Original Paper

Acute behavioral and biochemical responses of sheep to S/C ivermectin injection

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This study was designed to compare between the effects of subcutaneous injection of ivermectin at the left neck region versus behind the left elbow on the acute behavioural and biochemical responses of sheep, with the aim of selecting the most suitable injection strategy causing the least adverse effects on the animal health and welfare. Twenty-five sheep were assigned to 5 groups: one control group (C, without injection), and two groups injected with 0.9% NaCl either at neck (SN) or elbow (SE), and two groups injected with ivermectin (IVM) at a dose of 0.2 mg kg^{-1} BW either at neck (IN) or elbow (IE). Results reflected that head shaking and neck scratching showed significant increases in the IN group, while standing was significantly lower in the IE group compared to the C group. Pawing was significantly higher in both SE and IE groups compared to the C group. Plasma levels of cortisol, glucose and lactate were significantly increased in both IN and IE groups. There were no obvious changes in the levels of other stress markers among the different treated groups. In conclusions, the magnitude of acute stress reactivity was not significantly different between IVM injections behind the elbow and at the neck region.

Keywords: Ivermectin, sheep, behaviour, biochemical

1 Introduction

Ivermectin is a member of the macrocyclic lactones widely used as a broad-spectrum drug against a wide range of endo- and ectoparasites in ruminant animals (Gokbulut et al., 2008; Jameel et al., 2014). It is commercially available in different formulations including injections, oral, and rumino-reticular and cutaneous delivery systems (Mestorino et al., 2003). Although previous studies showed that subcutaneous (S/C) injection is a more efficient route for this drug in terms of drug bioavailability, efficacy, and persistency compared to other routes of administration (Lespine et al., 2005; Gokbulut et al., 2007), its irritability at the site of injection represents a major concern (Bokisch and Walker, 1986).

Given that much attention has been paid to animal welfare, selection of injection site depending on the satisfaction of this demand is of outmost significance. However, it is well established that the preferred site for S/C injections in sheep is the skin just behind the elbow; In Egypt, the preference of S/C injection at the neck region over that behind the elbow is a common trend among veterinarians without a well-established scientific basis. Therefore, this investigation sheds light on the potential difference between S/C injections at the neck region versus behind the elbow manifested by modulations in acute behavioural patterns and stress endpoints measured as a reflection of animal welfare.

2 Material and methods

2.1 Experimental groups

This experiment was conducted in the Hospital of Veterinary Medicine, Assuit University, Assuit, Egypt. Twenty multiparous, non-pregnant and non-lactating adult ewes and five native Egyptian breed rams (*Ovis aries*) weighing (35–45 kg) and aged 3–5 years, were used in this experiment. The sheep were randomly assigned to five groups [each had 4 ewes and one ram]; namely, a control group (C, without injection), two saline groups (SN and SE) (injected with 0.9% NaCl) and two IVM groups (IN and IE) [IVM at a dose of 0.2 mg kg^{-1} BW, 1% solution (Ivomec® injection Merk & Co. Inc., Rahway, NJ, USA)]. The injection was at the left neck region or behind the left elbow. The animals were penned individually for all ethological observations and collection of all data and samples.

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Table 1 The animal numbers, average body weight, and age of different experimental groups

Group / Data	C	SN	SE	IN	IE
Ewe numbers	4	4	4	4	4
Rams number	1	1	1	1	1
Number of pens	5	5	5	5	5
Average body weight/kg	40.20 ±1.16	39.00 ±1.30	40.60 ±1.63	40.00 ±1.48	40.80 ±1.24
Age/years	3.80 ±0.37	3.80 ±0.49	3.80 ±0.20	3.60 ±0.40	3.60 ±0.24

C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

2.2 Behavioural pattern recording

Before the time of injection, the animals were housed in a single large room. However, directly after the injection, each individual animal was hold inside a small separate pen with visual and auditory contact with other sheep. Each group had five animals which were housed in five different pens and each animals pen was used as statistical unite. The behavioural activities of individual sheep were observed continuously throughout the first thirty minutes post-injection. The frequency of sheep behavioural patterns including standing, walking, pawing, biting fleece, head shaking and neck shaking were recorded and quantified as the number of occurrences of these behaviours. The frequency of a particular behaviour in a 30-minute session was calculated. The sheep were considered to be in a normal standing position if animal in a straight-back posture with the head raised higher than the level of the back line. A hunched back posture with the head lower than the highest level of the back was considered abnormal. Locomotion was recorded as normal, stiff walking and running. Pawing was registered when the sheep pawed at the ground. Biting fleece was presented at biting the sides of the body, back line and sometimes the upper part of the legs. Head shaking was noted as the period of violent shaking and nodding of the head. Neck scratching was defined as sudden scratching to neck skin with the hind leg (occasionally including head), or rubbing the neck against solid surrounding objects.

2.3 Biochemical indicators of stress

Blood samples (20 mL) were collected from each sheep via the jugular vein immediately after 30 minutes behavioural observation. The blood samples were transferred to sterile test tubes with an anticoagulant and allowed to set for about 30 minutes in the refrigerator (4 °C). Then, all blood samples were centrifuged at 3000 × g for 25 minutes. The plasma samples were transferred to plastic Eppendorf tubes and stored frozen at -83 °C until the analysis.

Cortisol measurements were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Immunospec Corporation, USA) according to the manufacturer's instructions and protocol previously published (Watts and Tindall, 1988). Total cholesterol, triglycerides and lactate were estimated based on the published enzymatic colorimetric methods (McGowan et al., 1983; Young et al., 1975; Field et al., 1966) using various commercially available reagent kits (Egyptian Company for Biotechnology, Cairo, Egypt). Total protein was assessed based on the reaction of copper with the peptide bonds of proteins in alkaline medium to form a pink to purple biuret complex in which colour intensity is directly proportional to the protein concentration in the sample (Gornall et al., 1949). Urea was measured according to modified urease berthlot method (Shephard and Mazzachi, 1983) and creatinine and was analyzed by the colorimetric method with deproteinization (Bowers and Wong, 1980).

2.4 Statistical analysis

The data were represented as means ± standard error of the mean. The differences between groups were statistically analyzed by one-way analysis (ANOVA) of variance followed by Duncan posthoc after testing the data for normality using Anderson Darling test and for variance homogeneity to be sure that the data are normally distributed and variances would be homogenous using SPSS program version 16 (SPSS, Richmond, VA, USA). Behavioural data was log transformed before analysis because it was not normally distributed. Differences were considered statistically significant at $P<0.05$.

3 Results and discussion

3.1 Behavioural changes

3.1.1 Mobility behaviours

The overall relationships between the sites of S/C injections and mobility behaviors, including standing and walking were presented in Figure 1. The results show that S/C injection of saline or IVM at the neck region or

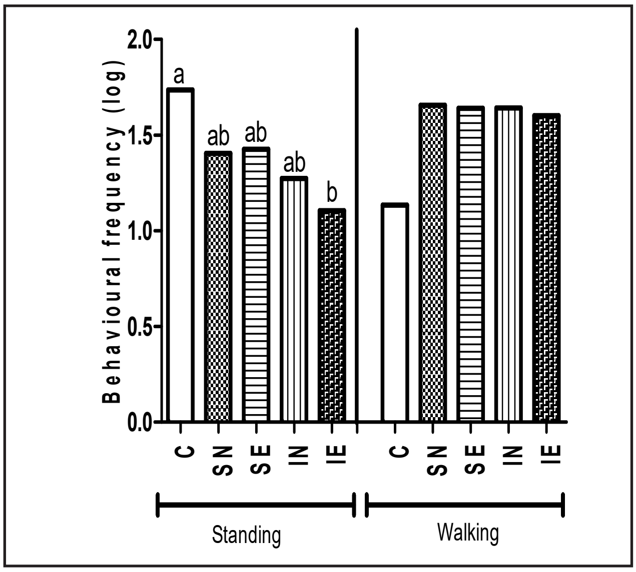

Figure 1 The frequency of standing and walking activities in the different experimental groups
Results are represented in logs of sheep activities frequency/30 minutes [of five sheep per group (n = 5)]
Different letters indicate a significant difference at P <0.05 (one-way ANOVA by Duncan posthoc)
C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

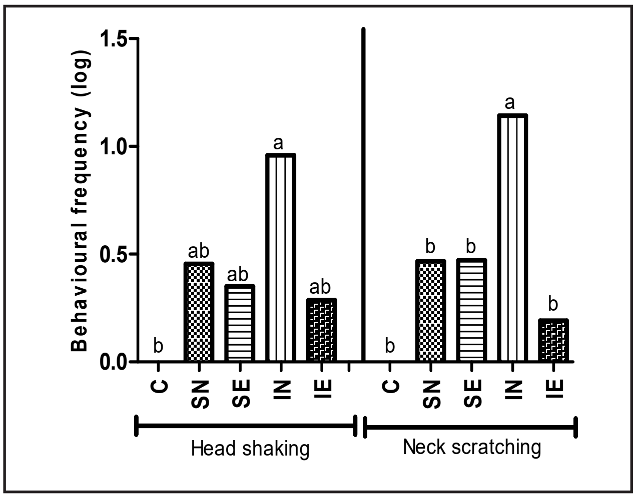

Figure 2 The frequency of head shaking and neck scratching in the different experimental groups
Results are represented in logs of sheep activities frequency/30 minutes [of five sheep per group (n = 5)]
Different letters indicate a significant difference at P <0.05 (one-way ANOVA by Duncan posthoc)
C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

behind elbow joint did not significantly (P >0.05) affect the walking activities. However, the results of standing activities show that S/C injection of IVM behind the elbow joint significantly (P ≤0.05) reduced the frequency of standing activities in sheep compared to the C group (Figure 1 and Table 2).

3.1.2 Head shaking and scratching the neck with hind legs

The results of the present study indicated that the neck scratching in the IN group was significantly (P = 0.015) higher than the rest of other groups, whereas head shaking in this group was significantly (P = 0.002) higher than that of the C group only (Figure 2 and Table 2).

