Land use × Slope position										
P-value	0.0010		0.1117		0.0790		0.0000		0.0000	

K – structure coefficient, *Kwsa* – structure coeficient of water-stable aggregates, *PAD* – percentage of macro-aggergate destruction, I_c – index of crusting, S_t – critical level of soil organic matter parameters, carbonate content and land use as well as the slope gradient have been studied, and now our attention was focused on soil structure parameters.

The actual structural state of soils based on structure coefficient (*K*) was strongly affected by both land use (p = 0.0000) and slope position (p = 0.0206). The interaction of land use and slope position had a strong tendency (p = 0.0010) to affect the actual structure state while the effect of land use type seemed be more pronounced than slope position (Table 1). Shein (2005) considered the *K* values ranging from 0.67 to 1.50 as favourable and *K* values lower than 0.67 as unfavourable structural state. In our case (in both land use and all slope position) the *K* values ranged from 1.60 to 2.74. Higher values represented better soil structure development (Šimanský et al., 2018). *K* values were about 82% higher on the slope used as the green fallow when comparing to intensively cultivated slope. Taking into account the slope position, *K* values indicated the worst actual structural state in flat (*F*) terrain under the slopes no matter the land use type. These results confirm the previous statements (Šimanský, 2011) as due to the erosion non-stabile aggregates (especially lower in size) are transported down along the slope and formation of stable soil structure depend on both external and internal factors (Amézketa, 1999; Bronick and Lal, 2005; Wiesmeier et al., 2012; Burdukovskii et al., 2019). When comparing the *K* values individually with dependence on land use and slope position, the effects differ depending on the slope form (Figure 1A). The *K* dynamics along the slope gradient from the upper to lower slope positions (p >0.05) have been expressed by quadratic polynomial trend in the best way (Table 2). Except actual structure state of soil (based on *K* values) water-stability of soil structure is very important to pay the attention because the ability of soil aggregates against water destruction is very significant especially from viewpoint of soil erosion (Six et al., 2004). The multifactor ANOVA analysis showed

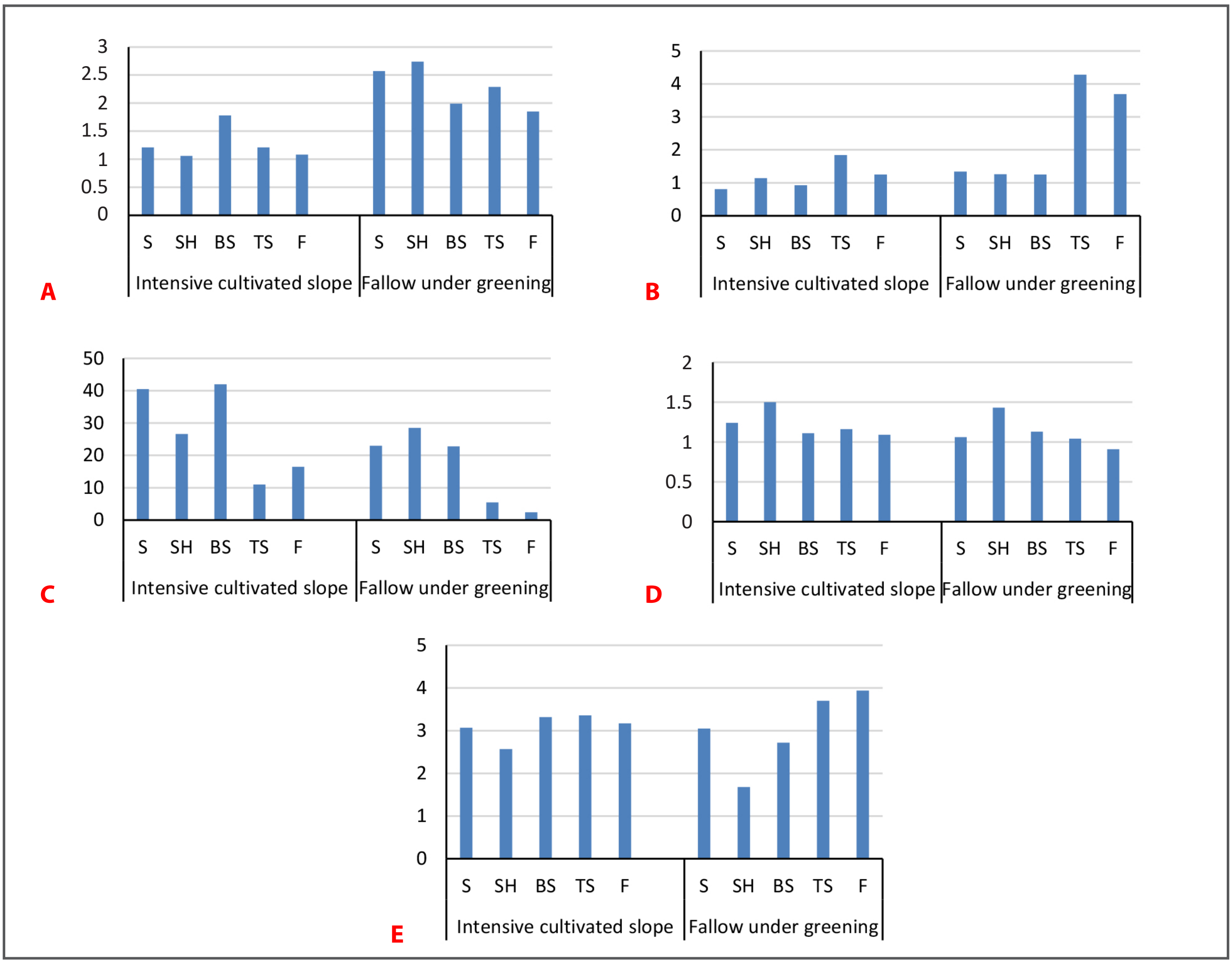

Figure 1 Soil structure parameterst along the slope gradient under intenisve cultivated slope and fallow under greening
A – structure coefficient, B – structure coeficient of water-stable aggregates, C – percentage of macro-aggergate destruction, D – index of crusting, E – critical level of soil organic matter
Notes: S – summit, SH – shoulder, BS – backslope, TS – toe slope, F – flat

significant differences between land use types in case of *Kwsa* (P = 0.0056) and *PAD* (P = 0.0009). The significant differences between slope positions for *PAD* (P = 0.0001) were also noted. The statistically significantly highest *Kwsa* values and opposite – the lowest percentage of macro-aggregate destruction (*PAD*) values were found for *G* compared to *IC* in terms of different land use type. Soils under the green fallow management as published Šimanský (2018) and Burdukovskii et al. (2019) have a significantly better developed structure than soils of intensively cultivated fields. There were no significant interactions between land use and slope position (Table 1).

Under both management types *G* and *IC* distinctive pattern have been observed. The highest *Kwsa* as well as the lowest *PAD* values were determined for samples collected from the lowest positions of the slopes (TS and F, Figure 1B and 1C). Although only in the *G* on the slope did the *PAD* show a statistically significant quadratic polynomial trend (Table 2) along the slope gradient (from the upper to the lower parts of the slope). The index of crusting (I_c), based on textural composition and soil organic matter content (Lal and Shukla, 2004), is a very important parameter of soil structure, as it is inversely related to clay and soil organic matter content, and directly to fine and coarse silt content. Formation of the soil crust depend on soil tillage and fertilization (Šimanský et al., 2008), as well as on the presence of sufficient supply of organic matter in soil (Špička et al., 1964; Šimanský et al., 2014). Soil under the green fallow was characterized with higher values of I_c (about 9% higher) when compared to intensive cultivation on the slope. Ic were also strongly affected by slope position (p = 0.0000). In both analysed slopes (*G* and *IC*), the highest I_c values were observed at SH position while the lowest at the flat terrain under the slope (Figure 1D). This results are surprising as we expected the highest formation of soil crust in flat terrain and the lowest values on the upper parts of the slope due to erosion processes. However, any statistically significant trend in I_c dynamics along the slope gradient in both *G* and *IC* were not observed (Table 2). The interaction of land use and slope position showed a strong tendency (p = 0.0000) to affect I_c values. The impact of the land use type tended to be more pronounced than slope position (Table 1). Critical soil organic matter content (S_t) according to Pieri (1991) is another important parameters of soil structure stability as the soil structure is significantly affected by soil organic matter content (Bronick and Lal, 2005; Czachor et al., 2015; Šimanský and Jonczak, 2016). The S_t was strongly affected by both land use type (p = 0.0114) and slope position (p = 0.0000). The values of S_t were statistically significantly dependent on the interaction of land use and slope position (Table 1). The most stable soil structure according to determined S_t values were observed at TS and F positions of slopes under both of

Table 2 Trends of soil structure parameters along the slope gradient

Land use	Soil structure parameter	Model	R^2
Intensive cultivation	*K*	$y = -0.0893x^2 + 0.5247x + 0.676$ (Quadratic polynomial)	0.3247
	Kwsa	$y = 0.8203x^{0.349}$ (Power model)	0.4972
	PAD	$y = 53.941e^{-0.268x}$ (Exponential)	0.5330
	I_c	$y = 1.4139e^{-0.051x}$ (Exponential)	0.3964
	S_t	$y = -0.0064x^2 + 0.1376x + 2.756$ (Quadratic polynomial)	0.2448
Fallow under greening	*K*	$y = -0.0121x^2 – 0.1161x + 2.77$ (Quadratic polynomial)	0.6365
	Kwsa	$y = 0.1443x^2 – 0.0937x + 1.058$ (Quadratic polynomial)	0.6994
	PAD	$y = -2.0514x^2 + 5.8966x + 21.324$ (Quadratic polynomial)	0.8631
	I_c	$y = -0.0564x^2 + 0.2696x + 0.926$ (Quadratic polynomial)	0.6141
	S_t	$y = 0.2257x^2 – 0.9743x + 3.458$ (Quadratic polynomial)	0.6751

K – structure coefficient, *Kwsa* – structure coeeficient of water-stable aggregates, *PAD* – percentage of macro-aggergate destruction, I_c – index of crusting, S_t – critical level of soil organic matter

Table 3 Correlation coefficients between soil parameters on the analysed slopes

	Sand	Silt	Clay	$CaCO_3$	pH	SOC	CL	C_{HWD}	CHS	CHA	CFA	HA : FA	QHS	QHA
Intensive cultivation														
K	0.185	-0.366	0.281	-0.171	-0.239	0.455	0.773*	0.049	0.582	0.318	0.665*	-0.629	-0.329	-0.308
Kwsa	-0.321	0.319	0.047	-0.597	-0.459	0.475	0.276	0.228	0.403	0.655*	0.163	0.216	-0.477	-0.529
PAD	0.484	-0.415	-0.184	0.589	0.455	-0.347	0.038	0.073	-0.173	-0.499	0.079	-0.416	0.357	0.394
I_c	-0.185	0.650*	-0.763*	0.704*	0.882***	-0.888***	-0.400	0.100	-0.852**	-0.698*	-0.814**	0.592	0.915***	0.882***
S_t	0.305	0.641*	0.528	0.660*	0.809**	0.908***	0.582	0.218	0.953***	0.766**	0.922***	0.685*	-0.946***	-0.936***
Green fallow														
K	-0.798**	0.841**	0.412	0.556	0.509	-0.567	-0.546	-0.591	-0.614	-0.624	-0.555	-0.537	0.605	0.520
Kwsa	-0.686*	-0.524	-0.826**	-0.618	-0.649*	0.676*	0.658*	0.700*	0.649*	0.677*	0.554	0.536	-0.734*	-0.860**
PAD	-0.877***	0.767**	0.824**	0.769**	0.799**	-0.838**	-0.842**	-0.856**	-0.855**	-0.864**	-0.783**	-0.619	0.868**	0.935***
I_c	-0.897***	0.912***	0.542	0.942***	0.890***	-0.965***	-0.959***	-0.903***	-0.973***	-0.952***	-0.951***	-0.658*	0.909***	0.747*
S_t	0.925***	-0.868**	-0.731*	-0.961***	-0.953***	0.993***	0.974***	0.915***	0.993***	0.988***	0.939***	0.712	-0.974***	-0.892***
Bothl land use types evaluated together														
K	-0.330	-0.111	0.719***	-0.511*	-0.493*	-0.241	-0.277	-0.431	-0.110	-0.068	-0.165	-0.029	-0.296	-0.429
Kwsa	0.361	-0.422	0.025	-0.640**	-0.649**	0.536*	0.437	0.399	0.604**	0.680***	0.392	0.541*	-0.642**	-0.680***
PAD	-0.139	0.259	-0.153	0.719***	0.684***	-0.510*	-0.346	-0.280	-0.553*	-0.638**	-0.334	-0.564*	0.646**	0.672**
I_c	-0.541*	0.816***	-0.312	0.765***	0.828***	-0.829***	-0.661**	-0.433	-0.860***	-0.813***	-0.824***	-0.305	0.903***	0.797***
S_t	0.745***	-0.673**	-0.243	-0.486*	-0.557*	0.981***	0.909***	0.767***	0.974***	0.927***	0.920***	0.411	-0.714***	-0.570**

