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DE GRUYTER OPEN

Estimating the size of plants by using two parallel views

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ABSTRACT

This paper presents a method of estimating the size of plants by using two parallel views of the scene, taken by a common digital camera. The approach relays on the principle of similar triangles with the following constraints: the resolution of the camera is known; the object is always in parallel to the camera sensor and the intermediate distance between the two concessive images is available. The approach was first calibrated and tested using one artificial object in a controlled environment. After that real examples were taken from agriculture, where we measured the distance and the size of a vine plant, apple and pear tree. By comparing the calculated values to measured values, we concluded that the average absolute error in distance was 0.11 m or around 3.7 %, and the absolute error in high was 0.09 m or 4.6 %.

Key words: digital image processing, size, digital camera, pixels, similar triangles

INTRODUCTION

In agriculture, as well as in other areas, it is important to know the size of the objects (Stajnko et al. 2004, Ehlert et al. 2009, Marcon et al. 2011) we are observing. But it is not always possible to measure them with conventional tools, such as tape measure, and special equipment, such as laser range finders, is rarely available. The only peace of equipment that is almost always with us is a mobile telephone with integrated digital camera. So why not measure distances with its help?

Such readings are also used when estimating sizes of the trees to calculate biomass (Ehlert et al. 2009), the size of leaf area to estimate the productivity of the crop (Marcon et al. 2011), the size of a fruit (Stajnko et al. 2004) to make a prognosis of the yield at the end of the harvest. The biomass is usually estimated by measuring the diameter of the tree trunk and assessing the height with the help of a trained eye. The usual way to measure leaf area involves destructive steps as the leafs have to be removed from the tree and put inside a scanning device. Marcon et al. on the other hand suggest less accurate but non-destructive computer vision supported approach. The last example, the size is measured with simple calliper measure. In combination with the number of the fruit on a tree is important to know when making a prognosis about the yield at the end of the harvest, to prepare enough storage, find potential buyers, set the right price, when fruit is only in the developing stages.

When making snapshots, the digital camera takes an image of the scene, transforms it to pixel or spatial space (Gonzales et al. 2008). There the metric information is lost. If we take an image at closer range, the object is bigger than if we take it further away. By taking a closer look at the images, we see that information is stored as a set of colour or grey pixels that describe the scene as well as the object. By taking a look at two such images of the same scene, taken at different distances, we see that the ratios are preserved (Videc 2015). This is a key property that can help to reconstruct metric information otherwise lost when capturing images.

This paper is organised as follows. In the second section mathematical background on how to construct a spatial to metric transformation is presented. Section three then evaluates the approach, by first using examples from a controlled environment in order to calculate the necessary parameters. The second part of section three then presents three examples from agriculture, used to estimate the distance

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to as well as an object. Section four concludes the paper by suggesting some possible future improvements.

MATERIALS AND METHODS

In order to estimate the metrics of an observed objects, at least two digital snapshots from two different distances of the scene must be captured. Of course, the position of the sensor must be in parallel to the object, with a known intermediate distance between the two capturing steps and known resolution of the camera. With these three constrains a pixel to metric space can be made when analysing the two images.
The pixel – metric space transformation is based on the use of similar triangles (Burger et al. 2009). The two triangles are similar if the ratio of the sides is the same and all their corresponding angles are equal. This relation is applied to transformation if one side of the triangle is considered as a distance between the object and the sensor and another as height of the object. By using two triangles from different images and comparing the known intermediate distances with the difference in pixel – height the rest of metric information can be computed (Videc 2015).
Fig. 1 depicts two similar triangles, with e1 as the distance between the object and the camera sensor and z_1 as the corresponding object height. On the next triangle, the distance and height are represented as e_2 and z_2. The Δz is used as a known intermediate distance between the two capturing steps.

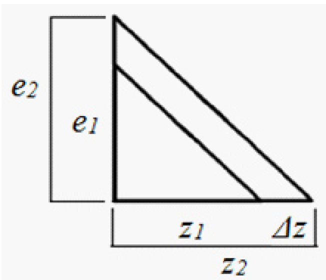

Fig. 1: Two similar triangles.

Distance between the camera and the object

Triangular symmetries from Fig. 1 can be summarized by Eq. (1) as:

$$\frac{e_1}{e_2} = \frac{z_1}{z_2} \quad (1)$$

If z_1 is an unknown distance, then z_2 can be written as a sum of z_1 and intermediate distance Δz, defined as shown by Eq. (2) and Eq. (3).

$$\frac{e_1}{e_2} = \frac{z_1}{z_1 + \Delta z} \quad (2)$$

$$z_1 = \frac{e_1 \cdot \Delta z}{e_2 - e_1} \quad (3)$$

The left side of Eq. (1) defines the ratio that can represent metric (real world) or pixel (spatial domain) distance. As the first is unknown at this point, the simple Euclidian pixel distance (Gonzales et al. 2008) is used for e_1 in e_2 measured from the image pare. Once z_1 is known, computing z_2 is a straightforward step, but the height of the object defined as Euclidian pixel distance requires an additional step of determining pixel-metric relation at a calculated distance where first the viewing angle of a camera must be known.

Viewing angle

The viewing angle or angle of view of a camera can defined with the help of the border points of the scene that lay at the opposite sites and are still visible on the image. The third point, the origin, is an imaginary point where the camera is located. All three define a viewing angle of an extent the scene is visible by the camera. The horizontal and vertical viewing angles of the cameras are usually different and depends on the lenses.

If the viewing angles of the camera are unknown, they can be measured and calculated. For instance, if an object of known size spans from one edge of the image to the other and the distance of the object is known, simple trigonometric equations can be used to compute it. Fig. 2 depicts the principle and Eq. (4) and Eq. (5) the mathematical background.

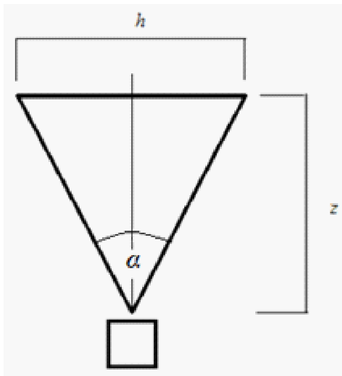

Fig. 2: Viewing angle of a camera.

If *h* from Fig. 2 is the width of an object and *z* the distance from the camera, then angle *α* can be computed as follows:

$$\frac{\alpha}{2} = arctg \frac{\frac{h}{2}}{z} \quad (4)$$

$$\alpha = 2 \cdot arctg \frac{h}{2 \cdot z} \quad (5)$$

Size of an object

If the distance between the camera and the object and the viewing angle are known, or computed as shown in subsections 2.1 and 2.2, respectively, then the size of an object can be computed using spatial – metric transformation. The spatial domain is build from pixels and their number depends on the resolution of the sensor that is used in the camera. Each pixel describes a part of the scene but the size in metric units depends on the distance.

So the first step is to calculate the height of the area that is summarized by one pixel at a given distance. The corresponding metric size of an area is defined by Eq. (6).

$$\Delta y = \frac{d}{r_y} \tag{6}$$

with d as Euclidian pixel distance between two farthest points on the image, r_y the resolution of an image on the y axis and Δy as the height of an area on the current axes in metric space.

In the second and final step the number of pixels or Euclidian pixel distance between two farthest points of an object (d) and the metric size of one pixel at a given distance (Δy) are used to compute the height of an object. This is done with a simple multiplication as shown by Eq. (7), with s_h as the height.

$$s_h = d \cdot \Delta y \tag{7}$$

The same approach can be used to calculate the width of an object, but using a different resolution constant that corresponds to the resolution of the sensor.

RESULTS

The results were captured in two steps. The first step was taken to calculate necessary parameters from subsection 2.2 and 2.3., with an additional goal to verify the approach on static object in indoor environment. Indoor environment is more suited to test the approach as it it not interfered by changing conditions that occur in nature, e.g. over and under illuminated scenes, moving caused by wind, etc. The second step used three real world scenarios, where digital images were used to estimate the height of an apple and peach tree as well as a vine plant.

Test phase – controlled environment

The test phase started with the search for camera viewing angle (α from Eq. (5)). For this step an object of known sizes was used, where its height was observed on different images taken from different distances; from 0.25 m to 2 m with 0.25 m step. For each distance an angle was calculated according to Eq. (5) and used to calculate an average value in order to minimize errors caused by human error. The results are summarized on Tab. 1.

Table 1: Viewing angle at different distances from the object.

Distance from the object [m]	Height of the object at given distance [m]	Viewing angle [°]
0.25	0.25	53.13
0.50	0.49	52.21
0.75	0.72	51.28
1.00	0.96	51.28
1.25	1.19	50.91
1.50	1.42	50.66
1.75	1.66	50.75
2.00	1.90	50.82
	Average:	51.38 ± 0.86

Next, a static object was placed in front of the camera at different distances, so it surface was in parallel with the camera. The object is depicted on Fig. 3.

Fig. 3: Static object positioned 0.25 m from the camera.

For the test phase the static object from Fig. 3 was positioned 0.25 m to 3 m from the camera in 0.25 m and 0.5 m from the camera. The step was longer in cases where the camera was positioned farther from the object, because the approach is less accurate at bigger distances due to the low number of pixels that describe it. For all images the height of the object was measured on images to get the Euclidian pixel distance from coordinates from the location of the farthest pixel on top and on the bottom. The measurements are shown in Tab. 2.

Next, according to Eq. (3) two consecutive Euclidian distances from Tab. 2 were used to calculate the distance between the object and the camera. The distances are summarized on Tab. 3 along with real, measured distances and an average error.

As shown by the results from Tab. 3 the accuracy of the calculated distance for the eight measurements falls within 0.22 m ± 0.25 m. In general, the greater the distance, the bigger the error. This is caused by the low number of pixels,

Table 2: The height of an object in pixels at different distances.