3.1.3 Pawing and biting fleece

Results show that S/C injection of IVM or saline behind the elbow joint significantly (P = 0.025) increased the pawing activities in comparison with the C group. However, the biting fleece activities have not been affected by the different treatments (Figure 3 and Table 2).

3.2 Biochemical changes

As shown in Table 3, plasma cortisol and glucose levels were significantly increased in the IN and IE groups when compared to the SN, SE and C groups. The SN and SE

Table 2 The frequency (Log) of different behavioural patterns in different experimental groups

Group / Pattern	C	SN	SE	IN	IE
Walking	1.134	1.655	1.639	1.642	1.601
Standing	1.736^{a}	1.404^{ab}	1.425^{ab}	1.274^{ab}	1.105^{b}
Scratching the neck	0^{b}	0.467^{b}	0.472^{b}	1.143^{a}	0.192^{b}
Pawing	0.320^{b}	1.028^{ab}	1.088^{a}	0.585^{ab}	1.325^{a}
Biting fleece	0.320	0.492	0.219	0.184	0.524
Head shaking	0^{b}	0.455^{ab}	0.351^{ab}	0.959^{a}	0.287^{ab}

Results are represented as means (logs) of sheep activities frequency/30 minutes [of five sheep per group (n = 5)]. Different letters indicate a significant difference at P <0.05 (one-way ANOVA by Duncan posthoc)
C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

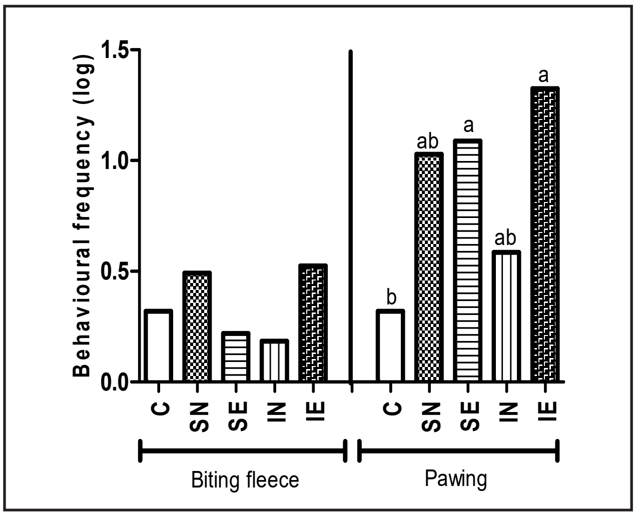

Figure 3 The frequency of biting fleece and pawing in the different experimental groups
Results are represented in logs of sheep activities frequency/30 minutes [of five sheep per group ($n = 5$)]
Different letters indicate a significant difference at $P < 0.05$ (one-way ANOVA by Duncan posthoc)
C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

groups had a significant hypoproteinemia in comparison with C, IN and IE groups. A significant hypolactemia was observed in the SN, SE, IN and IE groups compared to the C group. The levels of other stress markers such as total cholesterol, triglyceride, urea and creatinine have not been changed significantly between all the experimental groups.

Up to our knowledge the acute effects of S/C injection on the behavioural activities and biochemical changes in sheep have not been investigated before. In the current study the behavioural and biochemical responses were assessed in sheep undergoing S/C saline or IVM injection either at the neck region or behind elbow. Our findings showed IVM injection behind the elbow significantly reduced standing activities and increased pawing activities compared to the C group. In contrast, IVM injection at the neck region significantly increased head shaking and neck scratching. On contrary, the differences between the C, SN and SE groups were not significant except for pawing activities which were significantly increased in the SE group. These findings may indicate that IVM injection is more stressful than saline injection and at the same time, the altered type of behavioural activities and severity of alteration depend on the site of S/C injection. The behavioural changes observed in the current study may be attributed to the fact that injection of S/C irritant substance like IVM may stimulate inflammation, and cause swelling, itching and pain (Pulliam and Preston, 1989). Moreover, the increased pawing and reduced standing activities in both SE and IE groups may be explained based on the knowledge that the injection site behind the elbow in sheep is richly supplied with superficial nervous plexus (Ghoshal and Getty, 1967) along with suggested noceiceptive abundance given a solid-based rationality for more susceptibility for pain expression, irritation and neurological distress following injection. During the observation session the ingestive (feeding, drinking and rumination) and resting activities were not considered. The sheep had not been observed long enough to see the ingestive behavioral changes associated with injections, since they were observed for only 30 minutes immediately post-treatment.

Higher cortisol and glucose levels following IVM injection at the neck or behind the elbow than the saline or C groups reveal stress-induced difference between the treatments with a higher degree of discomfort and pain in case of the former. The ability of IVM injection to

Table 3 The levels of biochemical stress indicators in the plasma of different experimental groups

Group / Parameter	C	SN	SE	IN	IE
Cortisol (nmol/l)	97.32±4.17[b]	64.14±2.02[b]	65.66±2.34[b]	180.83 ±9.24[a]	227.30±54.64[a]
Glucose (mmol/l)	3.55 ±0.11[b]	3.41 ±0.32[b]	3.86 ±0.24[b]	5.84 ±0.35[a]	6.14 ±0.51[a]
Lactate (mmol/l)	5.40 ±0.27[a]	1.67 ±0.10c	1.49 ±0.25c	2.70 ±0.20[b]	2.83 ±0.42[b]
Total protein (g/dl)	3.50 ±0.12[a]	2.08 ±0.07[b]	2.15 ±0.06[b]	3.66 ±0.10[a]	3.49 ±0.06[a]
Total cholesterol (mmol/l)	3.02 ±0.17	4.03 ±0.73	2.78 ±0.72	3.70 ±0.27	3.42 ±0.52
Triglyceride (mmol/l)	2.99 ±0.71	3.38 ±0.52	2.48 ±0.48	2.58 ±0.34	3.16 ±0.85
Urea (mmol/l)	5.41 ±0.15	3.93 ±0.09	4.17 ±0.32	4.40 ±0.72	5.26 ±0.81
Creatinine (mmol/l)	0.13 ±0.01	0.98 ±0.01	0.09 ±0.01	0.09 ±0.01	0.13 ±0.03

Results are represented as means ±SEM of five sheep per group ($n = 5$). Different letters indicate a significant difference at $P < 0.05$ (one-way ANOVA by Duncan posthoc)
C – control group; SN – group injected saline at the neck region; SE – group injected saline behind the elbow; IN – group injected ivermectin at the neck region; IE – group injected ivermectin behind the elbow

augment cortisol secretion is compatible with a previous finding recorded in cow (Sadek and Shaheen, 2015) and could be attributed to pain-induced stimulation of the hypothalamic-pituitary-adrenal axis (Embi and Scherlag, 2014).

The hyperglycemia-associated IVM injection may be secondary to increased cortisol secretion during stress (Lakshmi and Sudhakar, 2010; Al-Qarawi, 2004). It is well known that cortisol inhibits insulin secretion through a genomic action in β cells, activates the key enzymes involved in hepatic gluconeogenesis, inhibits glucose uptake in peripheral tissues, and increases the glucogenic precursors as amino acids through muscle proteolysis (Lambilotte et al., 1997; Shpilbert et al., 2012; Dungan et al., 2009; Yoshioka et al., 2005; Larsen and Kristensen, 2013). In addition, epinephrine and norepinephrine may also contribute to the hyperglycemic status by enhancing glycolysis and gluconeogenesis. Moreover, stimulation of lipolysis by norepinephrine supplies glycerol which channels towards the glucogenic pathway (Marik et al., 2015). Contrary to these findings, Jin et al. (2013) reported that IVM displays antidiabetic activities in mice by reducing blood glucose and cholesterol levels, and also by improving insulin sensitivity in FXR dependent manner.

In contrast to previous data in the literature (Min et al., 2016; Tian et al., 2015), the marked hypolactemia following S/C injection of either saline or IVM represents a major surprise in this study and explanation of this outcome needs more investigation.

Hypoproteinemia in the saline-injected groups reflected the obvious debilitating action of stress (Khazadihe et al., 2014). The increase in the stress response-related hormones has adverse impacts on protein kinetics by inducing proteolysis (Paddon-Jones et al., 2006; Bessey and Lowe et al., 1993; Brillon et al., 1995). Owing to the increased protein turnover rate, greater inclusion of glucongenic amino acids could provide another metabolic building block for the gluconeogenic process in the liver (Chevalier et al., 2006).

4 Conclusions

The findings of the current study suggested that site of S/C injection of IVM significantly affected the behavioral activities of the sheep, while there were no differences in the biochemical indicators of stress between S/C injections of IVM at the neck region or behind the elbow joint. However, further investigation is still required to give information about the chronic effect of S/C injection at the neck region and behind the elbow comparing between the behavioural and biochemical indicators of stress before and after injection in the same animal.

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Original Paper

Replacement value of cassava vinasse meal for maize on growth performance, haematological parameters and organoleptic properties of Japanese quails (*Coturnix japonica*)

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Two hundred and twenty eight (228) one day old quails were used to assess the suitability of cassava vinasse meal (CVM) as a replacement for maize at varying inclusion levels of 0.0%, 5.0%, 10.0% and 15.0%. The birds were fed an adequate starter diet for the first week before being randomly alloted into 4 dietary group of 3 replicate of 19 birds each. The feeding trial lasted for six weeks. The crude protein, crude fibre, ether extract, ash, moisture, nitrogen free extract and metabolizable energy of the dehydrated cassava vinasse were 19.26%, 7.96%, 3.72%, 9.33%, 5.68% and 12.17 MJ kg^{-1} ME respectively. The results showed that final weight, average daily weight gain and feed conversion ratio were significantly (P <0.05) influenced as inclusion level of cassava vinasse meal increases. Significant differences were observed on the haematological parameters such as haematocrit and mean corpuscular volume (P <0.05). The analyzed panelist response on organoleptic parameters showed that tenderness, juiciness and texture were significantly different (P <0.05) with birds fed 10.0% CVM having the least values. In conclusion, 10.0% CVM (21% replacement for maize) in the diet of quails had no deleterious effect on the feed conversion ratio, haematological parameters and meat acceptability. Moreover, further research could be geared towards the use of exogenous enzymes and the performance of other poultry species including broiler chicken.