K – structure coefficient, *Kwsa* – structure coeeficient of water-stable aggregates, *PAD* – percentage of macro-aggergate destruction, I_c – index of crusting, S_t – critical level of soil organic matter, $CaCO_3$ – carbonate contents, pH – soil pH, SOC – soil organic carbon, C_L – labile carbon, C_{HWD} – hot water extracted carbon, CHS – humic substances carbon, CHA – humic acids carbon, CFA – fulvic acids carbon, Q_{HS} – colour quotients of humic substances, Q_{HA} – colour quotients of humic acids the examined land use types (Figure 1E). Despite the fact of any statistical significance the S_t dynamics is quite well expressed by the quadratic polynomial trend (Table 2).

The relationships between the soil structure parameters and texture as well as SOM were evaluated as shown in Table 3. The soil structure is affected by many factors (Amézketa, 1999; Bronick and Lal, 2005; Wiesmeier et al., 2012; Czachor et al., 2015; Šimanský and Jonczak, 2016; Šimanský et al., 2019), what has been confirmed with our findings. However, obtained results clearly show that not every factor influencing soil structure was influenced the same way. Many significant interactions were observed between investigated parameters. When samples from both land use types and all slope positions were assessed together, positive significant correlations were observed between silt and carbonate contents and I_c, while SOM and organic matter parameters did not have any effect on formation of soil crust what is surprising because as it was reported by Špička et al. (1964) and Maïga-Yaleu et al. (2013) as well as Šimanský et al. (2014) higher SOM content impede formation of soil crust. On the other hand, the S_t values were strong effected SOM quantity and quality. We also determined better soil structure state (based on S_t) with higher humus stability (Table 3). Differences were noted when the relationships were assessed separately depending on the land use. For instance, in *IC*, positive correlations between labile carbon (r = 0.773, P <0.01), carbon of fulvic acids (r = 0.665, P <0.05) and K were observed. Soil crust formation have been supported by higher contents of silt, carbonates and lower humus quality and stability. A higher silt and carbonate contents as well as higher content of SOM and better humus quality resulted in higher S_t values in intensive cultivation on the slope. In general, tillage disturbance has been recognized as one of the major causes of erosion. The erosion rates in conventionally tilled cropland are on average one- to two-fold greater than the rates of soil production (Montgomery, 2007). Reduction of soil tillage improves soil properties including texture, structure and organic matter content (Ba et al., 2016). Overall, the highest number of statistically significant correlations between soil structure parameters and texture as well as SOM parameters were observed in soil under green fallow than on intensively cultivated slope (Table 3). In *G*, the K values positively correlated with silt and carbonate contents. In opposite, SOM parameters did not affect the K values. Higher labile carbon content as well as higher humus quality and stability improved soil structure development evaluated on the base of *Kwsa* on *G* slope. Aggregate destruction (*PAD*) was more intensive due to higher sand and carbonate contents as well as due to lower humus stability. In *G,* formation of soil crust have been supported by higher silt and carbonate contents and on the other hand also by lower humus stability. With higher contents of silt, clay and SOM values of S_t were increasing.

Conclusions

Based on the results of this study we proved that the selected soil structure parameters of silt loam soil was different due to both: the land use type and the slope position. Moreover, it can be concluded that not all of the examined soil structure parameters were equally influenced by pedogenic factors. The assumption that the soil structure could be affected by the interactions between internal and external factors has been confirmed. Overall, the highest number of statistically significant correlations between soil structure parameters and texture as well as SOM parameters were observed in the green fallow than in intensive cultivation on the slope. The results indicate that land use can significantly affect the relationships between texture, SOM and soil structure development.

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Original Paper

Identification and relative abundance of native arbuscular mycorrhizal fungi associated with oil-seed crops and maize (*Zea mays* L.) in derived savannah of Nigeria

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A field survey was conducted to assess root colonization, spore densities and relative abundance of native arbuscular mycorrhizal fungi (AMF) based on morphological aspects. Roots and rhizosphere soil samples were collected from established fields of selected oil seed crops [soybean (*Glycine max* L.), sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*)] and maize (*Zea mays* L.) grown in derived savannah agro-ecology of Southwest Nigeria. The mean percentage of AMF colonization across all crops was 60.8%, ranging from 34% to 87.5%, with highest root colonization observed in soybean. The spore densities retrieved from the different rhizospheres were relatively high, varying from 124 to 298 spores per 50 g dry soil, with highest spore densities observed in maize rhizosphere soils. The spore densities in the soil significantly correlated ($r = 0.52$, and $P < 0.05$) with the root colonization. A total of 4 morphologically classifiable genera (*Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora*) of AMF within the phylum Glomeromycota were detected. The dominant genus was *Glomus* in all the crops with highest relative abundance of 60.9%, followed by *Acaulospora* (21.3%) and *Scutellospora* (12.8%), with lowest relative abundance of AM spores observed for *Gigaspora* (5%). This study could contribute significantly to a better understanding of AMF community structure in derived savannah agro-ecology of Nigeria.

Keywords: k Arbuscular mycorrhizal fungi, community structure, oil-seed crops, root colonization, spore density

1 Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread member of the soil biota and are important components of agricultural ecosystems. AMF form symbiotic relationships with most agricultural crops including maize, sesame, soybean and sunflower (Brundrett 2002; Smith and Read, 2008). AMF belong to the phylum Glomeromycota and about 250 species have been described mostly based on the morphology of their spores with recent molecular study indicating that the real number of AMF species may be much higher, comprising many uncultivated taxa (Schüßler et al., 2001; Ohsowski et al., 2014). They are generally essential for many important ecosystem functions and processes, including nutrient cycling, plant productivity and sustainability (Verbruggen and Kiers, 2010). The bidirectional exchange of nutrients between plants and AMF often results in a nutritional benefit for both partners. The host plant provides the fungus with carbohydrates, while in return the plant obtains rather immobile mineral nutrients such as phosphorus (P) from the fungus (Smith and Read 2008). AMF may also enhance host growth and survival by improving tolerance to drought and to some root pathogens and nematodes (Azcón-Aguilar and Barea, 1997; Yamato et al., 2009). They contribute to soil aggregate stability and may help in reducing salinity effects (Rillig and Mummey, 2006; Evelin et al., 2009). The widespread benefits of AMF may be critical to increasing crop yields and productivity in a sustainable agriculture.

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AMF have been reported to share a long history of coevolution with plants in various ecosystems, resulting in their adaptation to specific areas (Gosling et al., 2006). The majority of research on AMF symbiosis involves laboratory or greenhouse experiments, in which plants are cultivated in sterilized soil, with particular AMF species. They ignore indigenous AMF species that could alter plant responses or compete with the AMF inoculant (Munkvold et al., 2004). A number of factors have been shown to act as environmental filters, structuring AMF communities, such as host plants, land use, fertilization and soil pH (Lin et al., 2012; Oehl et al., 2010; Peyret-Guzzon et al., 2016). Host preferences have also been demonstrated to exist to a certain extent in AMF (Pivato et al., 2007), but strict host specificity seems to be rare. However, it is well established that individual AM fungal species can differ in their associations with different plants (Rillig, 2004). Most studies on the AMF community structure have been conducted at a small scale, with only a few authors reporting AMF diversity at the regional scale or larger (Hazard et al., 2013). Some AMF taxa have been reported to be surprisingly widespread (Davison et al., 2015), however, many cannot yet be directly linked to a certain set of agricultural practices or environmental conditions. Therefore, the understanding of the geographical distribution of these fungi remains somewhat limited.

Colonization by native AMF species in crops has been reported earlier (Maiti et al., 1995; Sawers et al., 2008; Campos-Soriano et al., 2010; Cosme et al., 2011). Despite its important role, there is little information on the distribution and abundance of the different mycorrhizal fungi species associated with some agricultural crops in derived savannah of tropical soil of southwest Nigeria. The derived savannah of Southwest Nigeria is characterized with tropical soils of low P availability. Under such environmental conditions, symbioses between plants and AMF may be an important factor for plant adaptation and survival. Given the paucity of information of AMF with different oil seed crops and maze in the derived savannah of Nigeria, a field survey was performed to assess the intensity of AMF root colonization, spore densities and relative abundance of native AMF genera in rhizosphere soils of oil-seed crops and maize. The study tested the hypothesis that AMF colonization and community structure would differ among the crops.

2 Material and methods

2.1 Study site

The study area is located in the Teaching and Research Farms, Federal University of Agriculture Abeokuta, Ogun State, Nigeria (Latitude 7° 15′ N, Longitude 3° 28′ E, altitude of 75 m a.s.l.).

2.2 Field sampling

Field soil and root samples were taken from the agricultural fields of the research farms in year 2012. Oil seed crops [soybean (*Glycine max* L.), sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*)] and maize (*Zea mays* L.) were grown on the fields in a continuous cropping system established in 2008. The crops were cultivated under organic farming system through the application of organic fertilizers (Aleshinloye) with no pesticide application. The samples were collected at 4, 8 and 12 weeks after planting (WAP) from the experimental fields during the growing period of the crops in late cropping season (August – December 2012). The soil samples were collected from depth of 0–20 cm from several points on the fields. At each sampling point, four subsamples (250 g) were collected from each field and mixed to produce composite soil samples. The collected samples were kept in sterilized plastic bags after removing large particles, broken roots and stone and stored in the refrigerator at 4 °C until processing.

2.3 AMF spore isolation and identification

AMF spore extraction was done in triplicates for individual soil sample. The spores were extracted by the modified wet sieving method of Giovannetti and Mosse (1980). A sample of 25 g of air-dried field soil was mixed with distilled water. The resulting mixture was passed through 250, 150 and 40 µm sieves. The fraction retained in the 500 µm sieve was checked for large spores, spore clusters, sporocarps and organic matter debris. Soil materials retained by the 150 and 40 µm sieves were washed into centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4,000 rpm for 2 minutes. The supernatants were decanted and subjected to sucrose centrifugation (70% (w/v)) gradient and centrifuged at 4,000 rpm for 2 minutes. The supernatant was passed through the 40 µm sieve, washed with distilled water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were recovered and counted at 40× magnification. For identification, spores were picked under the dissecting microscope with a glass micropipette and subsequently mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1 : 1 (v/v); Brundrett 2002) to get permanent slides for spore observation and identification under a compound microscope at up to 400× magnification. The spores were identified at the genus level on the basis of size, spore-wall structure, Melzer's reaction, colour and presence or absence of subtending hyphae and compared with descriptions of fungal genera according to taxonomic criteria (Shenck and Perez, 1990). The relative abundance was calculated based on percentage:

$$Relative\ AMF\ abundance = \frac{number\ of\ spores\ of\ a\ genus}{total\ number\ of\ spores\ in\ the\ rhizosphere} \times 100$$

2.4 Staining and estimation of AMF root colonization

The roots of the respective crop species were carefully freed from adhering soil and immediately fixed in 50% ethanol. Roots in ethanol were rinsed thoroughly in tap water, cut into approximately 1 cm segments and cleared in hot KOH solution (10% w/v, at 90 °C) for 1 hour. The bleached roots were rinsed to remove excess KOH and stained in acidic glycerol containing methyl blue lacto-glycerol (1 : 1 : 1 : 0.5 g) at 90 °C for 30 minutes (Phillips and Hayman, 1970). The stained root segments were mounted on microscopic slides and examined for AMF structure under light microscope to determine percentage root colonization:

$$Percentage\ root\ colonisation = \frac{number\ of\ root\ infected}{total\ number\ of\ roots} \times 100$$

2.5 Statistical analysis

Spore density and root colonization (%) were subjected to $\log e(x+1)$ transformation and square root transformation respectively for normalization of the data. Analysis of Variance was conducted to determine significant differences among the means at 5% probability level. Significant means were separated using Least Significant Difference (LSD). Pearson correlation analysis was used to detect the relationship between spore densities, percent root colonization and AMF relative abundance using the statistical package Genstat 12th Edition.