Distance from the object [m]	Euclidian pixel height [pixel]
0.25	2664
0.50	1344
0.75	896
1.00	672
1.25	552
1.50	461
2.00	361
2.50	289
3.00	241

Table 3: Calculated vs. measured distances.

Measured distance [m]	Calculated distance [m]	Absolute error [m]
0.50	0.48	0.02
0.75	0.71	0.04
1.00	0.90	0.10
1.25	1.75	0.50
1.50	1.53	0.03
2.00	2.55	0.55
2.50	2.01	0.49
3.00	3.02	0.02
	Average:	0.22 ± 0.25

that makes it hard to pinpoint the precise top / bottom pixel and is caused by human error.

The information about the distance, view angle and resolution of the camera makes it possible to calculate the size of objects that are parallel to the camera by measuring the Euclidian pixel distance. For the test object from Fig. 3 the results are summarized in Tab. 4.

The results from Tab. 4 prove that it is possible to use the approach from section 2 to calculate distance to the object as well as its height. The absolute distance error increases about 0.01 m for each 0.25 m of distance. On the other hand, this error does not effect the height measurements for the selected test distance (3 m) where the error is more or less constant.

Real world examples

For this subsection three different examples have been selected from agriculture, with an intent to calculate the height of the trees / plant and the distance from the camera, all compared to real, manually measured values. For these examples images of apple and pear tree as well as vine plant have been selected. In all three cases the Euclidian pixel distance was measured from the bottom to the top of the tree / plant for two consecutive images, taken with an intermediate distance of 0.5 m. Fig. 4 depicts all three selected examples, each with a measuring tape for reference.

In contrast to images from the previous subsection, in this case we have little influence on the capturing process. The objects are illuminated by the sun, with areas that can be completely white and other that are in the shade and are almost completely black. In addition, if there is some wind, the objects can move while taking the first and then the second image. Even if no wind is present it is not guaranteed that the object is in perfect alignment (in parallel) to the

Fig. 4: Three real world examples – pear tree (top), apple tree (middle), vine plant (bottom).

Table 4: Measured vs calculated distance and height of test object from Fig. 3.

Actual distance [m]	Calculated distance [m]	Abs. error - distance [m]	Actual height [m]	Calculated height [m]	Abs. Error - height [m]
0.25	0.24	0.01	0.25	0.24	0.01
0.50	0.48	0.02	0.25	0.24	0.01
0.75	0.72	0.03	0.25	0.24	0.01
1.00	0.96	0.04	0.25	0.24	0.01
1.25	1.20	0.05	0.25	0.25	0.00
1.50	1.44	0.06	0.25	0.25	0.00
2.00	1.92	0.08	0.25	0.26	0.01
2.50	2.41	0.09	0.25	0.26	0.01
3.00	2.89	0.11	0.25	0.26	0.01
	Average:	0.05 ± 0.03		Average:	0.008 ± 0.004

Table 5: Euclidian pixel distances for the tree selected examples.

	Measured distance [m]	Euclidian pixel distance [pixels]
Peach tree	3	2587
	3.5	2226
Apple tree	3	2642
	3.5	2259
Vine plant	2	2213
	2.5	1783

sensor, which means that the distance actually changes from one part of the object to the next. All this effect the results.

Tab. 5 shows the Euclidian pixel distances for all three selected examples, along with the measured distance to an object, for reference. The actual calculated distances and heights are summarized and compared to real distances in Tab. 6, 7 and 8, respectively.

As seen in Tabs. 6, 7 and 8, the calculated distance on average misses for 0.12 m for peach tree, 0.12 m for apple tree and 0.9 m for vine plant. The error of the calculated height is 0.15 m, 0.06 m and 0.07 m. In both cases the results have a bigger error rate for examples from the uncontrolled environment compared to the controlled from the previous subsection, which was expected. In order to evaluate the approach, all three examples are summarized by Tab. 9.

Table 6: Calculated vs measured distance and height for peach tree.

Measured distance [m]	Calculated distance [m]	Abs. error - distance [m]	Measured height [m]	Calculated height [m]	Abs. error – height [m]
3.00	2.89	0.11	2.35	2.49	0.14
3.50	3.37	0.13	2.35	2.50	0.15
	Average:	0.12 ± 0.01		Average:	0.15 ± 0.01

Table 7: Calculated vs measured distance and high for apple tree.

Measured distance [m]	Calculated distance [m]	Abs. error - distance [m]	Measured height [m]	Calculated height [m]	Abs. error – height [m]
3.00	2.89	0.11	2.60	2.54	0.06
3.50	3.37	0.13	2.60	2.54	0.06
	Average:	0.12 ± 0.014		Average:	0.06 ± 0.00

Table 8: Calculated vs measured distance and height for vine plant.

Measured distance [m]	Calculated distance [m]	Abs. error - distance [m]	Measured height [m]	Calculated height [m]	Abs. error – height [m]
2.00	1.92	0.08	1.35	1.41	0.06
2.50	2.41	0.09	1.35	1.43	0.08
	Average:	0.09 ± 0.007		Average:	0.07 ± 0.01

Table 9: The accuracy of the approach for all selected examples.

Tree / plant	Abs. error - distance [m]	Abs. error - height [m]
Pear	0.12 (3.6 %)	0.15 (6.3 %)
Apple	0.12 (3.6 %)	0.06 (2.3 %)
Vine	0.09 (3.8 %)	0.07 (5.2 %)
Average:	0.11 ± 0.02	0.09 ± 0.05

DISCUSSION

As described in section 2 and evaluated in section 3, the experiment from this paper showed it is possible to reconstruct metric information, which is otherwise lost when taking digital images of different objects. There are of course limitations to this approach; the object has to be in parallel to the camera, two (or more) accurately taken images with known intermediate distance have to be available and that information about the resolution of the images and the viewing angle of the lens is known. The last can of course be calculated, as presented in section 3.

The results from tab. 9 summarize the average absolute error in distance at 0.11 m or around 3.7 % and the absolute error in height 0.09 m or 4.6 %. This is more than enough for some agricultural applications, but not enough for others that require a higher degree of accuracy. In order to improve this, a better equipment could be used (e.q. camera with higher resolution) and more iterations (more snapshots from different views) could be made to minimize human error with the help of averaging.

Another possible approach to improve the results could be an introduction of image registration techniques. This way, by using corresponding pixels' pairs on consecutive images, it would be possible to select precisely the same corresponding pixels when measuring the Euclidian pixel distance and eliminate the error users make when selecting pixels on the opposite sides of an object.

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Ocenjevanje velikosti rastlin s pomočjo dveh vzporednih pogledov

IZVLEČEK

V delu je opisana metoda za ocenjevanje velikosti rastlin s pomočjo dveh vzporednih pogledov na isto sceno, zajetih s pomočjo digitalne kamere. Postopek temelji na principu podobnih trikotnikov z naslednjimi omejitvami: znani so podatki o ločljivosti uporabljene kamere; opazovani predmet je vedno vzporeden s senzorjem kamere; znan je podatek o vmesni razdalji med dvema korakoma zajema podatkov. Prvi korak v delu opisuje umerjanje metode ob pomoči umetnega predmeta v nadzorovanih pogojih. Drugi korak pa opisuje uporabo postopka na realnih primerih iz področja kmetijstva, kjer je bila ocenjena velikost vinske trte, jablane in hruške. Ob primerjavi dobljenih podatkov z izmerjenimi je bilo ugotovljena absolutna napaka v razdalji do predmetov v velikosti 0,11 m, oz. 3,7 % glede na celotno oddaljenost, absolutna napaka v velikosti v obsegu 0,09 m oz. 4,6 %.

Ključne besede: digitalno procesiranje slik, velikost, digitalna kamera, slikovni elementi, podobni trikotniki

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DE GRUYTER OPEN

Combining ability and breeding potential of oilseed rape advanced lines for some of important quantitative traits

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ABSTRACT

Information on estimates of combining ability of the promising lines of breeding material is important for evolving higher yielding varieties of oilseed rape (*Brassica napus* L.). An experiment was conducted to quantitatively examine the genetic parameters of phenological traits, plant height, pods on main raceme, pods per plant and seed yield for eight oilseed rape genotypes using a half-diallel crosses. The result of the diallel analysis revealed significant mean squares of general and specific combining abilities (GCA and SCA) for all studied traits, indicating the importance of additive and non-additive genetic effects for these traits. On the other hand estimation of high narrow-sense heritability estimates for days to flowering, duration of flowering and pods on main raceme, indicated the prime importance of additive genetic effects for these traits. L420 and L401 with significant negative GCA effects for days to flowering and days to maturity were suitable for yielding early maturity combinations. L41, Zafar and L22 with significant positive GCA effects for seed yield were superior parents for increasing seed yield. The crosses with significant positive SCA effects for seed yield had at least one parent with significant positive GCA effects for this trait. The crosses including L41×L22, L41×LF2, Zafar×L22 and Zafar×L420 with seed yield of 3421.7, 3400, 3348.1 and 3311.3 kg ha^{-1} could be promising for determination of superior recombinants for high seed yield coupled with other growth characters in advanced generations of segregation.

Key words: Additive genetic effects, Brassica napus, degree of dominance, combining ability, heritability, oilseed rape, yield.