Keywords: blood, cassava, quail, sensory properties, vinasse

1 Introduction

Vinasse is a by-product of the ethanol industry (Hidalgo, 2009). The demand for ethanol has resulted in the use of various feed stocks ranging from starch and sugars crops, and lignocellulosic biomass (Farrell et al., 2006; Hahn-Hägerdal et al., 2007; Mabee, 2007; Okano et al., 2010). Unfortunately, commercial viability of converting lignocelluloses to ethanol is still at the infant stage. Apart from the use of ethanol in pharmaceuticals, liquor, industrial solvents like paints and food industries, the use as biofuel has further heightened the demand. This is because fossil energy crisis and its related global environmental impacts have resulted in an urgent search for renewable energy sources (Kerr, 2007), and bioethanol is currently the most widely used, as it can replace liquid petroleum and thus help reduce greenhouse-gas pollution. Renewable Fuels Association (2016) reported that global fuel ethanol production increased from 49,676 million litres in 2007 to 97,217 million litres in 2015.

Cassava tubers accumulate starch and this makes it one of the richest fermentable substances for the production of ethanol. Although and Onabolu (1999) reported that cassava is the third largest source of carbohydrate class of food for human consumption in the world. According to Ajibola et al. (2012), cassava tuber contains starch with 30% of fermentable properties, which appears to hold more benefits when used for industrial ethanol production.

Nevertheless, vinasse pollutes the environment (Hui and Shuri, 2013). Tolmasquim (2007) reported that 58% of total ethanol production comes from cassava materials. This implies that more cassava vinasse will be generated. However, inadequate knowledge of the vinasse properties and mode of utilization in agriculture needs urgent investigation, since vinasse posses a great threat to underground water and other water sources. Overtime, vinasse had been disposed directly to the flowing water and these affects water quality, ecosystem

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and human health; also only few countries have improved upon these issues by making stringent amendments, while many countries have a long way to go about it (EPA, 2012).

Therefore, rather than vinasse becoming an environmental nuisance, the economic use as feed needs to be researched, which form the basis for this study.

2 Material and methods

2.1 Site of the Experiment

The experiment was carried out at the Poultry unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

2.2 Experimental Birds and Management

Two hundred and twenty eight (228) one day old quails of mixed sexes were purchased from VOM research institute outreach, Ikire, Osun state. On arrival, the birds were rapidly but gently unloaded into a pre-warmed pen and anti-stress solution was given. The birds were fed an adequate starter feed for the first week, thereafter they were randomly allotted into 4 dietary treatment group of 3 replicate of 19 birds each. The feeding trial lasted for six weeks.

2.3 Collection of Test Ingredients

The ingredient was sourced from a research institute in Ogbomoso. It was collected in the liquid form but was dehydrated at 85 °C until constant weight was obtained. It was then milled and referred to as Cassava vinasse meal.

2.4 Experimental Diet

A maize-soyabean based diet served as the control (T_1). Cassava vinasse meal was used to replace maize at 5%, 10% and 15% inclusion level (weight for weight) in diets T_2, T_3 and T_4 respectively. The quails were fed conventional diet for one week before introducing the test diet. The birds were offered feed and water *ad libitum* throughout the duration of the experiment.

2.5 Data collection and analysis

2.5.1 Proximate and energy composition

Proximate analysis was determined using the procedure of AOAC (2000) while the energy composition was calculated using the formulae M.E (kcal kg^{-1} = 37 × %CP + 81.1 × %Fat + 35 × %NFE (Pauzenga, 1985) before being expressed in mega joule (MJ).

2.5.2 Growth performance

Growth performance indices were monitored. The following data were collected during the study; Average daily feed intake (ADFI) (g bird day^{-1}) = total feed intake / (number of birds x number of days); Average daily gain (ADG) (g bird day^{-1}) = (final weight gain-initial weight) / number of days; Feed conversion ratio (FCR) = total feed intake (kg)/total weight gain (kg).

Table 1 Composition of the experimental diets

Ingredients (%)	T_1	T_2	T_3	T_4
Maize	48.60	43.60	38.60	33.60
Wheat offal	2.00	2.00	2.00	2.00
Soyabean meal	32.50	32.50	32.50	32.50
Csaasava vinasse	0.00	5.00	10.00	15.00
Fishmeal	12.90	12.90	12.90	12.90
Limestone	1.35	1.35	1.35	1.35
Bone meal	2.00	2.00	2.00	2.00
Salt	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
Crude Protein (%)	27.13	27.64	28.16	28.67
ME (MJ kg^{-1})	12.36	12.39	12.40	12.46
Crude Fibre (%)	3.38	3.68	3.98	4.28

ME = Metabolizable Energy

2.5.3 Haematological profile

On the 42nd day of the experiment, two birds were randomly selected from each replicate and blood samples were collected into ethylene diamine tetra-acetate (EDTA) bottles from the jugular vein of the birds through the use of sterilized needles and syringes and subjected to laboratory analysis for determination of haematological parameters and plasma proteins. Blood parameters such as Red Blood Cell (RBC) count and White Blood Cell (WBC) counts, haematocrit/Packed Cell Volume (PCV), Haemoglobin (Hb) content, absolute differential leucocyte counts namely neutrophil, eosinophil, lymphocyte, monocytes and basophil values were determined using the methods described by Ojediran and Emiola (2018) while Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Heamoglobin Concentration (MCHC) values were calculated using standard formulae described by Ojediran et al. (2015).

2.5.4 Organoleptic properties

Meat samples were taken from the breast of the quails used for haematological analysis. Sensory evaluation involved 10 untrained panellists but usual meat consumers. Sensory evaluation was carried out on colour, flavour, tenderness, juiciness and overall acceptability of the breast meat samples from each slaughtered birds per replicate on a nine point hedonic scale (1 = dislike extremely, 9 = like extremely) (Price and Schweigert, 1971).

2.6 Experimental design and statistical analysis

All data collected was subjected to analysis of variance in a completely randomized design using SAS (2000) software package and means was separated using Duncan multiple range test (Duncan, 1955) of the same package. Significant differences were at less than 5% probability level (p <0.05).

3 Results and discussion

The nutrient composition of cassava vinasse is as shown on Table 2. According to Marional et al. (2006) vinasse is consisting basically of water (93%). The vinasse used in this experiment was dehydrated before analyzing for the proximate composition. This may be responsible for the crude protein and metabolizable energy recorded. Meanwhile, Ahmed et al. (2013) reported that Sugarcane vinasse contained high moisture content (82.27%), ash (10.60%), proteins (6.20%), and very low carbohydrates content (0.93%). However, yeast residues will lead to the presence of amino acids and proteins in the wastewater, while, the wine characteristics depend mostly on the preparation, alcoholic fermentation system, types of yeast, distillation and separation. On the other hand, the chemical composition of ethanol vinasse is variable and depends on raw materials (Ahmed et al., 2013).

Table 2 Nutrient composition of cassava vinasse

Parameters (%)	
Moisture	5.68
Crude protein	19.26
Crude fibre	7.96
Ether extract	3.72
Ash	9.33
Nitrogen free extract	54.06
Metabolizable energy (MJ kg^{-1} ME)	12.17

The growth performance of quails fed varying inclusion levels of cassava vinasse meal (CVM) is presented in Table 3.The result shows that final weight (FW), average daily weight gain (ADWG) and feed conversion ratio (FCR) were significantly (p <0.05) affected as inclusion level of cassava vinasse meal increased. A linear decrease was observed in FW and ADWG as the inclusion level increases. The FW was higher in T_1 and lowest in T_4. This is also similar for ADWG in birds fed T_1 and T_4. A non-

Table 3 Growth parameters of Japanese quails fed varying levels of cassava vinasse meal

Parameters	T_1	T_2	T_3	T_4	SEM
IW (g b^{-1})	27.17	27.29	28.05	28.17	0.73
FW (g b^{-1})	152.87^a	144.02^b	140.91^{bc}	136.88^c	1.83
ADWG (g b d^{-1})	3.59^a	3.32^b	3.22^{bc}	3.10^c	0.06
ADFI (g b d^{-1})	18.08	17.95	18.42	18.69	0.20
FCR	5.05^b	5.41^{ab}	5.71^{ab}	6.02^a	0.14

abc – means on the same row with different superscripts are significantly different (p < 0.05)
IW – initial Weight, FW – final weight, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio, g b d^{-1} – gram per bird per day

significant increase was observed in ADFI from those fed T_1–T_4 (p >0.05). The FCR was seen to have a linear increase (p <0.05) with increasing inclusion level from T_1 to T_4. Birds fed T_1 had the least FCR which is significantly different (p <0.05) from those fed T_4 but comparable to birds fed T_2 and T_3. The significant linear decrease in weight as the cassava vinasse increases is contrary to the report of Agugu and Okeke (2005) when cassava root meal diet was fed to pullet chicks. Observation on the feed intake is in contrast of what was reported by Edache et al. (2005), when graded levels of yam peel was fed to quails but agreed with those of Guluwa et al. (2014) who reported no significant difference for Japanese quails fed graded levels of water soaked sweet oranges peel meal. The increased feed intake may be due to the need to meet up their energy requirement as observed by Akinfala et al. (2002) when intake increases as the level of cassava meal increases in broiler starter diets. Observation on the feed conversion ratio conform to the report of Guluwa et al. (2014).

Table 4 shows the haematological parameters of quails fed varying inclusion levels of cassava vinasse meal. Significant differences were observed on some of the haematological parameters such as haematocrit (HCT) and Mean corpuscular volume (MCV) (p <0.05). A significant linear decrease was observed in the HCT values. Birds fed T_1 had the highest value while the birds fed T_4 had the lowest value. MCV was observed to have a linear increase from T_1 to T_2 but showed a decrease from T_2–T_4. The haematological parameters showed that the birds were not anaemic and the immune system was not compromised. The non significant high values of WBC are indicative of the fact that they are capable of generating antibodies by the process of phagocytosis and have high degree of resistance to diseases (Soetan et al., 2013) and enhanced adaptability to local environmental and disease prevalent conditions (Iwuji and Herbert, 2012). The HCT values reported in this study could be attributed to cassava vinasse meal (Chineke et al., 2006). MCV, MCH and MCHC are red blood cell indices. The MCV shows the size of the red blood cell (Dacie et al., 1995). In most instances, a reduction in the value of MCV occurs in severe iron deficiency and is a fairly specific indicator of iron deficiency once thalassemia and the anaemia of chronic disease have been excluded (Aina and Ajibade, 2014).