3 Results and discussion

3.1 AMF root colonization and spore density in the rhizosphere

All root samples of the selected crops surveyed in this study were colonized by AMF. The percentage of AMF colonization ranged from 34% to 87.5% (Table 1). Soybean plants significantly had the highest AMF root colonization compared to other crops throughout the period of measurements at 4, 8 and 12 weeks after planting (WAP) (44, 87.5 and 85% respectively), but significantly comparable to maize root length colonization (40.5%) at 4 WAP.

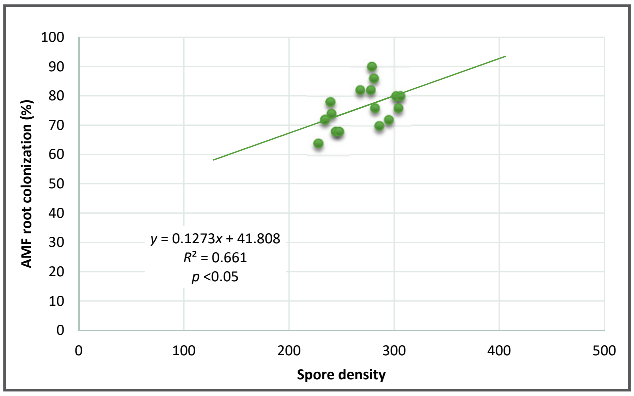

Figure 1 Pearson correlation between root colonization (%) and AMF spore density

Table 1 Percent root colonization of crops by AMF

Treatments	4 WAP	8 WAP	12 WAP
Crops			
Maize	40.5a	78.5b	78.0b
Sunflower	36.0b	69.0c	73.0b
Soybean	44.0a	87.5a	85.0a
Sesame	34.0b	64.5c	68.5c

Different letters within the same column indicate that treatment means are significantly different at $P < 0.05$; WAP = weeks after planting

Table 2 Spore density of AMF (50 g of soil) in rhizosphere of crops

Treatments	4 WAP	8 WAP	12 WAP
Crops			
Maize	164.8a	261.8a	298.5a
Sunflower	128.0c	203.5c	263.5b
Soybean	146.2b	235.0b	276.5ab
Sesame	124.2c	204.2c	239.8c

Different letters within the same column indicate that treatment means are significantly different at $P < 0.05$; WAP = weeks after planting

The spore densities (expressed as per 50 g dry soil) retrieved from different rhizosphere crops were relatively high, varying from 124 in sesame at 4 WAP to 298.5 in maize at 12 WAP (Table 2). AMF spore density was observed to be highest in maize throughout the period of measurement. The spore densities in the soil significantly correlated (r = 0.52, and P <0.05) with the root colonization of the crops (Figure 1).

3.2 AMF relative abundance

A total of 1056 AMF spores were identified and classified from the rhizosphere soil samples at 12 WAP. A total of 4 genera of AMF namely, *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora* were identified from the collected soil samples. Among the four genera of AM fungi observed, the most abundant genus was the *Glomus* with relative abundance of 60.9%, followed by *Acaulospora* (21.3%) and *Scutellospora* (12.8%), while lowest relative abundance of AM spores was observed for genus *Gigaspora* (5%) as shown in Figure 2 and 3.

The spore abundance of *Glomus*, *Acaulospora* and *Scutellospora* were positively correlated with root colonization with significant correlation observed with *Glomus* spore abundance (r^2 = 0.53, and P <0.01) as shown in Figure 4, 5 and 6. The spore abundance of *Gigaspora* was negatively correlated with root colonization (r^2 = 0.18, P >0.05) as shown in Figure 7.

In the present study, the results indicate variation in the spore density and root colonization of AM fungi naturally associated with the selected crops. Moreove, spore densities and root colonization were significantly

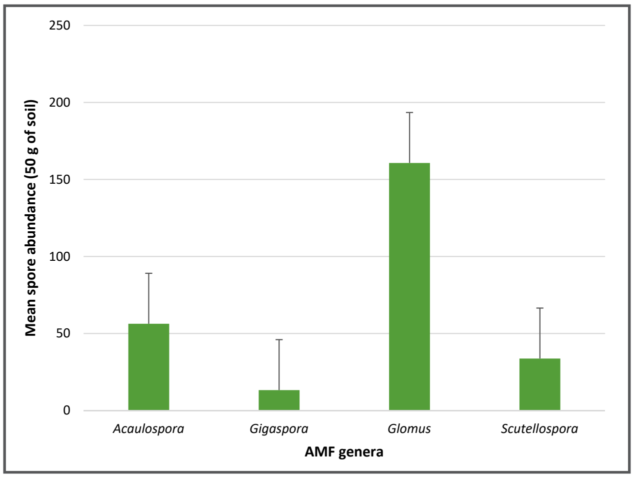

Figure 2 Mean spore abundance of AMF genera in rhizosphere soil of selected crops. Mean values were calculated from the data obtained in all plots. Bars in each column indicate standard error of means (±SE)

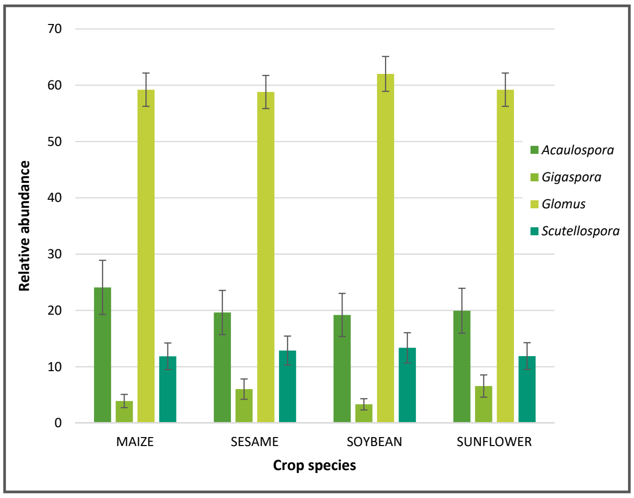

Figure 3 Relative abundance (%) of AM spores identified at genus level from the soil samples. Mean values were calculated from the data obtained in all plots belonging to the particular crop species. Bars in each column indicate standard error of means (±SE)

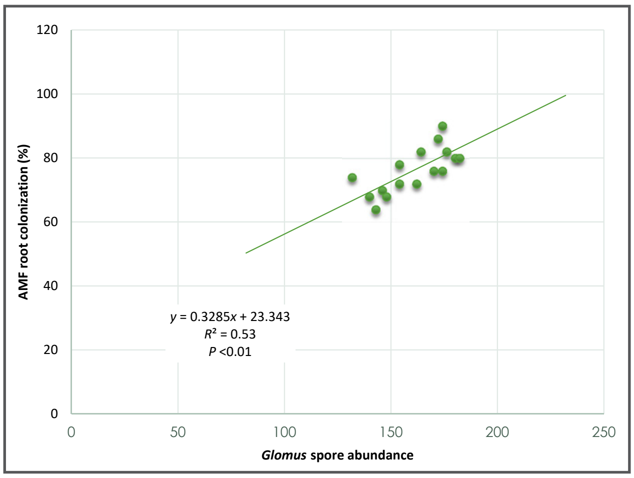

Figure 4 Pearson correlation between root colonization and *Glomus* spore abundance

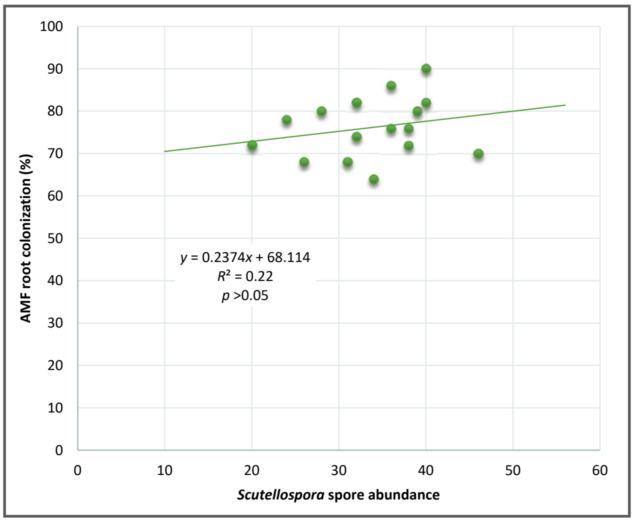

Figure 5 Pearson correlation between root colonization and *Scutellospora* spore abundance

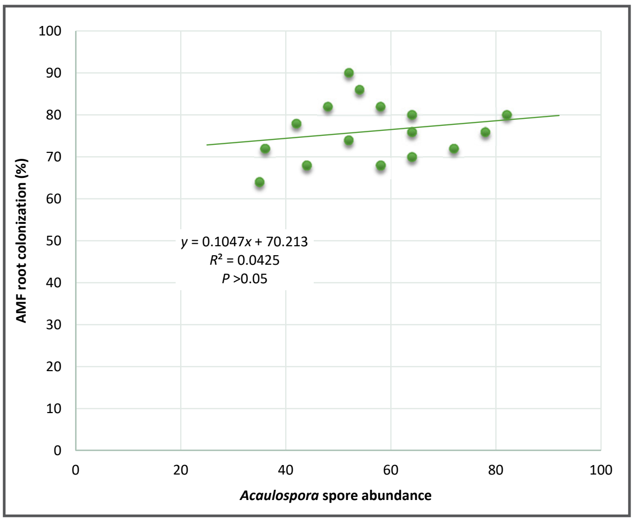

Figure 6 Pearson correlation between root colonization and *Acaulospora* spore abundance

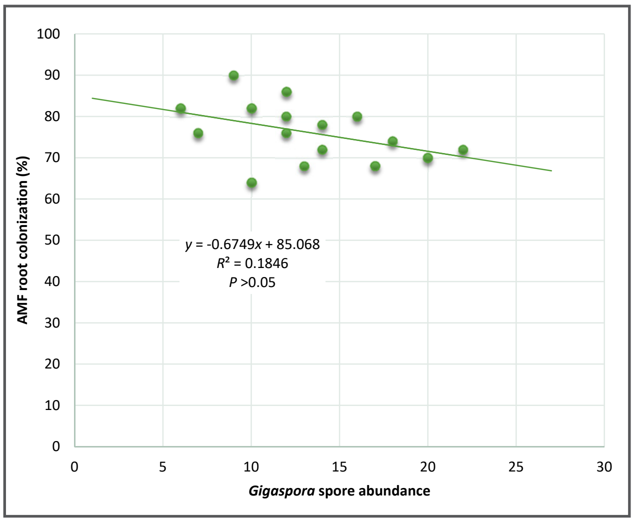

Figure 7 Pearson correlation between root colonization and *Gigaspora* spore abundance

higher in rhizosphere soils of tmaize and soybean. Host preferences have been demonstrated to exist to a certain extent in AMF, but strict host specificity seems to be rare (Pivato et al., 2007). This could possibly be attributed to the differences in rooting habits and nutrients demands of the crops (Douds et al. 2005) or amount of carbon transfer from the hosts to AMF. It has also been reported that AMF community composition depends on host plant species and, therefore, plant species may have varying degrees of selectivity on AMF species that range from selective specialists to non-selective generalists (Oehl et al. 2003; Scheublin et al. 2004). The relatively high root colonization in rhizosphere soil in this study could be due to the relatively low phosphorus in most tropical soils. In general, AMF spore density was found to be positively correlated with root colonization in this study. This could be due to the fact that some AMF species rely more on extensive formation of hyphal networks in roots and survival through spore formation as primary infective propagules in soils (Biermann and Linderman, 1983).