INTRODUCTION

Oilseed rape or canola (*Brassica napus* L.) is an important oil seed crop of the world (Downey and Rimer 1993) and due to its autumn cultivation in Iran and therefore low need of irrigation, it plays a major role in catering edible oil. Hence it is necessary to develop the new ideotype varieties or hybrids of oilseed rape with high yield components. Seed yield of canola (B. napus) is a quantitative trait, which is largely influenced by the different environmental effects and thus in most of the cases it has low heritability (Habekotte 1997; Diepenbrock 2000; Rameeh 2010; Rameeh 2015; Amiri-Oghana et al. 2016). Exploitation of genetic variability in any crop species is considered to be critical for making further genetic improvement in seed yield as well as other economically important traits (Mahmood et al. 2003; Ishaq et al. 2016). Inter- and intraspecies crosses of Brassica are suitable way to make genetic variations and develop new varieties (Brandle and McVetty 1990; Enqvist and Becker 1991; Qian et al. 2007; Rameeh 2011). Heterosis is commercially exploited in oilseed rape and its potential use has been demonstrated in turnip rape (B. rapa L.) and Indian mustard (B. juncea L.) for most agronomic traits (Teklwold and Becker 2005; Zhang and Zhu 2006). In oilseed rape breeding program for hybrid and open pollinated varieties, general and specific combining ability effects (GCA and SCA) are important indicators of the

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potential of inbred lines in hybrid combinations. By using diallel crosses manners, and other genetic designs significant GCA and SCA effects of phenological traits, seed yield and other yield associated traits were reported in oilseed rape (Shen et al. 2002; Nassimi, et al. 2006b; Wang et al. 2007). Flowering is the most critical developmental stage influencing the yield of this crop (Faraj et al. 2008). The unset of flower initiation can have strong influence on flower, pod and seed number (Diepenbrock 2000; Downey and Rimer 1993; Yasari and Patwardhan 2006; Rameeh 2017). Habekotte (1997) used a sensitivity analysis within a crop growth model to study options for increasing seed yield in winter oilseed rape. The most promising crop type for high seed yield combined late maturity with early flowering (Downey and Rimer 1993). Early flowering in Brassica can provide adequate time for seed formation process and can certainly cause early maturity and higher yields, therefore negative combining ability and heterosis are desirable for days to flowering. In earlier studies on spring cultivars of oilseed rape (Huang et al. 2010; Rameeh 2011; Liton et al. 2017) were stressed the important role of GCA and SCA effects for days to flowering but due to high heritability estimates for this trait in these reports, the prime importance of GCA effects were emphasized. Likewise, studies with winter cultivars of this species (Amiri-Oghana et al. 2009; Sabaghnia et al. 2010) showed both additive and dominance gene effects to have a significant role in the inheritance of flowering time. Significant negative GCA and SCA effects were reported for days to flowering and plant height. Significant GCA and SCA effects were reported for seed yield in oilseed rape (Rameeh, 2010) and other Brassica species (Teklwold and Becker 2005; Singh et al. 2010).

The objectives of the present study were to identify general and specific combining abilities and narrow-sense heritability for phenological traits, plant height and seed yield in adapted spring oilseed rape advanced lines.

MATERIALS AND METHODS

Eight diverse spring oilseed rape (B. napus) advanced lines including L41, Zafar, L56, L31, L22, LF2, L420 and L401 were crossed in half-diallel scheme during 2010-11 (Table 1). Twenty eight F1s along with their parents were grown in a randomized complete block design with three replications at Biekol Agriculture Research Station, located in Neka, Iran (53˚, 13′ E longitude and 36˚ 43′ N latitude, 15 m above sea level) during winter 2011-12. Each plot consisted of four rows 5 m long and 40 cm apart. The distance between plants on each row was 5 cm resulting in approximately 300 plants per plot, which were sufficient for F1 genetic analysis. The soil was classified as a deep loam soil (Typic Xerofluents, USDA classification) which contained an average of 280 g clay kg^{-1}, 560 g silt kg^{-1}, 160 g sand kg^{-1}, and 22.4 g organic matter kg^{-1} with a pH of 7.3. Soil samples were found to have 45 kg ha^{-1} (mineral N in the upper 30-cm profile). Fertilizers were applied at the rates of 100: 50: 90 kg ha^{-1} of N: P: K, respectively. All the plant protection measures were adopted to make the crop free from insects. Seed yield (adjusted to kg ha^{-1}) was recorded based on three middle rows of each plot. The data were recorded on ten randomly selected plants of each entry of each replication for days to flowering, duration of flowering and days to maturity and plant height, pods on main raceme and pods per plant. The combining ability analysis was performed using mean values their F1 generations along with parents by using Griffing's method 2 with mixed B model (Griffing 1956). The statistical t-student test was applied to examine the effects of general combining ability (GCA) and specific combining ability (SCA). All the analyses were performed using MS-Excel and SAS soft wares (Zhang and Kang 1997).

RESULTS AND DISCUSSIONS

Analysis of variance

Significant mean squares of genotypes for number of days to flowering, duration of flowering, number of days to maturity, plant height, number of pods on main raceme, number of pods per plant and seed yield indicated a sufficient genetic variation among the parents and their crosses for the studied traits and thus enabling the genetic analysis (Table 2). Significant mean squares of general and specific combining ability (GCA and SCA) mean squares for all the traits are indicating the importance of additive and non-additive genetic effects for controlling the traits. However. Significant ratio of GCA to SCA mean square and high narrow-sense heritability estimates for days to flowering, duration of flowering and number of pods on main raceme implied that inheritances these traits are strongly associated with additive gene actions. Similarly, earlier researchers (Huang et al. 2010; Sabaghnia et al. 2010; Rameeh 2011) reported the important

Table 1: Name and origin of oilseed rape genotypes.

Type	Origin	Pedigree	Genotype	Genotype no.
Spring-open-pollinated	Iran-Mazandaran	RGS003 × 308	L41	1
Spring-open-pollinated	Iran-Mazandaran	19H × Sarigol	Zafar	2
Spring-open-pollinated	Iran-Mazandaran	RW × RGS003	L56	3
Spring-open-pollinated	Iran-Mazandaran	SLM046 × 308	L31	4
Spring-open-pollinated	Iran-Mazandaran	Zarfam × 401	L22	5
Spring-open-pollinated	Iran-Mazandaran	Zarfam × 308	LF2	6
Spring-open-pollinated	Iran-Mazandaran	RGS003 × 420	L420	7
Spring-open-pollinated	Iran-Mazandaran	RGS003 × 420	L401	8

Table 2: Half-diallel analysis for phenological traits, yield components and seed yield in eight oilseed rape genotypes.

	df	(M.S)						
		No. days to flowering	Duration of flowering	No. days to maturity	Plant height	No. pods on main raceme	No. pods per plant	Seed yield
Rep	2	16.0 ns	10.5 ns	4.7 ns	275.3*	79.9 ns	78.7ns	1571000**
Crosses	35	913.3**	648.0**	424.8**	593.7**	359.4**	1417.3**	387481**
GCA	7	3979.2**	2581.1**	798.8**	1526.9**	1181.7**	1610.2**	975122**
SCA	28	146.8**	164.7**	331.3**	360.4**	153.9**	1369.1**	240570**
Error	70	7.6	13.5	2.7	151.1	27.5	103.8	64580
MS(GCA)/MS(SCA)		27.11**	15.67**	2.41*	4.24**	7.68**	1.18 ns	1.18 ns
.h^2N		0.84	0.76	0.32	0.43	0.60	0.32	0.32

ns,* and **: Not- significant, significant at 5% and 1% levels of probability, respectively.

role of additive genetic effects for phenological traits and also the importance of non-additive effects for seed yield.

General combining ability and performance means of the parents

Considering the negative GCA values for days to flowering, the genotypes L420 and L401 could be distinguished for its significantly different value. These parents had also a significant positive GCA effects for duration of flowering (Table 3). The mean values of the number days to flowering varied from 83 to 113 days in L420 and L22, respectively and the parents L420 and L401 with 83 and 84 days exhibited low mean values of this trait (Table 4). Significant positive correlation GCA effects of parents for the number of days to flowering with days to maturity and pods on main raceme (Table 5), concluded that, GCA effect of days to flowering is good criterion for improving GCA effect of days to maturity and pods on main raceme. Due to important positive effect of duration of flowering on pod formation, L420 and L401 with high mean values and also significant positive GCA effect of this trait will be preferred. Significant negative correlation was detected between GCA effect of parents for days to flowering and duration of flowering The parents with significant negative GCA effects for the number of days to flowering had also significant negative GCA effects of the number of days to maturity. Having studied several quantitative and qualitative traits of oilseed rape lines, cultivars and hybrids, Marjanović – Jeromela et al. (2007) reported different values of plant height. The parents L31, LF2 and L401 with significant negative GCA effects for plant height were suitable for the decreasing this trait and their mean performances for this trait were 149, 153 and 155 cm, respectively. The number of pods on main raceme ranged from 42 in L401 and 56 in L41 and Zafar and these parents had also significant positive GCA effects for this trait. The number of pods per plant as prime important yield component had considerable effect on seed yield, therefore the parents L41, Zafar and L22 with significant positive GCA effects of this trait were preferred. Among the parents, L41, Zafar and L22 had significant GCA effect for seed yield and most of parents with positive GCA effects of the number of pods per plant had positive GCA effect of this trait. This finding is in agreement with the results of Rameeh (2010) and Sabaghnia et al. (2009) reports for significant positive GCA effects of parents for seed yield in oilseed rape.

Table 3: General combining ability effects of half-diallel analysis for phenological traits, yield components and seed yield in eight oilseed rape genotypes.