The analyzed panellist response on organoleptics of quails fed cassava vinasse meal is shown in Table 5. Tenderness, juiciness and the texture were significantly different (p <0.05). The tenderness score ranges from 4.86 (T_3) to 6.86 (T_1) while juiciness recorded 6.57, 6.14, 4.57 and 4.57 in birds fed T_1–T_4 respectively unlike 5.14, 7.00, 4.71 and 5.29 for birds fed T_1–T_4 respectively for texture. The significant panellists result on tenderness, juiciness and texture shows that consumer preference was adversely influenced unlike the result of Ojediran and Emiola (2018), although, the meat colour or appearance goes a long way to influence the consumer preference. Consumers reject products in which the colour departs from the normal appearance (Qiao et al., 2001). However, colour of meat depends upon the pigment changes that take place during cooking (Price and Schweigert, 1971). According to Lawrie (1998), flavour could be influenced with the age of the animal while juiciness depends largely on the fat content of the carcasses (Lawrie, 1998). Intramuscular fat and water holding capacity of

Table 4 Haematological parameters of quails fed varying levels of cassava vinasse meal

Parameter	T_1	T_2	T_3	T_4	SEM
WBC (*10^9 l^{-1})	236.80	259.17	261.37	262.87	7.59
LYMPH# (*10^9 l^{-1})	117.87	118.60	112.27	119.43	0.75
GRAN# (*10^9 l^{-1})	32.80	31.13	31.60	31.60	0.34
HGB (g dl^{-1})	14.87	15.80	15.20	15.63	0.39
RBC (*10^{12} l^{-1})	2.84	3.22	3.84	3.85	0.28
HCT (%)	71.40^a	65.43^b	65.10^b	63.53^b	1.10
MCV (fl)	151.37^b	186.43^a	176.00^{ab}	173.37^{ab}	5.10
MCH (pg)	44.87	44.90	44.90	42.97	0.87
MCHC (g dl^{-1})	24.43	24.10	24.27	24.77	0.13
PLT (*10^9 l^{-1})	83.77	80.33	52.33	87.67	13.09
MPV (fl)	10.10	10.73	9.33	9.57	0.26

abc – means on the same row with different superscripts are significantly different (p <0.05)
WBC – white blood cells, LYMPH – lymphocytes, GRAN – granulocytes, HGB – haemoglobin, RBC – red blood cells, HCT – haematocrit or packed cell volume (PCV), MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, PLT – platelets, MPV – mean platelet volume

Table 5 The organoleptic indices of quail meat fed varying inclusion levels of cassava vinasse meal

Parameters	T_1	T_2	T_3	T_4	SEM
Colour	5.86	6.29	6.14	4.57	0.34
Flavour	3.29	4.00	3.57	4.14	0.24
Tenderness	6.86^{a}	6.43^{ab}	4.86^{b}	6.14^{ab}	0.27
Juice	6.57^{a}	6.14^{ab}	4.57^{b}	4.57^{b}	0.32
Texture	5.14^{ab}	7.00^{a}	4.71^{b}	5.29^{ab}	0.35
Over all	6.71	6.71	6.14	6.43	0.22

ab – means on the same row with different superscripts are significantly different (p <0.05)

meat is directly related to juiciness. Akinwunmi et al. (2013) reported that quail meat has the least water loss during cooking compared to other poultry birds which makes it more juicy and tender than others. This might be responsible for the significant effect on tenderness, texture and juiciness. Abu et al. (2015) recorded that the organoleptic values such as: juiciness, taste, colour and overall acceptability were influenced for birds fed cassava peels and leaf meal as replacement for soya bean meal up to 20% inclusion level.

4 Conclusions

In conclusions, 10.0% CVM (21% replacement for maize) in the diet of quails had no deleterious effect on the feed conversion ratio, haematological parameters and meat acceptability. Moreover, further research could be geared towards the use of exogenous enzymes and the performance of other poultry species including broiler chicken.

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Original Paper

Selected qualitative parameters above-ground phytomass of the Lenor-first Slovak cultivar of *Festulolium* A. et Gr.

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The aim of this experiment was to compare selected qualitative parameters in above-ground phytomass of the first Slovak cultivar of *Festulolium* A et. Gr. cv. Lenor in comparison to earlier registered cultivars Felina and Hykor. The pot experiment was conducted at the Demonstrating and Research base of the Department of Grassland Ecosystems and Forage Crops, Slovak Agricultural University in Nitra (Slovak Republic) with controlled moisture conditions (shelter) in 2017. Content of nitrogen, phosphorus, potassium, calcium, magnesium, crude fibre and water soluble carbohydrates were determined from dry above-ground phytomass of grasses. The significantly highest (P <0.05) nitrogen content in average of cuts was in above-ground phytomass of Felina (30.3 g kg^{-1}) compared to Hykor (25.4 g kg^{-1}) and new intergeneric hybrid Lenor (25.0 g kg^{-1}). The lowest phosphorus content was found out in hybrid Lenor (3.4 g kg^{-1}). In average of three cuts, the lowest concentration of potassium was in new intergeneric hybrid Lenor (5.8 g kg^{-1}). The lowest content of calcium was found out in hybrid Lenor (7.0 g kg^{-1}). Magnesium concentration ranged from 5.0 g kg^{-1} (Hykor) to 6.1 g kg^{-1} (Felina). Higher (P <0.05) fiber content in average of cuts was in above-ground phytomass of Lenor (27.0%) and Hykor (26.5%) than Felina (24.0%). The highest concentration of water soluble carbohydrates was found in Hykor and Lenor (3.5%).

Keywords: grass hybrids, festulolium, quality

1 Introduction

Festulolium A et Gr. is a natural or synthetic intergeneric hybrid between species of the genus *Festuca* L. and *Lolium* L. Breeding aim is a cumulating the positive, agriculturally important, parents properties in the newly created genome (Casler et al., 2002; Humphreys et al., 2003; Černoch and Groenbaek, 2015). Hybrids are divided into two groups. The first group consists of so called ryegrass type (*Lolium* L.) for example cv. Perun, Bečva and Lofa. Another group consists of so called fescue type (*Festuca* L.) for example cv. Felina, Fojtan and Hykor (Kováč, 2002; Humphreys et al., 2003).

x *Festulolium* had a broad spectrum uses. They are used in animal nutrition in the form of fresh phytomass, or as conserved fodder in the form of hay and silage. Among other things, they can also be used like a biogas plants. *Lolium* x *Festuca* grass hybrids have a good agronomical potential especially in adverse environments (Nesheim and Bronstad, 2000; Prochnow et al., 2009; Herkeľ et al., 2015; Hric et al., 2018).

Quality hay is a natural and irreplaceable feed for ruminants and horses (Bíro et al., 2014). Nutrient content in feeds from grasslands varies and their concentration, depending on several factors (Čunderlík and Martincová, 2013). Chemical composition and yield value of forage mainly depends on species, levels of soil fertilization, agro-technology, phenological phase, collection technology, conservation and storage (Gálik et al., 2016).

The aim of this experiment was to compare selected qualitative parameters of above-ground phytomass of the first Slovak cultivar of *Festulolium* A. et Gr. with different cultivars.

2 Material and methods

The experiment was established (sowed the seeds) on 21st March 2017. It was realized in the Demonstrating and Research Base of Department of Grassland Ecosystems and Forage Crops, Slovak Agricultural University in Nitra (Slovak Republic) with controlled moisture conditions in 2017.

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We watched out 3 cultivars of *Festulolium* A. et Gr.:

1. *Festulolium* A. et Gr. cv. Felina
2. *Festulolium* A. et Gr. cv. Hykor
3. *Festulolium* A. et Gr. cv. Lenor

Felina (registered in 1988) (*Lolium multiflorum* LAM. × *Festuca arundinacea* Schreb). was created by crossing *Lolium multiflorum* LAM. cv. Rožňovský and Tiara with *Festuca arundinacea* Schreb. ecotypes from the neighbourhood Hladké Životice (Czech republic) and backcrossing with selected varieties of the world assortment. Hybrid Felina is the first Czech variety of *Festulolium* A. et Gr. Felina is taller, persistent, resistant to frost and drought. It has a winter character. It is *Festulolium pabulare* and fescue type (Kováč et al., 2002).

Hykor (registered in 1991) (*Lolium multiflorum* LAM. x *Festuca arundinacea* Schreb.) was created by crossing *Lolium multiflorum* LAM. cv. Rožňovský and Tiara with *Festuca arundinacea* Schreb. ecotypes from the neighbourhood Hladké Životice (Czech republic) and backcrossing with selected varieties of the world assortment. Hykor is resistant to freezes, drought and lie down. It is *Festulolium pabulare* and fescue type (Kováč et al., 2002).

Lenor (registered in 2015) (*Lolium multiflorum* LAM. × *Festuca arundinacea* Schreb.) was created by crossing *Lolium multiflorum* LAM. cv. Jamaoba with *Festuca arundinacea* Schreb. cv. Sopline and backcrossing with selected varieties of the world assortment. Lenor is the first Slovak variety of *Festulolium* A. et Gr. It has a rapid growth in the spring. Lenor is resistant to frost and drought. It has fine leaves and it is suitable for grazing. The breeder is Graminex s.r.o. Levoča (Slovak Republic). It is *Festulolium pabulare* and fescue type (Bašta, 2017). In Slovak State Varieties Tests was Lenor was compared with Felina and Hykor.

Experiment was conducted in containers with volume 2 dm^3 in 3 replicates. Each container contained four individual seedlings. Chemical characteristics of applied substrate are documented in Table 1.