There was a prominent distribution of AMF genera among crop species, with higher relative abundance of *Glomus* genus and lower relative abundance of *Gigaspora* and *Scutellospora*. The genus *Glomus* was the most dominant and widely distributed followed by *Acaulospora* as also confirmed by this study. It has been previously reported that *Glomus* species are the most abundant among the glomeromycotan genera in tropical areas (Snoeck et al., 2010), regardless of the type of hosts and intensity of disturbance in the different ecosystems. Furthermore, *Glomus* species have the abiltiy to produce a relatively high number of spores within a very short period of time (Oehl et al., 2009). The significant reduction in relative spore abundance of *Gigaspora* and *Scutellospora* may be due to soil disturbances due to agricultural practices such as tillage (Boddington and Dodd, 2000). Furthermore, *Gigaspora*ceae have been reported to rely on spores as their primary infective propagules (Biermann and Linderman, 1983). Moreover, soil texture might also play a key role for their occurrence in tropical soils (Lekberg et al., 2007).

4 Conclusions

This study reveals the presence of different AMF genera and a high spore abundance in the rhizospheric soils of soybean, maize, sesame and sunflower grown in derived savannah of Nigeria. The crops regulates the intensity of mycorrhizal colonization and spore density. The information obtained from this study can be used to further investigate the impact of AMF symbiosis on productivity of the crops and could provide a primary basis for sustainable crop production in derived savannah agro-ecology of Nigeria.

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Original Paper

Effects of seed bed types and weed control methods on the vegetative parameters of long cayenne pepper (*Capsicum frutesens* L)

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The type of seed beds and weed control methods play significant role in determining sustainability in vegetable production. Field experiment was conducted during 2015 growing season to determine the effects of three seed bed types such as: raised bed (RB), flat bed (FB), ridged bed (RB) in combination with four weed control methods such as: hoe weeding (T_1), hand weeding (T_2), use of *Panicum maximum* as live mulch (T_3) and zero weeding (T_4) on the vegetative performance of long cayenne pepper (*Capsicum frutesens* L.) The experiment was arranged as split plot fitted into Randomized Complete Block Design (RCBD) with three replications. The growth parameters measured were the plant height (cm); number of leaves, numbers of branches, stem girth (cm), leaf area (cm^2), weed density (m^{-2}) and weed biomass (kg/ha). The result revealed that the seed bed types and the weed control methods had significant effect on the parameters measured. However, raised bed and hoe weeding (RBT1) enhanced all the parameters measured more than other treatment combinations by recording the highest mean value in all character and also proved to be more effective in reducing weed biomass than other weed control treatment combinations. It was concluded that sowing on raised bed and using hoe weeding as a means of controlling weeds should be recommended for effective performance of pepper for optimum growth.

Keywords: seed bed, weed control, Long cayenne pepper

1 Introduction

Long Cayenne Pepper (*Capsicum frutesens* L.) belongs to the family Solanaceae. It is one of the most important vegetables that is consumed worldwide after tomatoes and onions (Akinfasoye et al.2006) and was believed to have been introduced to Africa and Asia by Columbus from the new world (Alabi, 2006). It is best to start the seed in a good and warm environment.The progress faster that way because the seed of pepper is slow to germinate,taking up to 12–21 days or longer (NAERLS, 2006; Awalu and Mohamman, 2009). Although much of the greater part of the total hectarage of pepper is grown from transplant. Seeds can be sown directly in the open field, principally in some of the warmest part of the country (NAERLS, 2006). 10–12 seed can be planted at 45–50 cm apart on rows, 75 cm apart between rows (Grubben and Tahir, 2014). Cayenne pepper are usually a tapering group, 10–25 cm long, generally skinny, mostly red coloured, often with a curved tip and some what ripped skin, wich hang from bush as opposed to growing upright (Idowu et al., 2012).

Pepper production has increased in recent years in Nigeria and other sub-humid and semi-arid tropics as a result of its nutritional values. Ashenafi and Tekalign (2014) reported, that pepper contributes substantially to the Nigerian diet, accounting about 40 percent of the total vegetables consumed per day. It is a good source of vitamins A, C, E, B_1, B_2 and D (Auwalu and Muhamman, 2009). Also obtained from pepper are potassium, phosphorus and calcium (Idowu et al., 2012). USDA (2001) and Business day (2007) reported, that exportation of pepper in Nigeria has once been reported as a lucrative business, Nigeria being the largest producer of pepper in the world accounting for about 50 percent G.D.P of Africans production. Ashenafi and Tekalign (2014) and Gungula and Bayoso (2005) reported that in addition to pepper being easy to grow, it is easily processed and packaged for export. The cash income potentials being derived

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from it makes it suitable for use in poverty reduction and food security improvement programs. Although pepper is widely grown in Nigeria, yields obtained by farmers are often very low. This could be attributed to production constraints in pepper cultivation such as unpredictable climatic condition, weed infestation and improper seed bed preparation (Grubben and Tahir, 2004).

In crop production system prior to seedlings emergence, good seed bed preparation is one of the most important approaches to soil amendment and improvement (Samuel and Ajav, 2010). Zaragoza (2002) equally reported seed bed preparation as an effective step for increasing space, efficiency and yield of vegetable crops. Weed control is one of the most serious concerns of both commercial and subsistence pepper growers (Fitzroy, 2011). Weed found growing in pepper field compete with the crop for light, moisture, air, water, space, and nutrient. This competition decreases plant vigour, quality and yield. Peter et al. (2014) and Mark (2014) reported, that pepper plant unlike other vegetable crops like tomatoes and lettuce, has shallow root system which makes it more vulnerable to weed competition and this is detrimental to its establishment and growth.

Generally, plants require an environment where nutrients and other resources that support its growth and development are in abundance and they do best when their roots are able to extract these resources (Adigun, 2001). Zaragoza (2002) equally reported, that vegetables grown on seed beds do better because those soils are usually light and loosed with good tilt which makes root penetration easy. This helps in nutrient absorption in plants and thus improves crop emergence and growth.

Weed control is vital to achieving good crop performance and effective weed control strategies are limited for *Capsicum* producers. Over a long period, there was no herbicides registered for broad leaf weed in Nigeria (Shaikia et al., 2004). Also, proper soil management practices like good seed bed preparation is useful for improving soil condition for enhancement of crop growth (Zaragoza, 2002). Incidentally, many farmers do not give desired attention to the manner in which they prepare their seed beds and the best method of controlling weeds. To increase the performance of pepper, proper seed bed management preparation and weed control practice becomes an important option. Therefore, this research was designed with the objective of determining the appropriate seed bed types and weed control methods that is suitable for the establishment and growth of long cayenne pepper.

2 Material and methods

2.1 Experimental site

The experiment was carried out at the National Horticultural Research Institute (NIHORT) Vegetable Experimental Field in Ibadan (Lat, 7° 22° N, Long 3° 50° E). The study area is in the tropical wet and dry climate with a bio modal rainfall pattern having long rainy season which usually start in late March to September and to early November after a short dry spell in August. The average minimum and maximum temperature from 21 to 37 °C, with an annual rainfall of about 1,250–1,500 mm and average relative humidity of about 70%.

2.2 Nursery operation

Long cayenne pepper seedlings were raised in seed boxes each measuring 1 × 1 m (L × B) and 1 × 1 m height. The nursery boxes were filled with sterilized top soil, seeds were sown by broadcasting, watered and monitored for six weeks before transplanting to the permanent beds.

2.3 Pre – weed sampling

Weed samples were harvested within the net plot area before planting to know the predominant weeds on the experimental field, using 0.25 × 0.25 m quadrate which was placed thrice at random arrangement and the weeds within the quadrate were identified and recorded.

2.4 Land preparation and plot layout

The experimental field was cleared of its vegetation, debris were burnt, the field was ploughed and then the bed types and ridges were constructed. The experimental field was divided into 12 plots that comprised the 3 seed bed types: raised beds, ridge beds and flat beds that constituted the main plots while the weeding treatments (hoe weeding, hand weeding, live mulch and zero weeding) were randomly assigned to the beds as sub-plots and these treatments were replicated thrice giving a sum total of 48 plots. Each bed size was 2 × 2 m with 1 m gap between the beds. The blocks were spaced out 1 m apart with 1.5 m dimensions of the beds to ease movement during farming operation and the total land area was 227.5 m2.

2.5 Treatments and experimental design

The experiment was arranged as split plot fitted into Randomized Complete Block Design (RCBD) with three replications. The three seed bed types were allotted to the main plots while the four weed control methods were allotted to the sub-plots. The three seed bed treatments were: Raised Beds (RB), Flat Bed (FB), Ridge Bed (R). The four weed control methods were: T_1 – Hoe weeding, T_2 – Hand weeding, T_3, – Use of live much (*Panicum maximum*) and T_4 – Zero weeding.

2.6 *Planting and cultural practices*

At 6 weeks after sowing, one seedling was planted per hole at a spacing of 50 × 50 cm and 20 plants were planted per seed beds giving a total plant population of 1,440 plants per ha. The weeding treatment was carried out at 4, 6 and 8 weeks after transplanting prior to each weeding at 2 weeks interval except mulching which was maintained throughout the study period and zero weeding where retained throughout the experiment. Data were collected on the fresh weight of the weed samples harvested from each of the 3 randomly selected net plots and recorded. The weeds were later oven-dried, weighed and the average weight of the biomass was determined.

2.7 *Data collection and statistical analysis*

The experiment was arranged as split plot fitted into Randomized Complete Block Design (RCBD) with three replications. Data collection on the growth characteristics of pepper commeranced at 4 WAP and was done forthnightly. The growth assesment of the test crop was made from 4 randomly selected tagged plants from the certral row of each plot. The recorded data were compiled and statistically analyzed via Analysis of Variance with Statistical Analysis Software (SAS) package, and the significant means were separated by least significant difference (LSD) at 5% level of significance (Gomez and Gomez, 1984).

3 Results and discussion

3.1 *Effect of seedbed types and weed control methods on growth parameters of pepper plant height*

The main and interactive effect of seedbed types and weed control methods on the pepper plant height is presented in Table 1. Seed bed types had no significant effect on pepper plant height except at 4 weeks after transplanting (WATP) with significantly (p <0.05) higher plant height value recorded in ridge seed bed type (22.24 cm) which was closely related to the value recorded in raise bed type (21.35 cm), while the least value was recorded in the flat bed type (19.20 cm).

Weed control methods had significant (p <0.05) effect on plant height of pepper throughout the study period. At 4, 6 and 8 weeks after transplanting (WATP), significantly (p <0.05) higher value of pepper plant height was recorded in hoe weeded plot (23.24 cm, 35.97 cm and 43.00 cm) compared with the values recorded in hand weeded plot (21.08 cm, 31.10 cm and 34.40 cm) while the least value were recorded in zero weeded plots (16.62 cm, 24.27 cm and 31.20 cm).

There was interactive effect of seedbed types and weed control methods on pepper plant height throughout the study periods. At 4, 6 and 8 weeks after transplanting (WATP), significantly (p <0.05) higher plant height values (24.80 cm, 36.40 cm and 44.67 cm) were recorded in raised bed hoe weeded plot closely followed by raised bed live mulch plots (22.84 cm, 34.43 cm and 43.40 cm) while the least value were recorded in flat bed zero weeded plot (14.10 cm, 21.00 cm and 27.43 cm).