Parents	No. days to flowering	Duration of flowering	No. days to maturity	Plant height	No. pods on main raceme	No. pods per plant	Seed yield
L41	5.4**	-4.53**	2.25**	6.34**	5.50**	10.72**	184.68**
Zafar	5.1**	-3.93**	1.28**	9.15**	5.93**	9.31**	147.26**
L56	8.6**	-6.33**	1.82**	4.51*	5.12**	-8.78**	27.47ns
L31	4.0**	-6.00**	5.62**	-10.37**	-1.20	0.33ns	-291.27**
L22	9.9**	-5.73**	5.32**	-1.50	5.64**	3.59*	187.30**
LF2	3.9**	-3.27**	-1.92**	-5.97**	-6.95**	-2.85ns	9.99ns
L420	-18.8**	13.77**	-4.98**	4.58*	-5.59**	-6.17**	-44.01ns
L401	-17.9**	16.03**	-9.38**	-6.76**	-8.44**	-6.16**	-221.41**

ns,* and **: Not -significant, significant at 5% and 1% levels of probability, respectively.

Table 4: Mean values of half-diallel analysis for phenological traits, yield components and seed yield in eight oilseed rape genotypes.

Parents	No. days to flowering	Duration of flowering	No. days to maturity	Plant height (cm)	No. pods on main raceme	No. pods per plant	Seed yield (kg ha^{-1})
L41	107	57	208	168	56	140	2929.2
Zafar	107	58	207	170	56	137	2925.0
L56	110	55	207	164	54	130	2866.7
L31	106	55	212	149	48	119	2187.5
L22	113	55	212	158	55	158	3120.8
LF2	106	58	205	153	44	112	2737.5
L420	83	76	199	164	44	130	2612.5
L401	84	78	198	155	42	94	2083.3
LSD(α=0.05)	4.41	5.88	2.63	19.67	8.39	16.64	414.99
LSD(α=0.01)	5.78	7.71	3.45	25.79	11.00	22.13	551.93

Table 5: Pearsons correlation coefficient among GCA effects of parents and SCA effects of crosses for phenological traits, yield components and seed yield.

GCA (n=8)							
	No. days to flowering	Duration of flowering	No. days to maturity	Plant height	No. pods on main raceme	No. pods per plant	Seed yield
No. days to flowering	1						
Duration of flowering	-0.98**	1					
No. days to maturity	0.87**	-0.91**	1				
Plant height	0.12 ^{ns}	0.11 ^{ns}	0.07 ^{ns}	1			
No. pods on main raceme	0.75*	-0.72*	0.76*	0.60 ^{ns}	1		
No. pods per plant	0.48 ^{ns}	-0.48	0.51 ^{ns}	0.37	0.61 ^{ns}	1	
Seed yield	0.51 ^{ns}	-0.43	0.35 ^{ns}	0.73*	0.69 ^{ns}	0.57 ^{ns}	1
SCA (n=28)							
	No. days to flowering	Duration of flowering	No. days to maturity	Plant height	No. pods on main raceme	No. pods per plant	Seed yield
No. days to flowering	1						
Duration of flowering	-0.75**	1					
No. days to maturity	0.59**	0.61**	1				
Plant height	-0.09 ^{ns}	0.38*	0.04 ^{ns}	1			
No. pods on main raceme	-0.15 ^{ns}	0.33 ^{ns}	-0.07 ^{ns}	0.71**	1		
No. pods per plant	-0.14 ^{ns}	0.08 ^{ns}	0.02 ^{ns}	0.56**	0.65**	1	
Seed yield	-0.18 ^{ns}	0.05 ^{ns}	-0.04 ^{ns}	0.27 ^{ns}	0.35 ^{ns}	0.66**	1

* and **: Significant at 5% and 1% levels of probability, respectively.

Specific combining ability effects of the crosses

Out of 28 crosses, 13crosses had significant negative SCA effects of No. days days to flowering (Table6). Most of the crosses with significant negative SCA effect of No. days days to flowering had at least one parent with significant negative GCA effect for this trait. The crosses L420×L401, Zafar×L401, L41×L420, L56×L420, L31×L420 and L31×L401 with 66, 78, 84, 84, 83 and 81 days to flowering had low mean values of this trait (Table 7), were also good combinations. SCA effects of combinations for No. days to flowering was significant negative correlated to No. days to maturity, therefore SCA effect of No. days to flowering is good selection criterion for improving No. days to maturity. The crosses L41×L401, Zafar×L401, L56×L401, L31× L420, L22

× L420, LF2×L401 and L420×L401 with 79, 90, 78, 77, 76, 78 and 88 days of flowering had also significant positive SCA effects for this trait were considered as merit combinations. The crossesincluding L41×L420, Zafar×L420, Zafar×L401, L56×L420 and LF2×L401 with low mean values of days to maturity (188, 188, 184, 189 and 178 days, respectively) also high significant negative SCA effect of No. days to maturity (-16.03, -14.39, -14.66, -13.93 and -17.13, respectively) were suitable combinations for improving this trait. The genotypes with low mean values of plant height will have high tolerance to lodging, therefore the crosses including L41×L31, L56×L401 and LF2×L401 with significant negative SCA effect for plant height were valuable combinations for improving this trait. The knowledge of yield components helps breeders to select genotypes and methods suitable for achieving specific objectives in rapeseed breeding. It is also desirable to know other traits and the genetic rules regulating their inheritance since these traits are associated with yield components (Teklwold and Becker, 2005; Zhang and Zhu, 2006). High positive SCA values of No of pods on main raceme, which were significantly different from the other values, were registered in L41×LF2, L41×L420, Zafar×L401 and L31×L401. Significant negative correlation exhibited between SCA effects of combinations for No. of pods per plant and seed yield (Table 5), therefore SCA effect of combinations for the No. of pods per plant is good selection criterion for improving seed yield of combinations. Seed yield per plant is a highly variable trait, mainly because it depends on all other yield components. This complex trait is controlled by a large number of genes, whose expression is strongly affected by environmental conditions. In this study, different values of seed yield per plant were registered, from 2196.7 kg ha^{-1} in L56×L420 to 3400 kg ha^{-1}L41×LF2. The crosses L41×LF2, Zafar×L22, Zafar×L420 and L56 ×L22 with seed yield of 3400, 3348.1, 3311.3 and 3278.4 kg ha^{-1} were high potential combinations for seed yield. Hybrid combinations L41×LF2, Zafar×L420, L56×L22, L31×L401and LF2×L420 had highest SCA values, which were highly significant in relation to the SCA values of the other hybrid combinations. The crosses L41 × LF2, Zafar × L420, L56 ×L22, L31×L401 and LF2×L420 with significant positive SCA effects for seed yield were suitable combinations for improving this trait. It had been observed before that crossing a parent with high GCA values with a parent with low GCA values may produce a hybrid combination with high SCA values (Marjanović – Jeromela, et al., 2007).

Table 6: Mean values of half-diallel analysis for phenological traits, yield components and seed yield of eight oilseed rape genotypes.

Crosses	No. days to flowering	Duration of flowering	No. days to maturity	Plant height (cm)	No. pods on main raceme	No. pods per plant	Seed yield (kg ha^{-1})
L41 × Zafar	114	46	214	167.2	55	145	2839.2
L41 ×L56	118	49	214	173.7	62	136	3180.8
L41 ×L31	111	47	213	141.1	48	138	2315.9
L41 ×L22	113	53	211	164.3	63	156	3412.7
L41 ×LF2	104	65	206	169.5	62	172	3400.0
L41 ×L420	84	73	188	188.1	57	162	3000.0
L41 ×L401	98	79	207	173.7	47	154	2908.3
Zafar × L56	121	46	213	183.6	62	139	3031.1
Zafar × L31	113	49	217	155.8	49	129	2673.8
Zafar × L22	113	56	212	162.4	62	145	3348.1
Zafar × LF2	114	57	211	165.6	54	146	2512.5
Zafar × L420	84	74	188	163.9	45	173	3311.3
Zafar × L401	78	90	184	192.5	65	177	2975.0
L56 × L31	119	48	215	156.2	56	139	2302.5
L56 × L22	117	51	212	171.4	61	148	3287.4
L56 × LF2	114	51	206	153.6	48	124	2915.4
L56 × L420	84	74	189	151.8	46	105	2695.8
L56 × L401	86	78	194	147.7	36	96	2196.7
L31 × L22	112	56	215	150.9	52	145	2626.9
L31 × LF2	112	49	215	149.8	44	172	2511.7
L31 × L420	83	77	189	147.6	34	112	2537.5
L31 × L401	81	68	214	144.5	47	166	2812.5
L22 × LF2	117	49	217	147.2	37	112	2727.7
L22 × L420	98	76	202	166.2	55	106	2413.7
L22 × L401	117	49	214	144.3	47	143	2883.3
LF2 × L420	94	66	207	151.9	39	131	3079.6
LF2 × L401	83	78	178	134.4	38	125	2546.4
L420 × L401	66	88	213	160.7	34	125	2365.4
LSD(α=0.05)	4.41	5.88	2.63	19.67	8.39	16.64	414.99
LSD(α=0.01)	5.78	7.71	3.45	25.79	11.00	22.13	551.93

CONCLUSIONS

In general pods Significant ratio of GCA to SCA mean square and high narrow-sense heritability estimates for days to flowering, duration of flowering and number of pods on main raceme implied that inheritances these traits are strongly associated with additive gene actions. Among the parents, L41, Zafar and L22 with significant positive GCA effects for seed yield were superior combiners for improving this trait. The crosses with significant positive SCA effects for seed yield had at least one parent with significant positive GCA effects for this trait. The crosses including L41×L22, L41×LF2, Zafar×L22 and Zafar×L420 with seed yield of 3421.7, 3400, 3348.1 and 3311.3 kg ha^{-1} could be promising for determination of superior recombinants for high seed yield coupled with other growth characters in advanced generations of segregation.

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Table 7: Specific combining ability effects of half-diallel analysis for phenological traits, yield components and seed yield in eight oilseed rape genotypes.