Grass plants were fertilized at a dose of 100 kg ha^{-1} N (1.44 g per a container) with NPK fertilizer (14-9-10). The whole dose of fertilizer was divided into two equal doses (2 × 50 kg ha^{-1} N). First fertilization was applied 21st April 2017 (month after sowing) and second fertilization was done after the first mowing. The plants were irrigated at the first signs of water scarcity. Irrigation dose was 200 ml water for each container.

Grasses were cut to 50 mm. There 3 mowing were realized in terms 23rd June, 14th August and 27th September 2017. Moved aboveground phytomass was drying at 105 °C to constant weight. Then were determined:

- N – by Kjeldahl,
- P – spectrophotometrically by phosphomolybdic method after wet mineralization with HNO_3 and $HClO_4$,
- K and Ca – flame photometry after wet mineralization with s HNO_3 and $HClO_4$,
- Mg – spectrophotometrically with titanium yellow after wet mineralization with HNO_3 and $HClO_4$,
- WSC – (water soluble carbohydrates) by method Luff-Schoorl,
- CF – (crude fiber) by two-stage hydrolysis in weakly acidic and weakly alkaline medium by Hennberg-Stohmann.

The results were evaluated by software STATISTICA 7.1 complete CZ analysis of variance (Fisher LSD test, $\alpha = 0.05$).

3 Results and discussion

Mineral content play a number of important roles in the animal organism. The content of mineral content is about 1.6% in plants. Their contents vary widely and their concentration depends on several factors (Bíro et al., 2016). *Poaceae* L. is characterized by different values of nutritional value Gibson (2009). According to Gibson (2009), the suitable range of mineral content in the grassland is as follows: N 10.0–53.0 g kg^{-1}, P 0.5–9.8 g kg^{-1}, K 2.1–49.3 g kg^{-1}, Ca 0.3–27.3 g kg^{-1}, Mg 0.3–7.9 g kg^{-1}.

Selected qualitative parameters in above-ground phytomass of *Festulolium* A. et Gr. cultivars are presented in table 2. The nitrogen (N) content decreased in all cultivars from 1st cut to 2nd cut. Only in hybrid Felina was the N content higher in the second cut (32.0 g kg^{-1}) compared to first cut (31.6 g kg^{-1}). The higher (P <0.05) nitrogen content in average of cuts was in above-ground phytomass on Felina (30.3 g kg^{-1}) than Hykor (25.4 g kg^{-1}) and Lenor (25.0 g kg^{-1}). Similar trend of the decrease of the nitrogen content in above-ground phytomass of *Festulolium* A. et Gr. with increased cut found Skládanka et al. (2010).

Also, the phosphorus (P) content gradually declined from 1st cut to 3rd cut. Only in hybrid Hykor was the P content higher in the third cut (3.5 g kg^{-1}) compared

Table 1 Substrate agrochemical properties

Nt	P	K	Ca	Na	Mg	Fe	C_{ox}	pH
mg kg^{-1}							g kg^{-1}	
4,067.07	71.53	538.78	6,720.00	556.52	716.29	39.43	4.41	6.70

to second cut (3.0 g kg^{-1}). In the total evaluation was not finding significant difference of P content between the evaluated cultivars. At the least content of phosphorus was found out in hybrid Lenor (3.4 g kg^{-1}). Jančovič et al. (2013) considers P content in the dry matter of grassland 2.8 g kg^{-1} and more.

Another element of assessment was potassium (K). Jančovič et al. (2013) considered the potassium content in the grassland dry phytomass from 20 to 22 g kg^{-1}. It is lower concentration than the physiological need for plants, but it is higher than the need for animals. The potassium concentration in above-ground phytomass had a decreasing tendency with other cuts. The exception was only the third cut of Felina In average of three cuts was the least concentration of potassium in new intergeneric hybrid Lenor (5.8 g kg^{-1}).

In first cut the calcium (Ca) content of above-ground phytomass was from 4.6 g kg^{-1} (Lenor) to 6.4 g kg^{-1} (Felina). In the next cut, the Ca concentration increased by almost twice compared to the first cut. In the last, the cut calcium content declined in all cultivars. In the average Ca concentration of above-ground phytomass was 7.0 g kg^{-1} and higher. The qualitative grassland feed should contain at least 7.0 g kg^{-1} calcium (Jančovič et al., 2013).

Magnesium (Mg) content in above-ground phytomass cultivars had similar development as calcium. In the total evaluation significant difference of Mg concentration between the evaluated cultivars was not found. Magnesium content was from 5.0 g kg^{-1} (Hykor) to 6.1 g kg^{-1} (Felina).

In ruminant nutrition the fibre has irreplaceable place. It participates in the proper digestion of feed, encourage chewing, rumen and intestinal peristalsis and ensures the mechanical saturation of the animal (Gálik et al., 2016). Štýbnarová et al. (2013) state that the minimum fibre content in feed intended for the proper functioning of the ruminant digestive system should be 18.0–20.0%. The authors further point out that the fibre content above 30%, declined the digestibility of the feed and therefore the energy value of the grasses. In the first cut was concentration of crude fiber (CF) as follows: 24.0% (Lenor), 23.1% (Hykor) and 21.3% (Felina). In the first cut Houdek (2010) found the 29.6% and 29.0% CF content of the hybrid Felina and Hykor, respectively. In the following cut the crude fiber concentration in Felina was 24.16%, in Hykor 27.02% and in Lenor 27.04%. Similar results in the second cut was measured by Houdek (2010). Also in the last cut new intergeneric hybrid Lenor reached the highest CF content (30.1%). The higher (P <0.05) fiber content of average of cuts was in above-ground phytomass in Lenor (27.0%) and Hykor (26.5%) as Felina (24.0%). Černoch et al. (2004) found the total fiber content of the cultivar Felina 26.4% and Hykor 25.3%. Skládanka et al. (2014) presented content of CF in Felina 27.9% and Hykor 27.0%.

Water soluble carbohydrates (WSC) are an important source of energy for the animals and for the rumen microflora. Digestibility is almost 100%. The plants accumulate sugars during the day and during the night they spend them. This suggests that the WSC content is the lowest in the morning and the highest at the end of the day (Hakl and Fuksa, 2011). Water soluble carbohydrates have a positive effect on animal production parameters. In work Miller et al. (2001) has proven effect of WSC from

Table 2 Selected qualitative parameters above-ground phytomass of *Festulolium* A. et Gr. cultivars

Order of cut and cultivar	N	P	K	Ca	Mg	CF	WSC
	g kg^{-1}					%	
1st cut Felina	31.6	4.6	8.8	6.4	5.0	21.3	4.0
2nd cut Felina	32.0	3.9	5.3	12.5	7.2	24.2	2.4
3rd cut Felina	27.3	3.1	5.7	8.9	6.0	26.6	1.2
Average of cuts	30.3^{a}	3.9^{a}	6.6^{a}	9.3^{a}	6.1^{a}	24.0^{a}	2.5^{a}
1st cut Hykor	30.4	4.6	7.8	5.9	4.2	23.1	4.2
2nd cut Hykor	23.7	3.0	5.7	11.7	6.2	27.0	1.9
3rd cut Hykor	22.1	3.5	5.3	6.3	4.6	29.5	4.2
Average of cuts	25.4^{b}	3.7^{a}	6.3^{a}	8.0^{a}	5.0^{a}	26.5^{b}	3.5^{a}
1st cut Lenor	30.1	4.5	7.4	4.6	3.9	24.0	4.2
2nd cut Lenor	24.4	3.0	5.5	8.6	6.6	27.0	2.7
3rd cut Lenor	20.5	2.8	4.4	7.8	6.4	30.1	3.5
Average of cuts	25.0^{b}	3.4^{a}	5.8^{a}	7.0^{a}	5.6^{a}	27.0^{b}	3.5^{a}

a, b statistically significant differences (Fisher LSD test, $\alpha = 0.05$), N – nitrogen, P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, CF – crude fiber, WSC – water soluble carbohydrates grasses on improve milk production. In the first cut was concentration of water soluble carbohydrates in Felina 4.0% and 4.2% in Hykor and new hybrid Lenor. In the first cut Houdek and Jambor (2010) found the WSC content of the hybrid Felina 12.2% and 11.9% Hykor. In the second cut declined WSC content on all cultivars (1.9–2.7%). In the second cut Houdek and Jambor (2010) found the 9.82% and 14.0% of water soluble carbohydrates concentration of the hybrid Felina and Hykor, respectively. In the last cut was reached the highest content of water soluble carbohydrates in Hykor (4.2%). In the total evaluation was not finding significant difference of WSC content between the evaluated cultivars. The highest concentration of water soluble carbohydrates has in Hykor and Lenor (3.5%). Skládanka et al. (2014) presented content of WSC in Felina 11.0% and Hykor 12.9%. Černoch et al. (2004) found the water soluble carbohydrates concentration of the cultivar Felina 7.2% and Hykor 10.3%.

4 Conclusions

On the basis of the results, it can be stated that new Slovak cultivar of *Festulolium* A. et Gr. Lenor had comparable values of qualitative parameters faboveground phytomass in comparison with earlier registered cultivars Felina and Hykor. Consisdering the evaluation of the content of nitrogen, phosphorus, potassium, calcium, magnesium, crude fiber and water soluble carbohydrates. Lenor was found suitable for animal nutrition.

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Original Paper

Analysis of coat quality of Chinchilla rabbit breed

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Between breeders, Chinchilla rabbit is very popular as it has standard body shape and high quality of typically coloured fur. The aim of this study was to analyse quality of Chinchilla rabbit fur. Coat samples were gathered at the National Animal Exhibition in Nitra. We collected samples from three different body areas – those being scapula, back and thigh. Altogether, we obtained 153 samples of different individual animals. We observed different parameters of the fur. Those were – thickness and length of coat, height and width of the undercolour, ticking and height of the guard hair. We found out that average thickness of coat in the examined population was 0.106 mm in the area of scapula; 0.104 mm in the thigh area and 0.113 mm from the back area. Length of the guard hair from area of scapula was 35.8 mm; 37.9 mm in the thigh area and 36.4 mm in the back area. Height of the undercolour was 26.9 mm in the back area, 26.4 mm in area of scapula and 27.6 mm in the area of thighs. Observed width of intermediate colour was 5.1 mm in thigh area; 4.3 mm in back area and 4.8 mm in scapula area. Measured height of terminal black line of hair was 4.7 mm in area of scapula; 5.3 mm in the back area and 5.3 mm in thigh area. The differences among the evaluated body areas were not significant.