3.2 *Number of leaves*

The main and interactive effect of seed bed types and weed control methods on the number of leaves of pepper is as presented in Table 2. Seed bed types influenced the number of leaves of pepper throughout the study period with significantly (p <0.05) higher values of number of leaves of pepper recorded in raised bed type (12.61, 25.55 and 35.00) compared with the values recorded in ridge bed type (12.39, 21.88 and 31.98) for 4, 6 and 8 (WATP) respectively, while the least values were recorded in flat bed type (9.94, 17.26 and 26.07).

Weed control methods had significant (p <0.05) effect on the number of leaves of pepper throughout the study period. At 4, 6 and 8 (WATP), hoe weeded plots recorded significantly (p <0.05) higher values of number of leaves (13.37, 23.53 and 32.49), closely followed by the values recorded in live mulch plots (12.43, 22.86 and 32.00) while the least values were recorded in zero weeded plots (10.25, 18.94 and 28.00).

There were interactive effects of seed bed types and weed control methods on the number of leaves of pepper throughout the study period. At 4, 6 and 8 (WATP) significantly (p <0.05) higher number of leaves of pepper was recorded in raised bed hoe weeded plot (14.60, 28.80 and 37.90) compared with the values recorded in raised bed hand weeded plots (12.08, 25.90 and 35.63) and ridge hoe weeded plots (14.25, 23.67 and 33.13) while the least value was observed in flat bed zero weeded plot (8.32, 15.53 and 24.00).

3.3 *Stemgirth*

The main and interactive effect of seed bed types weed and control methods on stem girth of pepper is as presented in Table 3. Seed bed types had significant effect on stem girth of pepper throughout the study period. Significantly (p <0.05) higher stem girth values (1.66 cm, 2.56 cm and 3.49 cm) were recorded in ridge bed type respectively while flat bed recorded the least values (1.44 cm, 2.30 cm and 3.28 cm).

Weed control method also had significant effect on stem girth of pepper throughout the period of study. At 4, 6 and 8 (WATP), significantly higher stem girth values were recorded in hoe weeded plots (1.79 cm, 2.91 cm and 3.70 cm) compared with the value recorded in live mulched plot (1.65 cm, 2.71 cm and 2.51 cm) while the

Table 1 The interactive effect of seed bed types and weed control methods on pepper plant height (cm)

Seed bed type	Weed control methods				Seed bed type mean
Plant height at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	24.80	21.04	22.84	18.41	21.35
Flat bed	22.40	19.93	20.38	14.10	19.20
Ridge	24.22	22.27	23.22	17.68	22.24
Weed control method mean	23.24	21.08	22.67	16.62	
LSD bed (B)	2.27				
LSD weed control method (W)	2.62			6	
LSD B × W	4.53				
Plant height at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	36.40	31.10	34.43	26.33	32.01
Flat bed	35.37	28.87	29.47	21.00	28.67
Ridge	36.13	33.57	34.80	25.47	32.49
Weed control method mean	35.97	31.10	32.90	24.27	
LSD bed (B)	3.28				
LSD weed control method (W)	3.78				
LSD B × W	6.55				
Plant height at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	44.67	34.07	43.40	27.13	37.30
Flat bed	41.47	37.23	40.47	27.43	36.80
Ridge	42.80	38.70	40.70	31.67	38.50
Weed control method mean	43.00	34.40	41.50	31.20	
LSD bed (B)	5.72				
LSD weed control method (W)	6.00				
LSD B × W	11.44				

least values were recorded in zero weeded plots (1.33 cm, 2.10 cm and 3.57 cm) respectively.

The main and interactive effect of seed bed types and weed control methods on pepper stem girth at 4, 6 and 8 after transplanting (WATP) was significant throughout the study period with significantly (p <0.05) higher values recorded in raise bed hoe weeded plot (1.79 cm, 2.91 cm and 3.70 cm) closely followed by ridge hoe weeded plot (1.74 cm, 2.84 cm and 3.60 cm) and raised bed live mulch treated plot (1.65 cm, 2.71 cm and 3.51 cm) respectively while the least values was recorded in flat bed zero weeded plots (1.41 cm, 1.92 cm and 3.78 cm).

3.4 Pepper branches

Table 4 presents the main and interactive effect of seed bed types and weed control methods on pepper branches. Seed bed types had significant effect on the number of branches of pepper throughout the study period with significantly (p <0.05) higher value of number of pepper branches recorded in raised bed type (1.88, 4.02 and 5.90) at 4, 6 and 8 (WATP), respectively compared with the value recorded in the ridge bed type (1.57, 3.12 and 4.92) while the least values were recorded in flat bed type (1.18, 2.57 and 3.27).

Weed control methods had significant effect on the number of pepper branch throughout the study period with significantly (p <0.05) higher value of pepper branches recorded in the hoe weeded plots (2.03, 4.02 and 6.20) respectively followed by the values of number of pepper branches recorded in hand weeded plot (1.41, 3.14 and 5.00) and live mulch plots (1.60, 3.46 and 5.29)

Table 2 The interactive effect of seed bed types and weed control methods on the number of leaves of pepper

Seed bed type	Weed control methods				Seed bed type mean
Number of leaves at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	14.60	12.08	12.52	11.60	12.61
Flat bed	11.25	9.43	10.75	8.32	9.94
Ridge	14.25	12.03	14.09	10.83	12.39
Weed control method mean	13.37	11.18	12.43	10.25	
LSD bed (B)	2.87				
LSD weed control method (W)	11.65				
LSD B × W	2.87				
Number of leaves at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	28.80	25.90	26.13	21.37	25.55
Flat bed	18.13	15.33	20.03	15.53	17.76
Ridge	23.67	21.30	22.63	19.93	21.88
Weed control method mean	23.53	20.92	22.86	18.94	
LSD bed (B)	2.66				
LSD weed control method (W)	3.08				
LSD B × W	5.33				
Number of leaves at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	37.90	35.63	36.13	29.73	35.00
Flat bed	26.40	24.94	28.93	24.00	26.07
Ridge	33.13	31.63	33.43	29.73	31.98
Weed control method mean	32.49	30.76	32.00	28.00	
LSD bed (B)	3.74				
LSD weed control method (W)	4.32				
LSD B × W	7.48				

respectively, while the least values were recorded in zero weeded plots (1.14, 2.19 and 3.62).

There was interactive effect of seed bed types and weed control methods had on the number of branches of pepper throughout the study period. At 4, 6 and 8 WATP, significantly higher values of number of branches of pepper were recorded in raised bed hoe weeded plots (2.67, 5.27 and 7.77) respectively, compared with the values recorded in raised bed live mulch plots (1.88, 4.63 and 6.53) while the least values were observed in flat bed zero weeded plots (1.03, 2.03 and 3.27).

3.5 Pepper leaf area

The main and interactive effect of seed bed types and weed control methods on the leaf area of pepper plot is as presented in Table 5. Seed bed types had significant effect on the leave area of pepper except at 6 weeks after transplanting. At 4 and 8 weeks after planting, significantly (WATP) (p <0.05) higher leave area values were recorded in raised bed type (5.11 cm^2, 7.99 cm^2, 10.51 cm^2) closely followed by the values recorded in ridge bed type (4.48 cm^2, 7.96 cm^2, 10.08 cm^2) while the least value was recorded in the flat bed type (4.40 cm^2, 7.60 cm^2, 9.59 cm^2).

Weed control methods equally had significant effect on the leave area of pepper throughout the study period at 4, 6 and 8 (WATP) after transplanting respectively, with significantly (p <0.05) higher leave area values recorded in hoe weeded plots (5.67 cm^2, 9.09 cm^2 and 11.11 cm^2) closely followed by the values recorded in the live mulch plots (5.29 cm^2, 8.33cm^2 and 10.19 cm^2) respectively,

Table 3 The interactive effect of seed bed types and weed control methods on the stem girth

Stem girth (cm)	Weed control methods				Seed bed type mean
Stem girth at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	1.79	1.51	1.65	1.41	1.66
Flat bed	1.69	1.43	1.53	1.00	1.44
Ridge	1.74	1.64	1.72	1.49	1.58
Weed control method mean	1.74	1.53	1.64	1.33	
LSD bed (B)	0.14				
LSD weed control method (W)	0.17				
LSD B × W	0.29				
Stem girth at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	2.91	2.69	2.71	1.92	2.56
Flat bed	2.49	2.23	2.34	2.13	2.30
Ridge	2.84	2.38	2.50	2.25	2.50
Weed control method mean	2.75	2.48	2.47	2.10	
LSD bed (B)	0.15				
LSD weed control method (W)	0.17				
LSD B × W	0.29				
Stem girth at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	3.70	3.43	3.57	3.78	3.49
Flat bed	3.48	3.29	3.31	3.08	3.28
Ridge	3.60	3.38	3.51	3.26	3.36
Weed control method mean	3.58	3.37	3.50	3.04	
LSD bed (B)	0.22				
LSD weed control method (W)	0.25				
LSD B × W	0.44				

while the least value was recorded in the zero weeded plot (3.53 cm^2, 6.45 cm^2 and 8.09 cm^2).

There was interactive effect of seed bed types and weed control methods on pepper leave area throughout the study period with significantly (p <0.05) higher leave area values recorded in raised bed hoe weeded at 4, 6 and 8 (WATP) after planting (10.13 cm^2, 12.72 cm^2 and 20.20 cm^2), while the least value was recorded in flat bed zero weeded plot (2.79 cm^2, 5.95 cm^2, and 7.42 cm^2).

3.6 Weed density in pepper plots

Seed bed types had no significant effect on the weed density value were recorded in pepper plots except at 8 weeks after transplanting with significantly (p <0.05) higher weed density values recorded in flat bed (18.83 m^{-2}) which was at pare with the value recorded in ridge bed type (17.17 m^{-2}) while the least value was recorded in raised seed bed type (16.83 m^{-2}). Weed control methods had significant effect on weed density values recorded in pepper plot throughout the study period. Significantly (p <0.05) higher weed density values were recorded in zero weeded (22.45 m^{-2}, 18.56 m^{-2} and 14.67 m^{-2}) compared with the values obtained in live mulched plot (12.67 m^{-2}, 14.11 m^{-2} and 15.89 m^{-2}) while the least value was recorded in the hoe weeded plots (11.67 m^{-2}, 12.33 and 13.78 m^{-2}) (Table 6).

There was interactive effect of seed bed types and weed control methods on weed density values recorded in pepper plot throughout the study period with significantly (p <0.05) higher weed density value recorded in flat bed zero weeded plots (12.72 m^{-2}, 20.00 m^{-2} and

Table 4 The interactive effect of seed bed types and weed control methods on the number of branches

Pepper branches	Weed control methods				Seed bed type mean
Pepper branches at 4 weeks after planting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	2.67	1.77	1.88	1.30	1.88
Flat bed	1.33	1.10	1.27	1.03	1.18
Ridge	2.10	1.33	1.77	1.10	1.57
Weed control method mean	2.03	1.41	1.60	1.14	
LSD bed (B)	0.53				
LSD weed control method (W)	0.62				
LSD B × W	1.07				
Pepper branches at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	5.27	3.97	4.63	2.20	4.02
Flat bed	3.40	2.13	2.73	2.03	2.57
Ridge	3.80	2.67	3.67	2.33	3.12
Weed control method mean	4.02	3.14	3.46	2.19	
LSD bed (B)	1.02				
LSD weed control method (W)	1.18				
LSD B × W	2.03				
Pepper branches at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	7.77	5.3	6.53	3.67	5.90
Flat bed	5.07	4.07	4.67	3.27	3.27
Ridge	5.77	4.40	5.57	3.93	4.92
Weed control method mean	6.20	5.00	5.29	3.62	
LSD bed (B)	1.10				
LSD weed control method (W)	1.23				
LSD B × W	2.20				

27.30 m^{-2}) while the least values were recorded on raised bed hoe weeded plots (7.29 m^{-2},11.00 m^{-2} and 13.33 m^{-2}).