Crosses	No. days to flowering	Duration of flowering	No. days to maturity	Plant height	No. pods on main raceme	No. pods per plant	Seed yield
L41 × Zafar	1.36	-7.35**	4.04**	-8.70	-6.25**	-12.08*	-278.1*
L41 ×L56	2.16	-1.95	3.84**	2.41	1.48	-3.36ns	183.2 ns
L41 ×L31	-0.54	-4.28*	-1.29	-15.28*	-6.36**	-9.84ns	-362.9**
L41 ×L22	-4.47**	1.79	-2.99**	-0.98	2.30	4.51 ns	255.3 ns
L41 ×LF2	-7.11**	11.65**	-0.43	8.69	13.43**	26.51**	420.0**
L41 ×L420	-5.17**	2.62	-16.03**	16.74*	7.90**	20.23**	74.0 ns
L41 ×L401	8.33**	5.69**	8.04**	13.71*	0.55	11.79*	159.7 ns
Zafar × L56	5.46**	-5.21**	3.47**	9.56	1.45	0.84 ns	71.0 ns
Zafar × L31	2.09	-2.88	3.34**	-3.43	-5.29*	-17.80**	32.4 ns
Zafar × L22	-3.84**	3.85*	-1.36	-5.63	0.81	-4.69 ns	228.1 ns
Zafar × LF2	2.86*	3.05	4.87**	2.01	5.83*	2.35 ns	-430.1**
Zafar × L420	-4.87**	2.69	-14.39**	-10.21	-5.13*	32.37**	422.7**
Zafar × L401	-11.71**	16.42**	-14.66**	29.70**	17.69**	36.22**	263.8 ns
L56 × L31	3.89**	-0.81	1.14	1.65	2.88	10.22 ns	-219.1 ns
L56 × L22	-3.71**	1.92	-1.89	7.94	0.24	16.13**	287.3*
L56 × LF2	-0.67	-0.88	0.01	-5.39	0.30	-1.39 ns	92.6 ns
L56 × L420	-8.41**	4.75*	-13.93**	-17.67**	-3.43	-17.27**	-73.0 ns
L56 × L401	-7.24**	7.15**	-5.19**	-10.50	-10.71**	-26.12**	-394.8**
L31 × L22	-4.41**	5.92**	-2.36**	2.36	-1.70	3.96 ns	-54.5 ns
L31 × LF2	2.29	-3.21	4.54**	5.76	2.52	36.93**	7.6 ns
L31 × L420	-4.11**	7.75**	-18.06**	-7.05	-9.00**	-19.75**	87.4 ns
L31 × L401	-6.94**	-3.51	11.34**	1.22	6.75**	34.30**	539.8**
L22 × LF2	1.03	-3.81*	6.84**	-5.78	-11.75**	-26.29**	-255.0 ns
L22 × L420	4.63**	6.82**	-4.76**	2.71	5.03*	-28.14**	-515.0**
L22 × L401	22.79**	-22.45**	11.97**	-7.85	-0.12	8.11 ns	132.1 ns
LF2 × L420	6.66**	-6.31**	7.14**	-7.15	1.95	3.20 ns	328.2*
LF2 × L401	-5.17**	3.42	-17.13**	-13.32*	3.77	-2.68 ns	-27.5 ns
L420 × L401	0.76	-3.61	20.94**	2.50	-1.29	-0.23 ns	-154.6 ns

ns,* and **: Not- significant, significant at 5% and 1% levels of probability, respectively.

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Potencial razmnoževanja selekcioniranih linij oljne repice na nekatere pomembne kvantitativne lastnosti

IZVLEČEK

Informacije o ocenah združevanja lastnosti potencialnih linij plemenskega materiala so pomembne za razvoj boljših sort oljne repice (Brassica napus L.). S preiskavo smo proučevali kvantitativne genetske lastnosti fenološko pomembnih lastnosti (višina rastlin, št. strokov na socvetju/rastlino in pridelek semena za osem genotipov polovično dialelnih križancev oljne repice. Rezultat dielektrične analize je pokazal značilne srednje kvadratne odklone splošnih (SPLO) in specifičnih (SPEC) lastnosti za vse proučevane lastnosti. To kaže na pomembnost delovanja aditivnega in ne-aditivnega učinka na proučevane lastnosti.

V ozkem smislu je pokazala heritabiliteta za število dni do cvetenja, trajanja cvetenja in strokov na glavnem socvetju, da je glavni aditivni učinek na proučevane lastnosti. Liniji L420 in L401 sta z značilnimi negativnimi učinki na SPLO lastnosti za število dni do cvetenja, starosti ko rastline doseže zrelost sta bili primerni za tvorjenje zgodaj zrelih kombinacij oljne repice.

Linije L41, Zafar in L22 z značilno pozitivnimi učinki na SPLO lastnosti za količino semena so bili primerni za starševsko linijo za povečanje količine semen. Križanci s statistično značilnim pozitivnim SPEC lastnostmi imajo vsaj enega starša z značilno pozitivno lastnostjo za to SPLO lastnost. Možne najboljše kombinacije križanj so se pokazali križanci L41×L22, L41×LF2, Zafar×L22 in Zafar×L420, ki so imeli pridelek semena 3421,7, 3400, 3348,1 in 3311,3 kg ha-1.

Omenjeni križanci so najboljši rekombinanti za visok pridelek semena in druge rastne lastnosti v naslednjih generacijah segregacijske selekcije.

Ključne besede: oljna repica, križanci, aditivni učinki, stopnja dominantnosti, sposobnost združevanja lastnosti, heritabiliteta, pridelek

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Blood profile and gut microbial load of broilers fed siam weed (*Chromolaena odorata*) leaf meal in their diets

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ABSTRACT

The responses of 96 day old broilers fed *Chromolaena odorata* leaf meal (COLM) on blood profile and gut microbial load were studied for 49 days. The birds were randomly assigned to four treatment groups and replicated three times in a completely randomized design. The birds were fed four experimental diets formulated at 0%, 2%, 4% and 6% for the starter phase and 0%, 4%, 8% and 12% of COLM at the finisher phase for T1, T2, T3 and T4 respectively. All the haematological parameters were similar (P>0.05) across the treatment groups except MCH and WBC which were significantly (P< 0.05) higher and better in the treatment groups than the control group. The results on serum chemistry showed significant (P<0.05) difference for total protein, albumin, creatinine and cholesterol across the treatment groups. Cholesterol was significantly (P<0.05) reduced with increasing levels of COLM. Results showed that *coliforms* and *E.coli* were consistently higher (P<0.05) among the T1 birds than those fed T2, T3 and T4. It was concluded that, COLM enhanced adequate haematocrit and immune status, hypoglycaemic ability, suppresses the growth of gut pathogenic microorganisms and enhanced the growth of beneficial microorganism in broilers.

Key words: broilers, COLM, leaf meal, gut health, blood profile, medicinal plant

INTRODUCTION

Broiler production is one of the most important sources of animal protein all over the world today. This could be attributed to the high rate of feed utilization efficiency and fast growth rate of the birds (Jiwuba et al. 2016a). Poultry production has been challenged by poor physiological functions, microbial infections and unavailability of good quality feeds on a sustainable basis at stable prices. The escalating cost of conventional feedstuff has stimulated research into alternative feedstuffs with the aim of reducing the cost of poultry production, maintaining the feed quality, increasing the production rate and improving the health status of the birds.

Several alternative feedstuffs have been successively used as possible replacement either partially or wholly of conventional feedstuff. However, the alternative feed of interest in this study is the Siam weed (*Chromolaena odorata*). *C. odorata* belongs to the family *Asteraceae* and known by diverse names such as "Obiarakara", "Awolowo", "Diochie", "Independence", "ewe Akintola" triffid weed and bitter bush in most parts of Nigeria. It is an important perennial Tropical "obnoxious weed" because of its ability to spread to new lands within a short period of time. Earlier reports (Akinmoladun et al. 2007, Igboh 2009, Ikewuchi and Ikewuchi 2011) on *C. odorata* have noted its multipurpose medicinal properties. Dried leaves of *C. odorata* have also been reported (Moses et al. 2010) to be used as mosquito repellant, antimicrobial agent against *Bacillus cereus* and antifungal agent against *Aspergillus*

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niger. The juice from the fresh leaves when applied to cuts or wounds may help reduce bleeding considerably. Other traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic (Vital and Windell 2009). The offensive odour of the fresh leaves could be given off when the plant is disturbed by mere touching or bruising of the leaves and this odour is greatly reduced when the leaves are sun dried for subsequent milling (Fasuyi et al. 2005). Not much has also been written on the biochemical, haematological and gut microbial population implications of siam weed leaf meal on poultry diets.

Sajise et al. (1974) reported that the importance of *C. odorata* as a cause of poisoning and death in livestock is due to its conversion either in the foodstuff or within the alimentary canal into nitrite. Nitrite converts haemoglobin of the blood to methaemoglobin which is unable to act as an oxygen carrier and this might possibly lead to the death of the animals through tissue anoxia (Fasuyi et al. 2005). Another biochemical implication with the utilization of *C. odorata* leaf may be specifically when used in combination with cassava by-products, is the affinity which methaemoglobin has for cyanide leading to the formation of cyanmethaemoglobin which is another oxygen depleting compound in the blood (Fasuyi et al. 2005). Although nutritive research on *C. odorata* leaves and its use in broiler diets remain scanty, it has been however reported to be relatively high in crude protein, dry matter, vitamins and minerals (Apori et al. 2000). This study was therefore designed to determine the serum biochemical, haematological profile and gut microbial population of broilers fed *C. odorata* leaf meal in their diets.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the poultry Unit of Federal College of Agriculture, Ishiagu, Ivo Local Government Area, Ebonyi state, Nigeria. The College is located at about three kilometres (3km) away from Ishiagu main town.