Keywords: fur, quality, hair, rabbit, Chinchilla

1 Introduction

Chinchilla is originally a breed that came from France. It was originally bred by N.J. Dybowski who was originally a Pole. It was exposed for the first time in Saint-Maur in 1913. In 1915 it got to England, later in years 1920 and 1923 to Switzerland, respectively to Czechoslovakia where it was initially exposed at exhibition in Pardubice by breeder named F. Majer. Commercial possibilities of using the Chinchilla rabbits is described by Brumwell (1928).Fashion trends have big effects on the demand for rabbit hairs and therefore this cause variation in world prices. Deedrick and Koch (2004) describe the basic structure of hair. Hair can be defined as a slender, thread-like outgrowth from a follicle in the skin of mammals. They have presented that animal hairs are classified into three basic types (guard hair, fur or wool hairs and tactile hairs or whiskers) and that animal hairs can be classified into three major groups on the basis of their microscopic appearance (deer family and antelope, commercial fur animals and domestic animals. Chinchilla produces very esthetical and quality fur. Glossy, well laid-down and flexible fur is the characteristic of healthy individual. The flexibility of fur is based on thickness of individual guard hair to which we give extra attention. The aim of the study was to analyse the quality of coat and structure of fur of Chinchilla rabbit.

2 Material and methods

Material and samples were gathered over the course of five years at International exhibition in Nitra and we gathered fur samples of 153 rabbits of Chinchilla. Individual samples were collected from three different parts of body – scapula (1), back (2) and thigh (3). We used the samples to measure and weigh the thickness of guard hair, total length of guard hair, height of the undercolour, width of ticking and length of terminal black line of the guard hair which creates a typical black ticking for this breed. During review of the animals we evaluated flexibility of fur and ticking, as well as focused on main faults of this breed.

Width of guard hair was analysed by a method that uses stereomicroscope Olympus SZX 16 and software – QuickPhoto Micro (v.2.3, © Promicra, s.r.o. 2009).The methodology for measuring hair was developed on

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Figure 1 Structure of Chinchilla rabbit fur

the basis described by Fik et al. (2011). We measured 10 guard hairs from each of observed areas and found out the average thickness of guard hair. As the place of measurement, we used the area in which the guard hair was the thickest, approximately in the middle of its length. Length of hair as well as its other parts – height of undercolour, width of ticking and length of terminal black line of hair we measured by sliding ruler. We again gathered 10 hairs from each area and we found out the average value. Likewise, we observed elasticity of the fur of this breed, which is one of main assessment factors at National Animal Exhibition. We picked a rating scale from one to four – where the value 4 was assigned to sample which got completely back to the previous state after teasing. Value of one was assigned to fur which kept standing after teasing. Occurrence of ticking was evaluated by three people – we spectated its sufficiency or insufficiency. The results were compared with *T*-test. The spreadsheet calculator MS Excel was used.

3 Results and discussion

During our experiment the average width of guard hair over the course of 5 years in thigh area was 0.104 ±0.016 mm, in area of scapula it was 0.106 ±0.015 mm and in back area it was 0.113 ±0.019 mm. Kopański (1965) mentions width of guard hair 0.113 mm and thickness undercoat 0.014 mm in Chinchilla rabbit. Average length of guard hair in area of scapula was 35.8 ±5.254 mm, in the back area it was 36.4 ±5.321 mm and in the thigh area 37.9 ±3.947 mm. Chinchilla has typical rich dark blue colour undercoat and it is important that two thirds of hair should be as dark as possible. Undercolour height is an important trait for fur animal, which decides the wool hairs quality as the crucial index of evaluation (Huang et al., 2016). Average height of undercolour in the area of scapula was 26.4 ±5.619 mm, in thigh area 27.6 ±3.484 mm and in the back area we measured 26.9 ±4.523 mm. Intermediate colour of is important part of guard hair that creates so called "play of colours" which should have width of 5 mm. Intermediate colour based on our measurements reached values similar to the standard. In our measurement – intermediate colour in thigh are was 5.1 ±1.449, in area of scapula it was 4.8 ±1.363 mm and in the back are it was 4.3 ±1.336 mm. Terminal black line of guard hair plays main role in creation of black ticking. Measured height of terminal black line of hair in area of scapula was 4.7 ±2.296 mm, in back area it was 5.3 ±1.996 mm and in the thigh area it was 5.3 ±1.512 mm. During evaluation of fur elasticity that was rated on scale from 1 to 4 we rated the best fur as the one that got back to previous state after teasing. The highest quality fur was scored only in 26.4% of animals. A little bit lower tier rated

Table 1 Observed values of fur selected parameters

Parameters of the fur	Scapula area (mm)	Back area (mm)	Thigh area (mm)
Width of guard hair	0.106 ±0.015	0.113 ±0.019	0.104 ±0.016
Length of guard hair	35.8 ±5.254	36.4 ±5.321	37.9 ±3.947
Height of undercolour	26.4 ±5.619	26.9 ±4.523	27.6 ±3.484
Intermediate colour	4.8 ±1.363	4.3 ±1.336	5.1 ±1.449
Height of terminal black line	4.7 ±2.296	5.3 ±1.996	5.3 ±1.512

with number 3 was in 37.7% of animals. Even less flexible fur (marked 2) had 30.2% of animals. The least – inflexible coat where hair kept on standing vertically was observed in 5.7% of animals. Another typical sign of Chinchilla fur is black ticking formed by dark tip of the guard hair. Almost in all of the animals the ticking was marked sufficient – which means it was significant. Only 11, 3% of individuals had the ticking less visible and it was caused by brighter colouring of the body.

We saw many faults in our specimen of Chinchilla breed during the course of 5 years. Most frequent being brighter colouring of the body on the sides of corpus – which occurred in 24.5% of animals. Other noticed faults were deviations in colours of fur on the level of undercoat. The spectated brighter colour of undercoat in 5.7% out of total count of rabbits was detected. (In 9.4% of rabbits there was a very low transient colour as of which the colour gradation was hardly sufficient.). In 3.8% of animals (we seen misalignment in colours – caused by darker guard hair on the head. Some other rabbits had too soft structure of fur (3.8%). Fur is part of skin system, that way we also marginally focused on main fault connected with skin of the animals and that being loose skin in the neck and breast area which occurred in 20.8% specimen. Similar tendency of hair quality indicators in Chinchilla rabbits also describes Mamojková (2012) and Mamojková (2014).

Rabbit hair is a natural fibre, not contaminated in the processing. It is environmentally safe material with special morphological structure, it is a unique stylish garment and the demand for rabbit fur keeps on growing (Zhang, 2011). Mengüç et al. (2014) described physical properties of Angora Rabbit fibres. There are three types of hair that are known to be produced by the Angora rabbit – those being: guide hair, down hair and guard hair. Length of guard hair is usually around 80 mm and they are known to have rough points that lock together and lie over the down hair by which they seal off. Guide hairs usually grow up to 110 mm in length and have covering function for other hair and they guide the direction of their growth. Down hairs are smooth part of fur that tends to develop to length of 60 mm with small amount of cuticle scales. Down hairs are one of the finest animal materials used in production of textiles because of their diameter which is only 14 µm. (Franck, 2001)

Length of guard hair has most importance in furriers, during the processing of furs. For this reason the highest straightness of fur is required that way no additional pruning is required because that way typical attributes of fur for the chosen breed can be lost. Average length of guard hair is 3 cm based on previous measurements by different authors. According to Dahiya and Yadav (2013) the structure of the animal hairs is different accordingly to which body region is the hair from. We are capable of distinguishing 3 regions in guard hairs. Firstly, the region closest to skin that being fine shaft or proximal portion closest to the skin. Secondly, a much wider distal section which is shield-shaped and flat in looks of it. And lastly, the one that tapers down to a very fine point. The considerable variety of guard hair rests in thickness and length but generally their length is longer than the rest of coat fibres and that way they basically shine through the undercoat. A single medulla composed of longitudinal series of cavities is usually contained in lower sections of guard hair as well as the tip of the guard hair and even the fur fibres. In the place where the guard hairs become thicker and obtain shield shaped shape in that place the medulla is created by multiple series of cavities – those being from two up to six. Only few of highest quality fur fibres (<8 µm) and the terminal ends of guard hair are solid protein – this type of medulla layout is a known rabbit characteristic (Rogers et al., 2006).

Verhoef-Verhallen (2000) characterizes Chinchilla as small and wildly coloured rabbit with completely vanished red and yellow pigment in fur. Colour of fur is grey with significant ticking, formed by black terminal lines of individual guard hair. Bottom part of body is always white, undercoat being dark blue. Important part of fur colouring is white intermediate colour which creates a ring typical for the breed. According to Covriget al. (2013) experts believe that the best chinchilla colours are produced with either an albino or Himalayan gene paired with the chinchilla gene. Even though the standard of the breed is strictly defined not every specimen fits it. Width of fur is equal for entire population even if sometimes guard hair is occasionally significantly thicker than they should be, and that way thick guard hair can have effect on softness of fur. Length of fur is less uniform. While we examined this parameter, we noticed that there was higher variability in body where the longest fur occurred in the thigh area. Body colouring of Chinchillas is fairly uniform even though it is occasionally affected by darker rabbits brought from abroad. Best known fault of body colouring of Chinchillas is the different colouring when head or breast area is brighter than rest of the body. Uniformity has been reached in colouring of the undercoat – it was reached by selection where have been only individuals with dark undercolour. Another fault could be changes in intermediate colour which is often bright and that way usually less bounded and there could also be a problem with its width. Some specimen also had brownish colouring which can be currently changed very hardly. Fur in this individual is usually quite dense. Dense and flexible fur usually has shorter length and with shorter length comes less expressed veil – that way sometimes come to contact with individuals whose typical tenting completely disappeared. Comparing the results with other authors was not possible because we have not been able to find a work about evaluation of quality of coat and fur in Chinchilla breed. Given parameters are only stated in breeding standard.