3.7 Weed biomass in pepper plot

The main effect of seed bed types and weed control methods on weed biomass value in pepper plot is as presented in Table 7. Seed bed types had no significant effect on the weed biomass in pepper plots except at 8 weeks after transplanting with significantly higher weed biomass value (0.48 kg/ha) recorded in flat bed seed bed types closely followed by the values recorded in the ridge bed type (0.46 kg/ha) while the least value was recorded in the raised bed type (0.34 kg/ha).

Weed control methods had significant effect on the weed biomass values recorded in pepper plots throughout the study period. Significantly (p <0.05) higher weed biomass values were recorded in the zero weeded plots (0.26 kg/ha, 0.60 kg/haand 0.68 kg/ha) while the least values were observed in the hoe weeded plots (0.10 kg/ha, 0.14 kg/haand 0.16 kg/ha).

There was interactive effect of seedbed types and control methods on weed biomass values recorded in pepper plot throughout the study period. At 4, 6 and 8 weeks after transplanting (WATP), significantly (p <0.05) higher weed biomass values were recorded in flat bed zero weeded plots (0.20 kg/ha, 0.67 kg/ha and 0.90 kg/ha), followed by flatbed hand weed plots (0.25 kg/ha, 0.57 kg/ha and 0.77 kg/ha) while the least values were observed in raised bed hoe weeded plot (0.10 kg/ha, 0.13 kg/ha and 0.12 kg/ha), respectively.

Table 5 The main interactive effect of seed bed types and weed control methods on Pepper leaf area (cm^2)

Seed bed type	Weed control methods				Seed bed type mean
Leaf area of pepper at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	10.13	8.99	9.17	8.36	5.11
Ridge	8.00	7.60	7.63	7.67	4.84
Flat bed	5.13	3.53	4.13	2.97	4.40
Weed control method mean	5.67	4.47	5.29	3.58	
LSD bed (B)	0.50				
LSD weed control method (W)	0.57				
LSD B × W	0.98				
Leaf area at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	12.72	11.05	12.12	10.45	7.99
Ridge	9.80	6.89	6.63	6.27	7.96
Flat bed	7.43	6.45	6.75	5.95	7.60
Weed control method mean	9.09	7.53	8.33	6.45	
LSD bed (B)	0.61				
LSD weed control method (W)	0.70				
LSD B × W	1.15				
Leaf area at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	20.20	11.07	16.37	10.00	10.51
Ridge	10.42	9.77	10.34	9.70	10.08
Flat bed	9.70	8.09	9.55	7.42	9.59
Weed control method mean	11.11	10.11	10.19	8.09	
LSD bed (B)	0.80				
LSD weed control method (W)	0.95				
LSD B × W	1.56				

The effect of different seed bed types and weed control method affected the performance of pepper. The flat bed produced shorter plants while there was significant increase in the growth characteristics of pepper plant in the raised bed. This may be attributed to the deeper ploughing obtained on raised bed that allowed for proper intake of nutrient by the pepper plant. Similar observation was made by Zaragoza (2002), who reported that raised beds provides fine tilt for proper growth and development of pepper.

In a similar manner, there was significant effect of weed control methods on the seed bed types on the growth of pepper. Raised bed hoe weeded plots produced the highest number of leaves, highest number of branch, plant height, stem girt and leaf area than other treatments. Flatbed zero weed plot resulted into significantly weaker plant while raised bed hoe weed plot produced vigorously healthy plants compared to other treatment combinations. This may be as a result of its ability to control weeds more efficiently than other methods Achieved result agree well with the previous of work of Mustapha (2014), who reported better vegetative performance of sweet pepper on raised bed then flat bed. This result may also be attributed to the volume of soil accumulated under raised bed that allowed for accessibility to nutrients and other resources needed for better growth and development of pepper in those plots. This is in line with the work of Imoloame (2014); Adigun et al. (2018) and Kanton et al. (2000) who reported better performance of sorghum in raised then flat bed and highest performance of soya bean complimented with hoe weeding under different weed control methods.

Table 6 The main and interactive effect of seed bed types and weed control methods on the weed density (m^{-2}) pepper plots

Seed bed type	Weed control methods				Seed bed type mean
Weed population in at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	7.29	8.45	8.40	9.60	12.92
Ridge	9.70	10.34	9.77	10.42	13.08
Flat bed	10.45	12.13	11.05	12.72	13.17
Weed control method mean	11.67	13.22	12.67	14.67	
LSD bed (B)	1.35				
LSD weed control method (W)	1.40				
LSD B × W	3.12				
Weed population at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	11.00	14.67	11.67	13.33	11.67
Ridge	14.33	15.00	14.67	15.67	15.58
Flat bed	15.67	18.60	16.00	20.00	15.75
Weed control method mean	12.33	15.67	14.11	18.56	
LSD bed (B)	1.95				
LSD weed control method (W)	2.30				
LSD B × W	3.80				
Weed population at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	13.33	14.44	13.67	14.67	16.83
Ridge	16.33	17.67	16.67	18.67	17.17
Flat bed	18.68	20.67	19.30	27.30	18.83
Weed control method mean	13.78	18.33	15.89	22.45	
LSD bed (B)	2.65				
LSD weed control method (W)	2.83				
LSD B × W	4.65				

This result also reveals the effectiveness of the hoe weeding method to significantly reduce the weed cover thereby minimizing weed competition with the pepper plant thereby leading to proper assimilation of nutrients, and accessibilities of sunlight and moisture for proper growth. The flat bed zero weeded plots had the lowest number of leaves and number of branches. This observation was due to the competition of the crops with the weeds for growth resources since the weeds were allowed to grow unchecked. This implies that the plant could not produce more leaves to conserve the available nutrients and moisture for the critical stages. The same factor could be responsible for the reduction in the stem girth and leaf area recorded in the flat bed zero weeded plots. This result is in conformity with the findings of Kanton et al. (2000), who recorded highest value of growth of sorghum in raised bed than flat beds.

The efficiency of weed control methods and the seed bed type adopted in this study can be deduced from the weed density (m^{-2}) and the weed biomass (kg/ha). The highest weed density (m^{-2}) and weed biomass (kg/ha) in the flat bed zero weeded plots may be attributed to the open soil surfaces and niches available for weeds for free aggressive growth. Timely eradication of weeds in the raised bed hoe weeded plots and the availability of growth resources for use by crops under this treatment resulted better performance and growth of crops in those plots. This agreed well with the findings of Kanton et al. (2000), Abidkhan et al. (2012), Madukwe et al. (2012)

Table 7 The main and interactive effect of seed bed types and weed control methods on weed biomass in pepper plots (kg/ha)

Seed bed type	Weed control methods				Seed bed type mean
Weed biomass at 4 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	0.10	0.10	0.10	0.11	0.16
Ridge	0.12	0.16	0.14	0.16	0.17
Flat bed	0.20	0.25	0.23	0.30	0.17
Weed control					
Method mean	0.10	0.17	0.13	0.26	
LSD bed (B)	0.02				
LSD weed control method (W)	0.02				
LSD B × W	0.04				
Weed biomass at 6 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	0.13	0.16	0.16	0.17	0.30
Ridge	0.22	0.33	0.23	0.33	0.34
Flat bed	0.33	0.57	0.47	0.67	0.37
Weed control					
Method mean	0.14	0.30	0.22	0.60	
LSD bed (B)	0.24				
LSD weed control method (W)	1.17				
LSD B × W	0.24				
Weed biomass at 8 weeks after transplanting					
	Hoe	Hand	Mulch	Zero weeding	
Raised bed	0.12	0.17	0.12	0.20	0.34
Ridge	0.30	0.38	0.30	0.67	0.46
Flat bed	0.67	0.77	0.67	0.90	0.48
Weed control					
Method mean	0.16	0.33	0.24	0.68	
LSD bed (B)	0.15				
LSD weed control method (W)	0.14				
LSD B × W	0.27				

and Imoloame (2014) who reported that growing crops on seed beds and supplementing chemical control weed treatment with hoe weeding at intervals helped to reduce crop-weed competition and provide almost crop weed free environment and improved crops vigour.

5 Conclusion

On the basis of this result, it can be concluded that sowing of pepper on raised seed bed was superior to sowing on flat bed on the vegetative parameters. Raised bed hoe weeding methods reduced weed cover more than other treatments. Meanwhile, raised bed hoe weeding treatments out measured other treatment combinations in terms of plant height, number of leaves, number of branches per plants, stem girth and leaf area of pepper plant. The maximum reduction of weed density and weed biomass observed in raised bed and hoe weeding was not only effective in providing long season control of weeds but has promoted better vegetative growth and performance of pepper

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Original Paper

Changes in floristic composition of grassland affected by the different exploitation intensity

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The aim of the work was to analyze the influence of different intensity of grassland exploitation on the development of floristic composition. The experiment was carried out in 2017 and 2018 in the Žirany village (SW Slovakia) characterized by a mild climate with an average annual temperature of 9 °C. Before the experiment was established, the grassland was used for sheep grazing and dominated by *Lollium perenne* L. The monitoring period was 2017 and 2018. The experimental crops were mown 2× (variant 4), 3× (variant 3) and 4× (variant 2). We established also control variant (variant 1) which was not mowed and fertilized, as well. The floristic composition was evaluated before each cut. It follows from the results obtained at the beginning of the monitoring in 2017, grass species (*Lollium perenne* L., *Poa trivalis* L., *Poa pratensis* L.) predominantly prevailed and they maintained their dominant position during the whole vegetation period in 2017. Furthermore, other meadow herbs (*Achillea millefolium* L., *Cerastium arvense* L.) were found in higher proportion. Legumes were found in a lesser extent. In 2018, we reduced the proportion in the botanical groups of other meadow herbs and leguminous plants. Conversely, grasses increased their share compared to 2017. The cover has been reduced mainly in variant 3 (3× mowed) and variant 2 (2× mowed).

Keywords: floristic composition, mowing management, grasses, legumes, other meadow herbs, permanent grassland

1 Introduction

Grasslands exist and have been used by man since the very beginning of human existence (Gibson, 2009). Their overall meaning is characterized by a range. They account for approximately 26% of the total land area and 80% of the agricultural land. Most of these stands are located in developing countries, where they are particularty important for the livehood of approximately one billion poor people. They form a fodder base for grazing animals and thus a number of high quality foods (Boval and Dixon, 2012). At present, numbers of livestock declining in the Slovak Republic. For example, the beaf cattle numbers have decreased by more than 19.000 and in sheep the drop was over 22.000 individuals from 2014 to 2016. So it can be stated that finding of more ways to use the produced biomass is becoming more and more interesting. Alternative ways of using biomass as a source of energy are becoming increasingly prominent (Pollák et al., 2013). In addition to producing features, grasslands also include a large number of non-productive features, such as the creation of a biological diversity reserve, cultural and recreational use, but they also serve as a potential carbon capture to reduce greenhouse gas emissions (Boval and Dixon, 2012). Grasslands have the potential and play a key role in the mitigation of greenhouse gases, especially in global carbon storage and its further sequestration (O'Mara, 2012). Soil and grasslands capture about 34% of the world's terrestrial carbon, which is vital for providing a variety of ecosystem services such as climate regulation (Eze et al. 2018). The aim of the paper is to analyze the influence of different intensity of grassland exploitation for the development of floristic composition. We take into account lower demand for phytomass, intensification management and its impact on grassland.

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2 Material and methods

The experiment with various/different? intensity of exploitation was established in the cadastral area of the Žirany village at an altitude of 210 m n. m. (SW Slovakia). At the locality, the soil type of fluvisol prevails with a weakly acidic to acidic soil reaction. The chemical composition of the soil samled in experimental plots is presented in Table 1.

From an agroclimatic point of view, the area is located in a temperate zone on the continental and Atlantic-continental border. Long-term annual average o fair temperature is 9 °C and annual rainfall average is 600 mm (Economic and social development program, 2014).