Processing of COLM. The fresh leaves of green succulent unflowered *Chromolaena odorata* were harvested with the College environment. The leaves were later sundried for five days to obtain about 10% moisture. They were thereafter milled by passing through 1mm hammer mill, before been used to formulate the experimental diets.

Experimental Diets: Four experimental diets designated as T1, T2, T3, and T4 where formulated to contain 0, 2, 4, 6, levels of COLM at starter phase and 0, 4, 8, 12, levels at finisher phase. The compositions of the experimental diets are given in Table 1 and Table 2.

Experimental Animals, Housing and Management: Ninety six (96) day old Abor acre chicks were sourced from a Chi farms® in Ibadan, Oyo state, Nigeria. They were randomly assigned to four (4) experimental treatment groups, each replicated three (3) times with eight (8) birds constituting a replicate. The birds were assigned the four experimental diets in a Completely Randomized Design (CRD) and fed the experimental diets for 49 days. Each replicate was housed in

Table 1: Percentage composition of the experimental diets for starter birds.

Ingredients	T1	T2	T3	T4
Maize	52.00	52.00	51.00	51.00
Wheat offal	7.00	6.00	5.00	3.00
Soybean meal	35.00	34.00	34.00	32.00
Chromolaena odorata leaf meal	0.00	2.00	4.00	6.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	2.50	2.50	2.50	2.50
Limestone	0.50	0.50	0.50	0.50
Vitamin premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
TOTAL	**100**	**100**	**100**	**100**
Calculated values				
Crude protein %	22.91	22.66	22.4	22.4
Metabolisable energy kcal/kg)	2907.22	2902.8	2898.45	2891.31

Table 2: Percentage composition of the experimental diets for finisher birds.

Ingredients	T1	T2	T3	T4
Maize	55.00	53.00	51.00	55.00
Wheat offal	10.00	9.00	8.00	3.00
Soya bean meal	28.55	27.55	26.55	23.55
Chromolaena odorata leaf meal	0.00	4.00	8.00	12.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00
Vitamin premix	0.35	0.35	0.35	0.35
Salt	0.25	0.25	0.25	0.25
Lysine	0.35	0.35	0.35	0.35
Methionine	0.50	0.50	0.50	0.50
TOTAL	**100**	**100**	**100**	**100**
Calculated values				
Crude protein %	20.87	20.02	20.23	19.6
Metabolisable energy kcal/kg)	2931.68	2920.18	2911.65	2901.30

a concrete floor covered with wood shavings as litter material. Before the arrival of the day old chicks, the brooding house was washed and disinfected and allowed to dry. The brooder house was pre-heated for 6-12hrs to enable it reach the normal temperature (32°C) required by day old chicks. Fresh feed and water was provided *ad libitum*. Heat and light was provided for the first 14 days using stoves and electric bulbs. The birds were vaccinated against infectious bursal (Gumboro) disease at days 10 and 24 respectively, while the NDV - Lasota vaccine was given on day 1, 14 and 28 to protect against New Castle Disease.

Blood profile analysis: Blood samples (5ml) were drawn from one bird per replicate on the last day of the study. The birds were bled through the marginal wing vein. The samples were separated into two lots and used for biochemical and haematological studies. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentrations (MCHC) were calculated.

Microbial analysis: From the eviscerated organs i.e (1 birds/replicate), a section of the caecum and crop were cut and used for microbial analysis. Approximately 1g of the caecal and crop content wastes mixed with 9ml of pre-reduced sterile dilution blank solution (Bryant and Burkey 1953), and homogenized for 3 minutes individually. From the initial 10^1 dilution, 6-fold serial dilutions were made in sterile pre-reduced dilution blank solution for total coliforms, lactic acid bacteria (LAB's) and total microbes. For each dilution, 1ml was inoculated in medium which include MRS agar for LAB's, MacConkay agar for coliforms and Nutrient agar for total microbes. The serially diluted samples were inoculated into growth media for *coliform, Lactobacillus* and *Escherichia coli*. *Coliform* bacteria were enumerated on McConkey agar (Merck kgaa b4271). *Escherichia coli* were enumerated on fluorescent (VRB agar Merck Kgaa) while *Lactobacillus* was enumerated on M. R.S. (de Man Rogosa and Sharp) agar. All the inoculated plates were then incubated at 37-40°C between 24-48 hours. Total number of bacterial colonies was counted at the end of each incubation period using improved bacteria colony counter (Jin et al. 1998).

Chemical Analysis: Chemical analysis (dry matter, crude protein, crude fibre, ash, and ether extract) of different experimental diets and that of the test ingredient was carried out at the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Animal Nutrition Laboratory, using the methods of (AOAC 2000). The nitrogen free extract was derived by subtracting the sum of other components (crude protein, ether extract, ash, crude fibre) from 100 on dry matter basis.

Statistical Analysis: The results were analyzed using the SPSS Window 17.0. One - way analysis of variance ANOVA) was employed to determine the means and standard error. Differences between treatments means was estimated by Duncan Multiple Range Test.

RESULTS

Chemical analysis

The chemical composition of the experimental diets and *Chromolaena odorata* leaf meal are presented in Table 3. The experimental diets and COLM were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), ash, ether extract (EE) and nitrogen free extract (NFE). The DM contents of the experimental diets (starter and finisher diets) failed to follow a regular trend. The DM for the starter diets ranged between 90.71 and 92.75% while that of the finisher diets varied from 92.71-94.40%. The CP content of the starter diets decreased with increased levels of COLM with the highest value (22.36%) recorded for T1 with a corresponding lowest value of 20.52% recorded for T4. However, the CP for the finisher diets ranged between 19.38 and 19.68%. The CF of the starter diets maintained a particular trend, decreasing with an increasing level of COLM till T3. The value for T4 is numerically higher for T2 and T3 diets. The CF of the finisher diets ranged from 4.00-4.94%, and did not follow a specific trend. The ash values for the starter diets ranged between 12.53 and 13.30% while that of the finisher diets ranged from 15.84-19.19% and all did not follow a specific trend across the treatment groups. The EE content for the starter diets ranged between 2.15 and 5.00% while that of the finisher diets ranged from 2.45-5.45% and all failed to follow a specific trend across the treatments. The NFE contents of

Table 3: Chemical compositions of diets.

	Starter diets				Finisher diets				
Parameter	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T3**	**COLM**
Dry matter	90.71	92.30	91.26	92.75	94.40	93.46	92.71	94.15	95.70
CP	22.36	21.68	20.95	20.52	19.38	19.68	19.68	19.68	20.52
Crude fibre	3.63	3.04	3.30	3.54	4.30	4.00	4.94	4.00	5.58
Ash	12.60	12.53	13.30	12.69	15.84	18.70	19.19	19.03	9.29
Ether extract	2.15	3.85	5.00	4.50	5.25	5.45	2.45	3.85	6.10
NFE	46.97	49.20	47.71	50.04	49.63	45.79	49.45	48.59	53.94

CP = Crude protein; NFE = Nitrogen free extract; COLM = *Chromolaena odorata* leaf meal

Table 4: Haematology of broiler birds fed Chromolaena odorata leaf meal in their diets.

parameter	T1	T2	T3	T4	SEM
Packed Cell Volume (%)	38.97	44.57	33.10	41.20	1.45
Red Blood Cell (X10$^{12/l}$)	2.98	3.22	2.95	2.94	0.23
Haemoglobin (g/dl)	11.30	12.77	11.93	11.57	0.40
MCHC (%)	28.33	28.00	28.17	27.87	0.49
MCH (pg)	26.13^{b}	29.53^{a}	27.70^{ab}	26.87^{b}	0.51
Mean Corpuscular Volume (f)	90.03	91.47	93.41	96.74	1.90
White Blood (Cell X10$^{12/l}$)	11.24^{b}	16.80^{a}	18.84^{a}	19.35^{a}	0.45

$^{a, b}$ Means within the same row with different superscripts are significantly different P <0.05
MCHC = Mean corpuscular heamoglobin concentration: MCH = Mean corpuscular haemoglobin

the experimental diets (starter and finisher diets) also did not follow a regular trend and ranged from 46.97-50.04% and 45.79-49.63% for starter and finisher diets respectively. The COLM showed a high DM value of 95.70% with a higher CP value of 20.52% for leaf meals. It also showed a low CF value of 5.5% with a corresponding high ash content of 9.29%. NFE and EE content of 53.94 and 6.10% respectively were also recorded for the COLM.

Haematological analysis

The haematological parameters of broiler birds fed *Chromolaena odorata* leaf meal in their diets are presented in Table 4. The packed cell volume (PCV) did not follow a particular trend, but was numerically higher in T2, T4, T1 and T3 in that order. The PCV values were not significantly (P > 0.05) affected across the treatment groups. The red blood cell, haemoglobin, mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) values also followed the same trend as PCV showing no significant difference (P>0.05) across the treatments. The mean corpuscular haemoglobin (MCH) was significantly (P<0.05) influenced. However, T1 was not significantly (P>0.05) different from T3 and T4, but was significantly (P<0.05) different from T2. The white blood cell (WBC) was also significantly (P<0.05) influenced by COLM in the diets of the broilers. The WBC values followed a particular trend, increasing with increasing levels of COLM in the diets. T1 was significantly (P<0.05) lower than T2, T3 and T4.