4 Conclusions

The skin of rabbits is a visual parameter to their general state of health and the rabbit fibre has a good potential for producing textiles with special properties. Average length of guard hair in area of scapula was 35.8 ±5.254 mm, in the back area it was 36.4 ±5.321 mm and in the thigh area 37.9 ±3.947 mm. Average height of undercolour in the area of scapula was 26.4 ±5.619 mm, in thigh area 27.6 ±3.484 mm and in the back area we measured 26.9 ±4.523 mm. Intermediate colour in thigh are was 5.1 ±1.449, in area of scapula it was 4.8 ±1.363 mm and in the back are it was 4.3 ±1.336 mm. Height of terminal black line of hair in area of scapula was 4.7 ±2.296 mm, in back area it was 5.3 ±1.996 mm and in the thigh area it was 5.3 ±1.512 mm. In the end we can only say that there are no significant differences – parameters are on same level from all our collected samples collected over five years. The biggest problem that should be taken care of in future is the existence of variability in colouring of individual animals, parts of their body or even guard hair. The results can be used for further breeding of the breed. Based on hair quality results, it would be possible to evaluate sires like those who contribute to improving the quality of hair and those who do not. Not ending and consistent selection of breeding work could sustain, possibly even improve quality of fur, feathering and that way it could keep the typical colouring that characterizes this breed. Interest in regular analysis of the results of the hair quality of Chinchilla and breeds with a similar coat structure should have especially clubs of breeders.

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Short communication

Differences in soil properties and crop yields after application of biochar blended with farmyard manure in sandy and loamy soils

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In recent years, the importance of biochar application in world´s soils have increased tendency mainly due to its opposite effects. Therefore, the effort of many companies is based on the development of soil amendment which together improve properties and crop productivity in lot of soils. In this short study, we have verified the effectiveness of biochar blended with farmyard manure named *Effeco* on soil properties and crop yields in different textural soils (1. sandy soil in Dolná Streda and 2. loamy soil in Veľké Uľany). Our results showed that the *Effeco* increased soil pH in both soils. In sandy soil, the *Effeco* more significantly affected sorptive parameters and soil organic carbon content than in loamy soil. Water retention in capillary pores after *Effeco* application in sandy and loamy soils was higher by 22% and 4%, respectively compared to control. On the other hand, more significant effect of *Effeco* application on soil structure was observed in loamy soil. The total crop productions in sandy and loamy soils due to the *Effeco* application were higher by 82% and 16%, respectively, compared to control plots. All in all, we concluded that the effects of biochar blended with farmyard manure differ mainly on soil texture.

Keywords: *Effeco*, sorptive parameters, soil organic matter, water retention, soil structure, loamy soil, sandy soil

1 Introduction

Long-term and mainly intensive soil management practices have negative effects on soils properties and often results in their degradation include soil acidification, decrease of soil organic matter, soil structure stability, porosity, water retention etc. (Polláková et al., 2018; Kotorová et al., 2018). A fundamental factor which alter soil properties is soil organic matter (Szombathová, 2010) and therefore, the effective maintenance of soil organic matter in degraded soils can help preserve soil fertility.

In last time, biochar has becoming a great of interest as a mean for carbon sequestration, resulting from its high content of carbon and long-term persistence in soil (Dong et al., 2019). Biochar is the solid product of pyrolysis, designed to be used for environmental management (Lehmann et al., 2015). IBI (2013) defines biochar as: A solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment. Biochar can be used as a product itself or as an ingredient within a blended product, with range of applications as an agent for soil improvement, improved resource use efficiency, remediation and/or protection against particular environmental pollution and as an avenue for greenhouse gas mitigation. The biochar properties can be different in relation to type of feedstock source, temperature and time of pyrolysis, pressure and soil type where the biochar is applied (Jeffery et al., 2011; Wang et al., 2013; Ahmad et al., 2014). For example, biochar produced from grasses at temperatures 250–400 °C had higher mineralisation rate (Zimmerman et al., 2011) than biochar produced at high temperatures (525–650 °C) and from hard woods (Fischer and Glaser, 2012). Biochar produced from manure usually has smaller surface area, than biochar produced from wood. The higher temperature increases the content of carbon and the surface area in biochar while the content of oxygen and hydrogen decreases (Lopez-Capel et al., 2016).

Under above mentioned context is evident that biochar properties and its acts in different soil-climatic condition are different. For farmers is, however essential whether the application of biochar improves soil fertility, increases crop yields and brings economic profit. Manufacture of biochar that would improve all soil characteristics and also bring the economic effect is not an easy task.

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Scientific studies show that the efficiency of biochar can be improved by its combination with application with other organic fertilizers, composts, NPK fertilizers. For this reason, fertilizer manufacturers are working to create products that combine biochar with other soil fertility enhancers in one suitable for different soil-climatic conditions. For example, a scientific studies and own research activities of the company Zdroje Zeme a.s. helped to developed soil amendment for activation intensively used land named *Effeco* (combination biochar together with farmyard manure in volume 1 : 1) and within this short study, we have verified the effectiveness of *Effeco* on soil properties and crop yields in different textural soils.

2 Material and methods

Field experiments were performed at two sites with texturally different soils. Before established experiments, the soils in both localities were intensively used. The sites description is given in Table 1. For the purposes of this short study, soil samples were taken from two treatments: 1. Control (no fertilized) and 2. *Effeco* amendment (at rate of 20 t ha^{-1}) in the autumn 2018 in both study sites. In soil samples, soil pH, sorptive characteristics, soil organic carbon, physical and hydro-physical properties were evaluated by standard methods (Hrivňáková et al., 2011). Yields of crops were also evaluated.

3 Results and disscussion

El-Naggar et al. (2019) published that the role of biochar application in the enhancement of soil fertility and productivity can be categorized into aspects relating to nutrient cycling, crop productivity, soil pH, cation exchange capacity (CEC), nitrogen (N), microbial communities, water retention, and C sequestration and our results mentioned aspects also confirmed (Table 2). In sandy soil, original neutral soil pH increased to slightly alkaline due to *Effeco* application. The increase of soil pH by 0.26 pH unit was determined also in loamy soil. Ibrahim et al. (2013) also reported increases in pH in a biochar amended sandy and loamy soils. In our cases, a higher decrease of hydrolytic acidity after *Effeco* application in sandy soil than loamy soil was observed. Some differences between soils in values of sum of basic cation and CEC were as result of *Effeco* application. In sandy soil, the *Effeco* significantly increased sum of basic cations and on the other hand in loamy soil its effects were opposite. In sandy and loamy soils, the CEC values were very low and high, respectively. In sandy soil, the CEC values after *Effeco* increased by 30% compared to no fertilized plot. The main reason is related to particle size distribution (low sorption capacity of sand particles) and higher level of soil organic matter after *Effeco* application. Opposite situation in loamy soil was determined. The CEC values decreased. The decrease of CEC is related to negative charge in the *Effeco* surface and absorption of anions is preferred. These results confirmed the findings of Laghari et al. (2015) who reported increase of CEC due to potentially high surface functional group content of biochar mainly in sandy-textured soils.

Biochar addition has been shown to increase organic carbon in soils (Agegnehu et al. 2016). Soil minerals and organic matter associate with biochar tended to form aggregates in which the biochar turned occluded from

Table 1 Characteristics of studied sites

Site	Climatic	Soil	Soil management	Crop and previous crop	Establishment of experiment
	Conditions				
Dolná Streda	9–10 °C 520–600 mm	Haplic Arenosol (Arenic, Calcic) sandy soil	reduced soil management (disking to the depth 15 cm) – growing of market crops	sunflower hard wheat	autumn 2017
Veľké Ulany	10 °C 550 mm	Vermic Chernozem (Mollic, Loamic) loamy soil	intensive soil management, included drip irrigation (conventional tillage to the depth 20 cm) – vegetable growing	capsicum carrot	spring 2018

Table 2 Soil pH, sorptive parameters and soil organic carbon content

Sities	Treatment	pH	H	SBC	CEC	Bs	SOC
Dolná Streda (sandy soil)	control	7.24	4.02	36.0	40	90.0	0.90
	Effeco	7.43	3.21	48.8	52.0	93.8	1.13
Veľké Uľany (loamy soil)	control	7.63	2.53	492.3	494.8	99.5	1.85
	Effeco	7.89	2.19	488.5	490.6	99.6	1.88

H – hydrolytic acidity, SBC – sum of basic cations, CEC – cation exchange capacity, Bs – base saturation, SOC – soil organic carbon