Permanent grassland was used for sheep grazing prior to the experiment. From the floristic point of view, grasses predominated, and the largest species present is the sole *Lolium perenne* L. The field trial was based on a block of four retrials. The test area was limited and each variant was established in plot size 2 × 3 m.

We have watched out the following variants in the experiment:

Variant 1 – Variant 1 – original stand, not fertilized, unmowed, abandoned, sampling for production detection was done at the time of seed maturation.

Variants 2, 3, 4:
Fertilization

N (120 kg/ha) – 80 kg N/ha in the spring at the time of greening of vegetation, 40 kg N/ha after the first cut, P (40 kg/ha) – full dose in time of greening of vegetation in spring, *K* (80 kg/ha) – full dose in time of greening of vegetation in spring.

Mowing

Variant 2 – mowed 4× (1st mowing at the time of stalking, 2nd mowing after 45 days from the first, 3rd mowing after 45 days from the second and 4th mowing after 45 days from the third mowing).

Variant 3 – mowed 3× (1st mowing at the time of hay-mowing maturity, 2nd mowing after 60 days from first mowing and 3rd mowing after 60 days of mowing second).

Variant 4 – mowed 2× (1st mowing in hay-mowing maturity, 2nd mowing after 90 days since first mowing).

Characteristic of used fertilizers

N – LAD 27 nitrogen fertilizer containing 27% nitrogen and 4.1% total MgO, approximately 7% CaO total and 2% CaO water soluble in granular form.

P – Superphosphate 19% P_2O_5 in granular form.

K – Potassium sulfate 50% K_2O in granular form.

Floristic evaluation of each stand was done by the Regal Reduced Projective Dominance method (Regal, 1956). According to Jaccard´s qualitative similarity index (IS_J), we calculated the correlation of floristic composition according to relationship (Moravec et al., 1994).

$$IS_J = \frac{C}{A + B - C} \times 100$$

where:
A – number of species in frame A
B – number of species in frame B
C – number of common species

3 Results and discussion

The changes that occur are a reflection of the different doses and combinations of applied fertilizers, but also the frequency of use (Klapp 1971). The different exploitation system, especially the frequency of cutting, influenced the representation of individual botanical groups in variants. According to Jančovič and Vozár (2014), due to the greater number of cuts, changes in the content of organic and mineral substances are caused. However, they can see these changes as positive. Krajčovič (2004) considers setting the correct date of the first mowing as a priority and very important. Due to the different intensity of use and fertilization of grasslands, there are various changes in their floristic composition.

The changes in the floristic composition of the monitored stand in 2017 to 2018 are shown in Figure 1. The control was the original crop, which was not fertilized and cut, thus in no way managed and subject to natural changes over the years.

In the variants used, grasses accounted for the highest proportion of all plant species and their value increased in the annual average of mowing due to fertilization and mowing in all monitored variants. The biggest difference was found in variant 3, where the average proportion of grasses increased from 43.2% (2017) to 87.0% (2018). The

Table 1 Agrochemical composition of the soil before the start of the experiment (year 2017)

Element	N	P	K	Ca	Na	Mg	C_{ox}	pH
Unit of measure	mg/kg						%	
	2,457.84	27.73	192.49	1,186.32	55.56	88.53	2.69	5.78

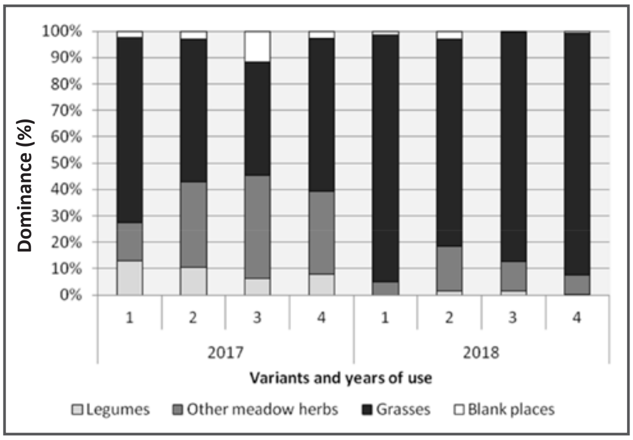

Figure 1 Year average values of floristic group composition of grassland (%)

lowest difference was recorded in variant 1, where the average annual cover increased from 70% (2017) to 93.5% (2018). A high proportion of grass was mainly *Lolium perenne* L., which in 2017 had the highest proportion of grass in variant 2 in the 2nd mowing, which was during this period 4× mowed (51.5%). In 2018, it was up to 68% in the 2nd mowing. The second dominant grass was *Poa trivialis* L., which in 2017 reached an average share of 7.5% (variant 4) in the first cut in 2018 to 45.5% (variant 4). Acordingly, *Poa pratensis* L. was an another widespread grass. The species achieved the highest proportion in the 1st mow of the variant 3 (38.0%) during initial year 2017 and in the 3rd mow of the variant 3 in 2018 (44%).

The average proportion of leguminoses was reduced year-on-year in all the observed variants (Figure 1). The biggest difference in the annual average of mowing was found in variant 1, where the average value of legumes matter decreased from 13% (2017) to the trace amount (2018). The lowest decrease was recorded in variant 3, where the average proportion of legumes decreased from 6.3% (2017) to 1.5% (2018). *Trifolium repens* L. (15%, variant 2) and *Lotus corniculatus* L.) (2.5%, variant 3) reached the highest proportion of all other species in the 3rd mowing in 2017. However, the proportion of these species notable decreased in 2018. The largest occurrence of *Trifolium repens* L. was found in the 1st mowing of the variant 2 (1.5%), while the *Lotus corniculatus* L. share decreased to up 0.5% in the 3rd mowing of the variant 3.

Similar as in the case of legumes, the average proportion of other meadow herbs was reduced by at least half in all evaluated variants. The largest difference in the annual average of weights was found in variant 3, where the average coverage of herbs decreased from 39% (2017) to 11.2% (2018). The smallest decrease was in the original crop (variant 1). There, the average proportion of herbs decreased from 14.5% (2017) to 5.0% (2018). Within those meadows plant species,the most common was *Achillea*

Table 2. Variant 1 – floristic composition and its changes for years 2017–2018 (%)

S.N.	*Name*	Year 2017	Year 2018
		1st cut	1st cut
Legumes			
1.	*Trifolium fragiferum* L.	–	r
2.	*Trifolium repens* L.	13,0	r
3.	*Trifolium aureum* Pollich	r	–
4.	*Lotus corniculatus* L.	r	r
5.	*Vicia sativa* L.	r	r
6.	*Vicia tetrasperma (*L.*)* Schreb.	r	r
	Together	13,0	r
Other meadow herbs			
7.	*Carduus acanthoides* L.	r	–
8.	*Cichorium intybus* L.	r	–
9.	*Stellaria graminea* L.	r	r
10.	*Daucus carota* L.	r	r
11.	*Cirsium arvense* (L.) Scop.	r	1,5
12.	*Taraxacum officinale* auct. non Weber	r	–
13.	*Convolvulus arvensis* L.	r	0,5
14.	*Achillea millefolium* L.	11,0	2,5
15.	*Cerastium arvense* L.	2,5	0,5
16.	*Plantago lanceolata* L.	r	–
17.	*Veronica chamaedrys* L.	–	r
18.	*Veronica verna* L.	1,0	–
Together		14,5	5,0
Grasses			
19.	*Festuca rubra* L.	r	–
20.	*Festuca pratensis* Huds.	0,5	–
21.	*Festuca ovina* L.	r	–
22.	*Poa pratensis* L.	30,0	6,0
23.	*Poa trivialis* L.	1,5	42,5
24.	*Lolium perenne* L.	24,5	1,5
25.	*Arrhenatherum elatius* subsp. *elatius*	3,0	11,5
26.	*Alopecurus pratensis* L.	7,5	27,5
27.	*Elymus repens* (L.) Gould	0,5	2,0
28.	*Elymus caninus* (L.) L.	–	r
29.	*Dactylis glomerata* L.	r	0,5
30.	*Bromus hordeaceus* L.	2,5	2,0
31.	*Phleum pratense* L.	–	r
32.	*Trisetum flavescens* (L.) P. Beauv.	r	–
Together		70,0	93,5
Blank places		2,5	1,5

r – rarus, trace occurrence, less than 1%. S.N. – serial number

millefolium L. and *Cerastium arvense* L., which in individual variations occurred in the initial year 2017 in higher quantities than in 2018. In 2017, *Achillea millefolium* L. reached the highest value in the 3rd mowing of variant 3 (52.5%), while in 2018 only 9%. *Cerastium arvense* L. had the highest share in the variant 3 in (7%) in 2017 whereas the highest value only of 0.5% was recorded in 2018.

Significant changes were also observed in the evaluation of blank places in all variants, but especially in the variant 3 (3×mowed), where the share decreased from an average of 11.5% to 0.3%.

At the beginning of the observation in 2017, the floristic composition of the individual variants of the experiment was relatively similar showing values in interval 46.43–76.47% (Table 2). In general, the lowest similarity was achieved comparing the variant 1 with the remaining variants where only one value reached a level higher than 50.00% (the variant 1 compared to the variant 4; 53.57%). Lower values were also observed comparing variants 1 and 3 (48.28%).

After a year of intensive use and fertilization, the differences in the similarity index (IS_J) of the species composition slightly decreased – the stands were homogenized in their floristic composition (Table 3). The biggest difference was found in variants 2 and 4 (56.25%). The largest year-to-year changes in floristic stand similarity were found between variants 3 and 4 (76.47% in 2017 versus 52.63% in 2018). We believe these results showed that a more significant intervention in the species composition is caused mainly by the different intensity of mowing.

Table 3 Variant 2 – floristic composition and its changes for years 2017 – 2018 (%)

S.N.	Name	Year 2017				Year 2018			
		1st cut	2nd cut	3rd cut	4th cut	1st cut	2nd cut	3rd cut	4th cut
Legumes									
1.	*Trifolium fragiferum* L.	–	–	r	0,5	–	–	–	r
2.	*Trifolium pratense* L.			r	r	–	–	–	–
3.	*Trifolium repens* L.	15,0	12,0	9,0	2,0	1,5	0,5	1,0	1,0
4.	*Lotus corniculatus* L.	1,0	1,0	1,5	0,5	r	0,5	0,5	1,0
5.	*Vicia sativa* L.	–	–	–	–	r	–	–	–
6.	*Vicia tetrasperma* (L.) Schreb.	r	–	–	–	0,5	–	r	r
Together		16,0	13,0	10,5	3,0	2,0	1,0	1,5	2,0
Other meadow herbs									
7.	*Carduus acanthoides* L.	1,0	2,0	3,0	2,5	0,5	1,0	r	r
8.	*Helianthemum nummularium* L.	–	–	–	–	–	–	–	2,0
6.	*Picris hieracioides* L.	–	–	r	–	–	–	1,0	r
7.	*Stellaria media* (L.) Vill.	–	–	–	–	1,0	–	–	r
8.	*Stellaria graminea* L.	1,5	r	–	4,0	3,5	4,5	1,5	3,0
9.	*Hieracium pilosella* L.	–	r	–	–	–	r	–	–
9.	*Daucus carota* L.	–	r	r	–	r	0,5	0,5	2,5
10.	*Chenopodium album* L.	–	–	–	–	–	–	r	–
11.	*Centaurea cyanus* L.	–	2,0	–	–	–	–	–	–
12.	*Selinum carvifolia* (L.) L.	–	–	–	–	–	r	–	–
13.	*Cirsium arvense* (L.) Scop.	–	r	r	–	–	r	r	r
14.	*Cirsium palustre* (L.) Scop.	–	–	–	–	r	–	–	–
15.	*Taraxacum officinale* L.	0,5	r	0,5	r	r	r	r	0,5
16.	*Convolvulus arvensis* L.	r	r	r	–	–	2,0	1,0	1,5
17.	*Achillea millefolium* L.	19,0	22,5	42,0	25,0	5,0	4,0	13,5	14,0
18.	*Cerastium arvense* L.	2,5	r	r	r	1,5	r	r	r
19.	*Plantago lanceolata* L.	r	r	0,5	0,5	–	–	r	0,5