Serum chemistry

The blood chemistry of broilers fed *Chromolaena odorata* leaf meal in their diets is presented in Table 5. The total protein was significantly (P<0.05) higher for the treatment groups (T2, T3 and T4) in comparison with the control group (T1). The albumen showed (P>0.05) similarities between T1 and T2 while T3 and T4 was significantly (P<0.05) different from T1. The globulin had a progressive increase across the treatments with incremental levels of COLM in the diets. Globulin also showed (P>0.05) similarities between T1 and T2 while T3 and T4 was significantly (P<0.05) different from T1. Urea, aspartate amino transferase (AST), and amino transferase (ALT) showed no significant (P>0.05) difference across the treatments and also failed to follow a particular pattern with increasing or decreasing levels of COLM. The creatinine showed significant (P<0.05) with T1, T2 and T3 showing no significant difference among each other, while T2 significantly differed from T1. The cholesterol values were significantly different (P < 0.05), and tended to decrease progressively with increasing levels of COLM in the diets of the broilers. The alkaline phosphate (ALP) values were not significantly different (P>0.05), but however increased linearly with increasing levels of COLM in the diets of the broilers.

Gut Microbial load

The gut microbial load of broilers fed *Chromolaena odorata* leaf meal in their diets is presented in table 6. The

Table 5: Blood chemistry of broilers fed *Chromolaena odorata* leaf meal in their diets.

Parameter	T1	T2	T3	T4	SEM
Total protein (g/dl)	5.00^{b}	6.13^{a}	6.64^{a}	6.52^{a}	0.25
Albumin (g/dl)	2.60^{b}	3.12^{ab}	3.41^{a}	3.22^{a}	0.11
Globulin (g/dl)	2.40^{b}	3.01^{ab}	3.23^{a}	3.30^{a}	0.08
Urea (mmol/L)	20.87	19.01	20.79	17.84	1.02
Creatinine (mmol/L)	51.30^{a}	50.97^{b}	51.14^{ab}	51.06^{ab}	3.05
Cholesterol (mmol/L)	95.18^{a}	90.12^{b}	82.47^{c}	68.76^{d}	3.17
ALP (U/L)	66.47	72.64	73.32	77.37	2.02
AST (U/L)	49.33	54.00	44.33	45.67	2.57
ALT (U/L)	45.64	63.33	52.00	58.67	6.21

$^{a-d}$ Means within the same row with different superscripts are significantly different (P < 0.05)

ALP=Alkaline phosphate, AST-Aspartate amino transferase, ALT=amino transferase coliform was significantly (P<0.05) higher for the control group in comparison with the treatment groups for the crop. The coliform at the crop also showed (P>0.05) similarities between T2 and T3 while T3 and T4 were also similar while T2 and T4 significantly (P<0.05) different each other and T1. The *Escherichia coli* showed (P>0.05) similarities between T1 and T2, and between T3 and T4. However, T1 and T2 significantly (P<0.05) differed from T3 and T4. There was a linear decrease of coliform and E. coli with increasing levels of COLM in the diets from T1 to T4. There was a significant (P<0.05) increase of *Lactobacillus* in the crop and caecum with incremental levels of COLM. At the caecum, coliform showed no significant (P>0.05) difference between T1 and T2, but however differed significantly (P<0.05) delite from the other treatment groups. E. coli showed significant (P<0.05) for T4 and other treatments (T1, T2 and T3) which are statistically similar (P>0.05) to each other. T3 and T4 also showed no significant (P>0.05) for E. coli in the caecum.

DISCUSSION

Chemical compositions

The proximate analysis of the COLM revealed a high dry matter value of 95.70% which was higher than 89.51%, 85.00% and 87.40% reported by Akinmutim and Akufo (2006), Bonsu et al. (2013) and Fasuyi et al. (2005) respectively. The crude protein content of 20.52% obtained in this present study for COLM is lower than the value of 22.80% reported by Bonsu et al. (2013) but higher than 15.27% and 16.20% reported by Ekenyem et al. (2009) and Igboh et al. (2009) respectively but however, comparable with 21.70% reported by Akinmutim and Akufo (2006) for the same leaf meal. The crude fibre (CF) content of 5.58% reported in this study is lower than the 11.49% and 20.10%, 13.72% and 11.67% reported by Bonsu et al. (2013), Ekenyem et al. (2009), Akinmutim and Akufo (2006) and Igboh et al. (2009) respectively. The relative low CF of COLM makes this leaf meal a potential feed stuff for monogastrics. The high ash content of COLM is an indication of high mineral profile of *Chromolaena odorata* leaves as attributed by Nwokolo (1987). However, the ash value of 9.29% for COLM in this study is lower than 11.50% reported by Bonsu et al. (2013) and higher than 3.63 and 6.17 reported by Igboh et al. (2009) and Fasuyi et al. (2005) respectively, but favourably compared with 9.50% and 9.64% as reported by Ekenyem et al. (2009) and Akinmutim and Akufo (2006) respectively. Jiwuba et al. (2016b) attributed variation in the proximate compositions of leaf meals to the age of the plants, the stage of development when the leaves were harvested, location, season, soil type, the dry matter content and processing method used. The dry matter, crude protein and crude fibre contents of the test diets compared with the control diet and are in agreement nutrient requirements for broilers as stated by NRC (1994).

Haematological studies

All the haematological parameters evaluated, fell within the normal physiological range for apparently healthy broilers as reported by Simaraks et al. (2004) and Jiwuba et al. (2017) indicating that the diets were nutritionally adequate in providing a sound plane of nutrition for the birds. The non significance and within normal range of Hb, RBC, MCHC, PCV and MCV gave a clear indication of absence anemia and feed toxicity. This further indicated that, all the birds had higher tendency to resist respiratory stress, due to the high level of Hb, which is carried on the RBC, and thus the oxygen carrying pigment. Jiwuba et al. (2016c) earlier stated that MCH is used as an index of toxicity and its reduction in blood concentration usually suggests the presence of toxic factor like haemaglutinin which has adverse effect on blood formation. Perhaps the MCH values obtained for all the treatment groups in this study fell within the normal physiological range of 25.3-33.40 (Pg) as reported by Jiwuba et al. (2017) for apparently healthy broilers. The COLM promoted significantly and numerically higher MCH values when compared to the control, indicating that the processing methods employed in this study eliminated the anti nutritional factors to a tolerable level by the birds. Adedapo et al. (2016) however observed that *Chromolaena odorata* leaves have anti-anaemic properties. The anti-anaemic property maybe attributed to the high iron content of its chlorophyll. The white blood cell counts were significantly (P>0.05) affected by the treatment diets, with the animals on the test diets having a higher and improved values than the control group. The significant increase in WBC counts of birds fed the COLM suggests high immunity since the WBCs are known to be the key actors in immune responses as they form the first line of defense against invading microorganism. The observed significant increase in the WBC observed in this study is in agreement with the results of Ekenyem et al. (2009) who studied the effect of partial replacement of *Chromolaena odorata* for soybean on haematological and serum chemistry of laying birds. Adedapo et al. (2016) noted that *Chromolaena odorata* leaf extract have immune-stimulating and prophylactic properties, which supports the result of this present study.

Serum chemistry

Serum biochemical studies are normally used for the diagnosis of organ diseases in farm animals. The values obtained in this study were within the normal range of serum protein 5.00-8.00 (g/dl) reported by Anon (1980) and 5.45-6.82g/dl reported by Ragab et al. (2012). Higher values of serum protein is an indication of enzyme hydrolysis of dietary proteins; demonstrating that the blood pool serves as a major source of amino acids needed for the synthesis of proteins. The albumin and globulin showed a significant difference with the test treatments having higher and better values than the control. The result of the present study however followed a similar trend with that of Ekenyem et al. (2009) for total protein, albumin and albumin for laying birds fed COLM. However, the values indicated nutritional adequacy of the test material in respect of protein, hence, high serum protein and albumin values are reflection of better quality and amount of protein in the diets (Jiwuba et al. 2016c). Globulin is an indicative of high immunity and good resistance to disease in

Table 6: Gut microbial load of broilers fed *Chromolaena odorata* leaf meal in their diets

Parameter	T1	T2	T3	T4	SEM
Crop					
Coliform (10^6)	7.67^a	6.33^b	5.00^{bc}	4.66^c	3.05
E. coli (10^6)	7.34^a	7.01^a	6.12^b	5.43^b	3.02
Lactobacillus 10^6)	7.10^d	8.53^c	9.81^b	11.09^a	3.01
Caecum					
Coliform (10^6)	8.11^a	8.02^a	6.71^b	5.19^c	3.94
E. coli (10^6)	6.34^a	6.07^a	5.31^{ab}	4.62^b	3.86
Lactobacillus (10^6)	8.00^d	9.67^c	10.67^b	12.33^a	3.64

experimental animals, the authors conclude. The increasing values of globulin observed in this study with increasing levels of COLM may be attributed to COLM due to its medicinal properties; a view corroborated by Akinmoladun et al. (2007), Vital and Windell (2009), Ekenyem et al. (2009) and Moses et al. (2010). Serum creatinine values ranged from 50.87 – 51.30mmol/L in this present study. The values obtained were within the normal range of 44 to 135mmol/L. Higher values than normal may signify kidney malfunction and extent of muscle wastage. The within range of the serum creatinine of the broilers in this study suggested that the birds were not surviving at the expenses of body reserves. Hence there was no weight losses observed in the study. The present findings also reveal that the COLM caused a significant and consistent reduction in the levels of serum cholesterol. Ekenyem et al. (2009) observed non significant effect of COLM cholesterol concentration in laying birds. Ikewuchi and Ikewuchi (2011) reported that COLM significantly decreased serum cholesterol, cardiovascular complications, due to dyslipidemic conditions, hypertension and obesity. The findings of Uhegbu et al. (2016) reported reductions in total cholesterol, glucose and triacylglycerol when rats were fed *Chromolaena odorata* leaf extract. Adedapo et al. (2016) reported hypoglycemic and hypolipidemic properties of *Chromolaena odorata* leaf extract. This reduction in serum cholesterol level of broilers fed the COLM diet may suggest a general decline in lipid mobilization and maybe suggest that COLM diet were capable of reducing serum cholesterol, hence assisting in the reduction and deposition of cholesterol in the muscle; thus enhancing the production of lean meat.