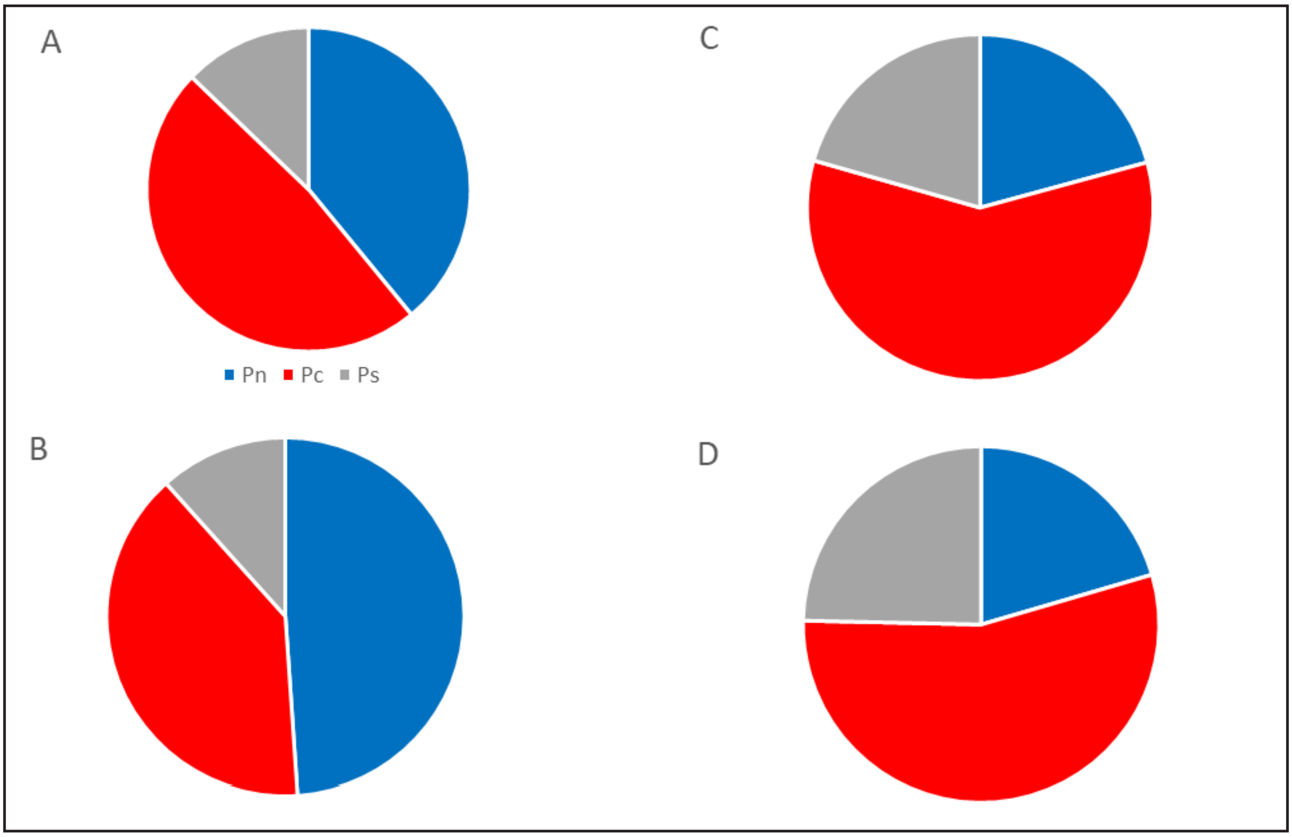

Figure 1 Volume of pores in sandy soil (A, B) and in loamy soil (C, D) after *Effeco* application (A, C) and in no fertilized treatments (B, D)
Pn – volume of non-capillary pores, Pc – volume of capillary pores, Ps – volume of semi-capillary pores

chemical degradation or transport (Brodowski et al., 2006), which could be the main reason of C increase in the aggregates and one of the most important mechanism of C sequestration in soils (Šimanský et al., 2017). In *Effeco* plot, the content of SOC was higher by 26% in comparison to control in sandy soil and the same trend but no significant was observed in loamy soil (Table 2).

In sandy soil, between treatments any significant differences were not determined for bulk density and total porosity, however, the volume of energetics pores differ on *Effeco* (Figure 1 A, B). In *Effeco* treatment, the volume of semi-capillary and capillary pores increased by 10 or 22% respectively on one hand, and decreased volume of non-capillary pores by 20% on the other. The volume of non-capillary, capillary and semi-capillary represented 39, 48 or 13% of the total porosity because of *Effeco* application, whereas in the case of control it was 49, 39 and 12% of the total porosity. In *Effeco* treatment values of capillary absorption, maximum capillary water capacity and retention water capacity were 32.6%, 27.4% and 24.4%, respectively, and in control these one was 27.8%, 23.2% and 20%, respectively. *Effeco* application almost one-time increased available water supply and also available water capacity compared to control. In loamy soil, the *Effeco* did not have any significant effects on energetics pore categories (Figure 1 C, D), capillary absorption, maximum capillary water capacity, retention water capacity and available water capacity (Table 3). On the other hand, *Effeco* applied to the loamy soil increased by 37% available water supply. Our results in both soils did not confirm positive biochar effect on decrease of bulk density on one hand and increase of total porosity on other (Ajayi and Horn, 2016; Obia et al., 2016), however, our results confirmed positive effects of biochar on water holding capacity (Haider et al., 2017; Omondi et al., 2016) mainly in sandy soil. Water retention in capillary pores after *Effeco* application in sandy and loamy soils was higher by 22% and 4%, respectively, compared to control. The potential of biochar addition for improving physical soil properties was mainly observed in coarse-textured and low fertility soils (Laghari et al., 2015; Omondi et al., 2016). From the soil structure point of view, these results were not obviously confirmed. As shown in Table 4, in sandy soil, the *Effeco* treatment had no significant effects on contents of water-stable aggregates in comparison to control. Despite this fact, the better soil structure (higher values of MWDw by 6% and K by 30%) after *Effeco* treatment than no fertilized (control) plot was determined. In case of loamy soil, after

Table 3 Physical and hydro-physical properties

Sities	Treatment	ρd	P	ΘKN	ΘMCWC	ΘRWC	ΘAWC	AWS
Dolná Streda (sandy soil)	control	1.26 0.16	50.7 ±6.08	27.8 ±2.33	23.2 ±3.18	20.0 ±2.76	13.7 ±3.11	5.01 ±2.59
	Effeco	1.26 0.13	50.7 ±4.88	32.6 ±0.57	27.4 ±1.34	24.4 ±1.56	17.4 ±1.84	9.07 ±3.15
Veľké Uľany (loamy soil)	control	1.36 ±0.08	46.0 ±3.54	38.3 ±2.55	34.6 ±1.48	31.6 ±0.21	24.5 ±0.99	11.6 ±0.21
	Effeco	1.36 ±0.02	47.4 ±1.20	38.7 ±0.85	34.6 ±0.99	33.0 ±1.06	25.6 ±2.19	15.9 ±1.77

Pd – bulk density, P – total porosity, ΘKN – capillary absorption, ΘMCWC – maximum capillary water capacity, ΘRWC – retention water capacity, ΘAWC – available water capacity, AWS – available water supply

Table 4 Soil structure parameters

Sities	Treatment	WSA_{mi}	WSA_{ma}	WSA_{ma} 0.5–3	MWDw	K
Dolná Streda (sandy soil)	control	18.2 ±2.04	81.8 ±2.04	43.7 ±1.86	0.53 ±0.04	0.82 ±0.01
	Effeco	19.5 ±4.67	80.5 ±4.67	40.0 ±4.06	0.56 ±0.09	1.07 ±0.06
Veľké Uľany (loamy soil)	control	41.2 ±6.83	58.8 ±6.83	15.1 ±0.49	0.42 ±0.05	0.70±0.07
	Effeco	21.6 ±4.95	78.4 ±4.95	36.8 ±10.8	0.92 ±0.12	1.22±0.04

WSA_{mi} – water-stable micro-aggergates, WSA_{ma} – water-stable macro-aggregates, MWDw – mean weight diameter for wet sieving, K – structure coefficient

Effeco application the situation was significantly better in all evaluated soil structure parameters compared to the sandy soil. The *Effeco* reduced the content of WSAmi on one hand, and increased content of WSAma and WSAma 0.5–3 on the other. In the *Effeco* treatment the values of MWDw and K were almost one times higher than control.

Biochar application to low fertility soils may also substantially enhance crop production (Laghari et al., 2015; Zhang et al., 2017) what confirmed our results (Figure 2). Application of *Effeco* (blended biochar with farmyard manure) at rate of 20 t ha^{-1} significantly increased grain yield of sunflower in comparison to no fertilized plots in sandy soil. The same effect was observed in case of loamy soil. The total yields of peppers were higher by 16% in *Effeco* treatment (20 t ha^{-1}) than control plot. During vegetation season of peppers, a total of three harvests of peppers have been done. The changes in yields between individual harvests are shown in Figure 2 B. In comparison to control, the *Effeco* application in 1st, 2nd and 3th harvests increased yield of peppers by 6, 15 and 20%, respectively. Differences between sandy and loamy soils in total yields of crops were observed too. The total crop productions in loamy and sandy soils due to the *Effeco* application were higher by 16% and 82%, respectively, as compared to control plots in both soils. As presented Laghari et al. (2015) but also Van Zwieten et al. (2010) the increase in crop productivity from biochar application is most commonly observed in nutrient-poor and degraded soils.

All in all, we concluded that the biochar blended with farmyard manure improved soil properties, but its effects differ mainly on soil texture. The results of this short study also indicate that the application of biochar in combination with farmyard manure can be useful

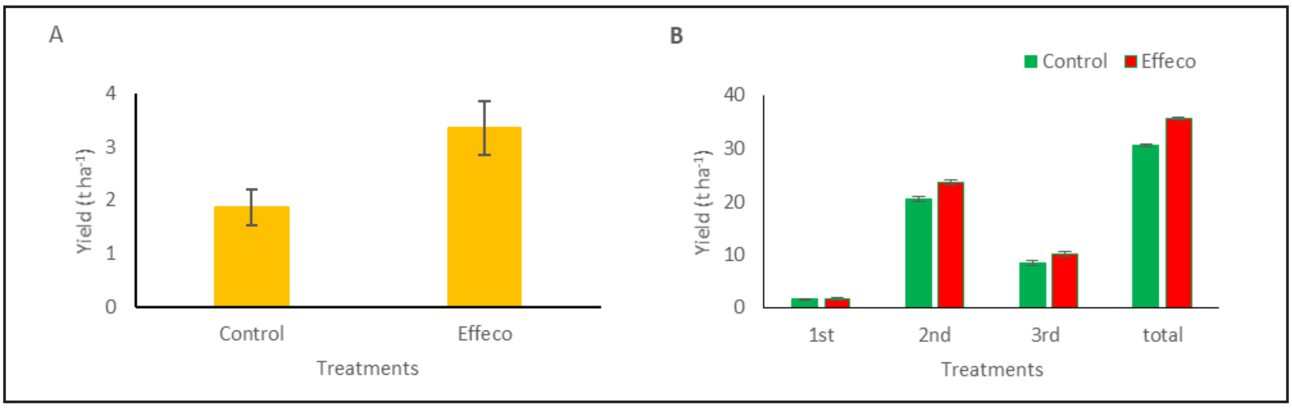

Figure 2 Yields of A) sunflower grains, and B) peppers fruits yield of pepper in 1st, 2nd, 3rd harvests

method for sustainable soil management in arable soils of Slovakia.

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