Continuation of Table 3

S.N.	Name	Year 2017				Year 2018			
		1st cut	2nd cut	3rd cut	4th cut	1st cut	2nd cut	3rd cut	4th cut
20.	*Veronica chamaedrys* L.	–	–	–	–	–	r	–	–
21.	*Glechoma hederacea* L.	r	–	–	r	r	r	r	3,0
Together		24,5	26,5	46,0	32,0	11,5	12,0	17,5	27,0
Grasses									
19.	*Festuca rubra* L.	0,5	4,0	7,5	1,5	–	r	–	–
20.	*Festuca pratensis* Huds.	0,5	0,5	–	1,0	2,0	1,5	–	–
21.	*Festuca ovina* L.	0,5	0,5	0,5	0,5	–	–	–	–
22.	*Festuca arundinacea* Schreb.	–	–	1,0	–	–	–	1,5	4,5
23.	*Poa pratensis* L.	17,5	0,5	–	21,5	27,5	r	28,5	47,0
24.	*Poa trivialis* L.	5,0	r	–	r	20,0	1,0	–	–
25.	*Lolium perenne* L.	33,0	51,5	26,5	38,5	26,0	68,0	30,0	2,5
26.	*Arrhenatherum elatius* subsp. *elatius*	r	r	r	r	r	2,5	1,0	1,0
27.	*Alopecurus pratensis* L.	2,5	0,5	r	r	11,0	2,0	6,5	3,5
	Agrostis capillaris L.	–	0,5	–	–	–	–	–	–
28.	*Elymus repens* (L.) Gould	–	–	0,5	–	–	11,5	12,0	1,0
30.	*Dactylis glomerata* L.	r	r	r	r	r	r	0,5	–
31.	*Bromus hordeaceus* L.	r	r	–	r	r	0,5	–	0,5
32.	*Trisetum flavescens* (L.) P. Beauv.	r	–	–	r	r	r	–	–
Together		59,5	58,0	36,0	63,0	86,5	87,0	80,0	60,0
Blank places		r	2,5	7,5	2	0	0	1,0	11,0

r – rarus, trace occurrence, less than 1%. S.N. – serial number

Table 4 Variant 3 – floristic composition and its changes for years 2017 – 2018 (%)

S.N.	*Name*	Year 2017			Year 2018		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Legumes							
1.	*Trifolium fragiferum* L.	–	–	r	–	r	–
2.	*Trifolium pratense* L.	–	r	–	–	–	–
3.	*Trifolium repens* L.	10,5	3,0	1,0	2,0	0,5	0,5
4.	*Lotus corniculatus* L.	0,5	1,0	2,5	r	0,5	0,5
5.	*Vicia sativa* L.	0,5	r	–	0,5	–	–
Together		11,5	4,0	3,5	2,5	1,0	1,0
Other meadow herbs							
6.	*Carduus acanthoides* L.	–	r	–	–	r	0,5
7.	*Cichorium intybus* L.	–	r	–	–	r	–
8.	*Picris hieracioides* L.	–	–	0,5	–	r	r
9.	*Stellaria media* (L.) *Vill.*	–	–	–	3,0	–	–
10.	*Stellaria graminea* L.	1,5	–	–	1,0	0,5	2,5
11.	*Knautia arvensis* (L.) Coult.	r	–	–	–	–	–
12.	*Hieracium pilosella* L.	–	0,5	–	–	–	–
13.	*Capsella bursa-pastoris* (L.) Medik.	r	–	–	–	–	r
14.	*Daucus carota* L.	r	1,0	1,0	r	0,5	0,5

Continuation of Table 4

S.N.	Name	Year 2017			Year 2018		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
15.	*Chenopodium album* L.	–	–	–	–	r	r
16.	*Potentilla argentea* L.	r	r	–	r	r	r
17.	*Centaurea cyanus* L.	–	r	r	r	r	r
18.	*Selinum carvifolia* (L.) L.	–	–	–	–	–	r
Other meadow herbs							
19.	*Pastinaca sativa* L.	r	0,5	r	0,5	1,5	0,5
20.	*Cirsium arvense* (L.) Scop.	–	r	r	–	r	r
21.	*Taraxacum officinale* L.	r	r	r	r	–	0,5
22.	*Convolvulus arvensis* L.	r	–	r	r	r	r
23.	*Achillea millefolium* L.	18,0	30,0	52,5	4,0	7,0	9,0
24.	*Cerastium arvense* L.	7,0	r	–	0,5	r	r
25.	*Plantago lanceolata* L.	0,5	1,5	2,5	r	1,0	0,5
26.	*Polygonum persicaria* L.	–	–	–	–	–	r
27.	*Glechoma hederacea* L.	–	–	–	r	–	r
Together		27,0	33,5	56,5	9,0	10,5	14,0
Grasses							
28.	*Festuca rubra* L.	1,0	20,5	3,0	0,5	2,5	–
29.	*Festuca pratensis* Huds.	0,5	r	r	0,5	0,5	–
30.	*Festuca ovina* L.	r	–	11,0	0,5	–	–
31.	*Festuca arundinacea* Schreb.	–	r	–	–	–	0,5
32.	*Poa pratensis* L.	38,0	0,5	8,0	r	r	44,0
33.	*Poa trivialis* L.	1,5	–	r	45,0	–	–
34.	*Lolium perenne* L.	9,5	15,5	6,0	11,0	24,0	0,5
35.	*Arrhenatherum elatius* subsp. *elatius*	3,0	1,5	0,5	20,5	28,0	–
36.	*Alopecurus pratensis* L.	4,5	0,5	0,5	6,5	13,5	11,0
37.	*Elymus repens* (L.) Gould	0,5	0,5	0,5	2,0	17,5	27,5
38.	*Elymus caninus* L.	–	–	–	–	0,5	1,0
39.	*Dactylis glomerata* L.	r	r	r	1,5	0,5	0,5
40.	*Bromus hordeaceus* L.	2,0	r	–	r	r	–
41.	*Phleum pratense* L.	–	–	–	–	1,0	r
42.	*Trisetum flavescens* (L.) P. Beauv.	0,5	–	–	r	–	–
Together		61,0	39,0	29,5	88,0	88,0	85,0
Blank places		0,5	23,5	10,5	0,5	0,5	r

r – rarus, trace occurrence, less than 1%. S.N. – serial number

Table 5 Variant 4 – floristic composition and its changes for years 2017 – 2018 (%)

S.N.	Name	Year 2017		Year 2018	
		1st cut	2nd cut	1st cut	2nd cut
Legumes					
1.	*Trifolium fragiferum* L.	–	–	r	–
2.	*Trifolium repens* L.	13,0	1,5	r	r
3.	*Trifolium aureum* Pollich	r	–	–	–
4.	*Lotus corniculatus* L.	0,5	0,5	r	0,5
5.	*Vicia sativa* L.	–	–	r	–
Together		13,5	2	r	0,5
Other meadow herbs					
6.	*Picris hieracioides* L.	–	r	–	–
7.	*Stellaria media* (L.) Vill.	–	–	r	–
8.	*Stellaria graminea* L.	r	–	1,5	0,5
9.	*Daucus carota* L.	r	0,5	r	–
10.	*Centaurea cyanus* L.	–	r	–	–
11.	*Cirsium arvense* (L.) Scop.	r	r	r	0,5
12.	*Taraxacum officinale* L.	r	1,0	r	r
13.	*Convolvulus arvensis* L.	r	1,0	1,0	3,5
14.	*Achillea millefolium* L.	11,5	47,5	2,0	5,5
15.	*Cerastium arvense* L.	1,0	–	–	–
16.	*Plantago lanceolata* L.	r	0,5	–	–
Together		12,5	50,5	4,5	10,0
Grasses					
17.	*Festuca rubra* L.	1,0	8,5	r	–
18.	*Festuca pratensis* Huds.	1,0	–	0,5	–
19.	*Festuca ovina* L.	r	–	r	–
20.	*Festuca arundinacea* Schreb.	–	r	–	1,5
21.	*Poa pratensis* L.	21,5	r	r	14,0
22.	*Poa trivialis* L.	7,5	–	45,5	–
23.	*Lolium perenne* L.	8,0	17,5	11,0	4,0
24.	*Arrhenatherum elatius* subsp. *elatius*	25,0	14,0	25,5	40,5
25.	*Alopecurus pratensis* L.	4,5	0,5	8,0	6,5
26.	*Elymus repens* (L.) Gould	r	1,5	2,0	1,5
27.	*Elymus caninus* (L.) L.	–	–	–	20,0
28.	*Dactylis glomerata* L.	0,5	r	1,5	0,5
29.	*Bromus hordeaceus* L.	5,0	–	0,5	–
30.	*Phleum pratense* L.	–	–	r	–
31.	*Trisetum flavescens* (L.) P. Beauv.	r	–	0,5	–
Together		74,0	42,0	95,0	88,5
Blank places		r	5,5	0,5	1,0

r – rarus, trace occurrence, less than 1%. S.N. – serial number

Table 6 Jaccard index IS_J of qualitative similarity 2017

Variant		Common species			
		1	2	3	4
IS_J	1	O	*13	*14	*15
	2	46.43%	O	*10	*10
	3	48.28%	55.56%	O	*13
	4	53.57%	55.56%	76.47%	O
	Species together	28	13	15	14

*number of common species

Table 7 Jaccard index IS_J of qualitative similarity 2018

Variant		Common species			
		1	2	3	4
IS_J	1	O	*10	*12	*12
	2	41.67%	O	*9	*9
	3	46.15%	47.37%	O	*10
	4	52.17%	56.25%	52.63%	O
	Species together	22	12	16	13

*number of common species

At the beginning of the observation, the individual variants were from the quality side (IS_J) similar to 46.43–76.47% (Table 6). In general, the lowest similarity with the remaining variations was achieved by variant 1, where only one value reached a level higher than 50.0% (compared to variant 4; 53.57%). Lower values were also observed when comparing variants 1 and 3 (48.28%).

After a year of intensive use and fertilization, the differences in the similarity (IS_J) of the species composition increased to 41.67–56.25% (Table 7). The biggest difference was between variant 3 and variant 4 (76.47% to 52.63%). The exception where the similarity increased and the difference was reduced between 2 – 4 (56.25%).

These results show that a more significant intervention in the species composition is caused mainly by the different intensity of mowing.

Klimeš et al. (2000), which observed the effect of mowing on a subxerophilic meadow found a significant increase in species wealth. He noted a linear increase in species in the 10-year period. These experiences suggest that, although the mowing of such meadows is a long-term process, it results in favourable results. In our experiment of extensively exploited meadows, due to different intensity of cuttings (2-cut, 3-cut, 4-cut), only grasses increased during the monitored period, other floristic groups such as legumes and other meadow herbs reduced by mowing and fertilization also reduced the share of blank places in the crops. The influence of different intensity of use on species diversity of grassland is not always clear and the results among authors may differ (Michaud et al., 2012, Briňák et al., 2013, Smith et al., 2008). Differences may be caused by different scientific methodologies or, for example, different site conditions (Štýbnarová – Dufek, 2016). In our experiment, the species together has grown in stands (Tables 6 and 7) that have been mowed three times a year (with the same fertilization), which contradicts the results achieved by Gaisler et al., 2011 or Kohoutek et al. In variant 1, which has not been mowed or fertilized more species were detected than in other variants 2, 3, 4.

Conclusions

In the monitored grassland, the impact of various type of management (different number of cuts per year, unmanaged stand) on floristic composition was evaluated. We found that the floristic composition of the stands was influenced by the number of cuttings. In the first year of 2017 grasses was predominant due to mowing and fertilization. Similarly in 2018 grasses reached the highest proportion of all observed plant species. Based on the similarity results, we found that the similarity of the floristic composition of the community was most evident in variant 3 of mowed 3 times and variant 4 which has been mowed 2 times.

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