Gut microbial population

The results showed that the broilers fed COLM in their diets had higher concentration of *Lactobacillus* compared to those fed control diet in the crop and caecum sections of the alimentary canal of the broilers. This suggests that the presence of COLM in the diets of broiler stimulated proliferation of *Lactobacillus*. *Coliforms* and *E. coli* were consistently higher in the birds fed the control diets than the birds fed the treatment diets in the crop and caecum. The overall results indicated that COLM diets stimulated the growth of *Lactobacillus* (beneficial bacteria). The stimulation of increased *Lactobacillus* populations by the COLM diets however resulted in the reduction of *coliform* and *E. coli* population in the crop and caecum respectively. This result thus suggested the efficacy of COLM in controlling pathogenic microorganisms and enhancing *Lactobacillus* population in the gut of broilers, thus an indicative of better nutrient availability for digestion and absorption. The inclusion of COLM in the diets of the experimental birds resulted in modulation of gut microflora towards proliferation of beneficial microflora. Jensen et al. (2003) further opined that the composition of the diet influences the species and number of bacteria in the gut.

CONCLUSIONS

The results of this study revealed that sun drying and subsequent milling as a processing methods, are the best for improving the nutritional value of COLM as no deleterious effects has been elicited on broiler chickens as evidenced by the within physiological range for all the haematological and serum biochemical indices studied. Similarly, the COLM diets stimulated *Lactobacillus* growth which in turn resulted to the reduction in *coliform* and *E. coli* populations. This establishes its potential usefulness in controlling pathogenic organisms, stimulation of erythropoiesis and immunity and influences metabolism in broilers.

From this study, it could be recommended that sun drying of *Chromolaena odorata* leaf meal and milling at 12%% level of inclusion can be used without any deleterious effect on the hematological and serum parameters and improved the population of beneficial gut microbe in broiler chicken. However, further researches are required by using other, processing techniques such as fermentation, biodegradation and higher inclusion levels.

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Krvna slika in mikroflora črevesja brojlerskih piščancev krmljenih z listno moko (*Chromolaena odorata*)

IZVLEČEK

Proučevani so bili odzivi 96-dnevnih piščancev, ki so bili 49 dni krmljeni z vključevanjem listne moke *Chromolaena odorata* (COLM) v obrok na profilu njihove krvi in mikroflore črevesja. Piščanci so bili naključno razdeljeni v štiri naključno oblikovane poskusne skupine in tri ponovitve. Piščanci so bili krmljeni s štirimi eksperimentalnimi dietami, s 0%, 2%, 4% in 6% COLM za prvo fazo pitanja - štarter krmo in s 0%, 4%, 8% in 12% COLM v drugi fazi – finišer krmo. Skupine so bile označene kot za T1, T2, T3 in T4.

Vsi hematološki parametri so bili statistično neznačilni (P > 0,05) med skupinami, razen pri MCH in WBC, ki so bili značilno (P < 0,05) višji v testiranih skupinah v primerjavi s kontrolo. Rezultati serumske analize so pokazali značilne razlike (P < 0,05) za testirane skupine za lastnosti kot so skupne beljakovine, albumin, kreatin in holesterol v primerjavi s kontrolno skupino. Holesterol je bil značilno (P < 0,05) manjši z naraščajočimi odstotki COLM v krmi piščancev. Rezultati analiz so pokazali, da so vsebnosti *coliformnih* mikroorganizmov in *E. coli* značilno višje (P < 0,05) pri piščancih iz kontrole T1 v primerjavi s T2, T3 in T4 skupino. Ugotovljeno je bilo, da je COLM spodbudno deluje na hematokrit in imunski status, na hipoglikemično sposobnost, zavira rast črevesnih patogenih mikroorganizmov in povečala rast koristnih mikroorganizmov v črevesju pitovnih piščancev.

Ključne besede: pitovni piščanci, COLM, listna moka, zdravje črevesja, krvna slika, zdravilna rastlina

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Yield performance and agronomic efficiency in oil pumpkins (*Cucurbita pepo* L. group *Pepo*) depending on production systems and varieties

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ABSTRACT

In 2013 and 2014, a long-term trial (which was established in 2007) was conducted at the University Agricultural Centre in Pivola near Hoče. It included different production systems (conventional, integrated, organic, biodynamic), carried out in a field trial with oil pumpkins. The aim of the research was to analyse the effects of different production systems, varieties (hybrid and population variety) and years of production, on formation of oil pumpkin yields. The agricultural practice has been carried out in accordance with the applicable legislations and standards for the individual production system. When sowing, before fertilizing with nitrogen in early June and after the harvest, the amount of soil mineral nitrogen was monitored. We evaluated the number and weight of harvestable, unripe and decayed fruits, and yield of oil seed pumpkins and calculated the agronomic efficiency of the applied nutrients. The results showed that the year of production, the production system and the variety have a significant effect on some fruit characteristics and the yield of oil pumpkin seeds. The content of soil mineral nitrogen in May and September was significantly influenced by the production system and the year. In June, only the production system had a significant effect. The hybrid significantly increased the yields of oil pumpkin seeds in all production systems, even in the year that was less suitable for production. The agronomic efficiency of the applied nutrients in the biodynamic and organic production system is higher or equal than in the conventional production system, similarly, agronomic efficiency is higher in the hybrid compared to the oldest population variety efficiently. A comparable oil pumpkin yield can be expected in biodynamic and organic production, when proper nutrition and well carried out cultivation practices are combined with a new variety, when compared to less sustainable production systems, which often cause damage to the environment.

Key words: production systems, variety, oil pumpkins, mineral nitrogen, agronomic efficiency, yield.

INTRODUCTION

Different agricultural production systems (PS), such as conventional (CON), integrated (INT), organic (ORG) and biodynamic (BD) farming, have different impacts on the environment, preservation of landscape and soil fertility (Wall et al. 2015). Agriculture has to provide food for an ever-increasing population, estimated to grow up to 10 billion in 2050, which will further intensify the production systems and increasingly impact the environment (Pingali 2012, Smith et al. 2013).

Consumer concern regarding possible adverse health effects of products and foods produced by intensive farming methods has led to a considerable increase in interest in the health benefits of organic production systems (ORG, BD). Nowadays, consumers expect a minimal environmental impact of PS and food produced in a way, that not only satisfies all their nutritional needs, but also positively impacts

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their health (Goetzke et al. 2014). Some authors (Specht et al. 2014, Touliatos et al. 2016, Muller et al. 2017) see the future of solving food and environmental problems in intensifying conventional farming and upgrading it to vertical farming or high-tech farming. Vertical farming offers a higher production yield with a growth index of 10 to 100 (CON = 1) in soil-less food production, low area use, very high-energy use (energy may be used from alternative sources), with low nitrogen losses, a controlled but poor biodiversity and high financial input and profit.

Convectional farming, which is the second biggest environmental polluter, is being increasingly criticised by the public, who demand a sustainable way of producing food, which does not include GMO or the use of synthetic materials (Bavec et al. 2009). Sustainable production systems guarantee agricultural products free from pesticide residue and other unwanted substances (Williams and Hammitt 2001), and with them less pollution of the environment. By applying soil biodiversity, they also provide benefits to human health because soil biodiversity can suppress disease-causing soil organisms and provide clean air, water and food, where conventional farming reduces all those benefits (Wall et al. 2015).

Quite a few studies comparing conventional and organic field crop production have been conducted lately (Connor 2008, Seufert et al. 2012, Meier et al. 2015, Shah et al. 2017), as well as studies comparing integrated and organic ways of production (Deikea et al. 2008, Muller et al. 2011, Nemecek et al. 2011), or comparisons among several PS (Pfiffner and Mäder 1997, Turinek 2011, Štraus 2012). The authors came to different conclusions concerning plant material and the applied agronomic measures. The effectiveness and appropriateness of the PS can be measured in different ways. Indicators of environmental influences like ecological footprint (Vukmanič 2016), consumed energy per area (Deikea et al. 2008) or the amount of yield per hectare (Turinek 2011) have been mentioned in the literature. The success indicator of a production system can also be the yield's response to the amount of applied nutrients (Fixen et al. 2015). That can be measured by the agronomic efficiency (AE) of N, P or K (AE_N, AE_P or AE_K), which results in the increase of yield in kg per kg of applied fertiliser (N, P_2O_5, K_2O). AE, in connection with estimated yield response, is becoming an important alternative indicator for supporting crop fertilization in some countries (Chuan et al. 2013, Zhou 2013).

There is very little research on the effect of PS on growth, development and yield of oil pumpkins (*Cucurbita pepo* L. group *Pepo*) in a sub-continental temperate climate in long-term experiments (Turinek 2011). The production of pumpkin seeds for oil is present in some European countries, the traditional producers being Slovenia, Austria, Hungary, Croatia and Serbia (Bavec 2000). A small portion of production is also starting to emerge in the Check Republic and Ukraine (Jariene at al. 2007). In Slovenia, oil pumpkins are produced on an area of 5410 ha, which is the biggest surface among oil plants (SURS 2017) and plays an important part in field crop rotation. The marketing of oil pumpkins and their products provides an important source of income for farms. Different production systems enable the producers to choose among different approaches of production, which includes changing up some of the techniques of production. Those may include using different overwintering and non-overwintering cover crops in strip till belts (Jakop 2010), or a combination of cover crops and roller crimpers (Bavec at al. 2014). Regardless of production system, a successful oil pumpkin production requires a well-chosen location, which has to be rich in humus, nutrients, sunlight and has a low weed population (Bavec and Jakop 1994, Kocjan Ačko 2015). The quantity of yield, regardless of production system, can be connected to the amount of N the production system receives either through mineral fertilizers (CON, INT), organic fertilizer (animal manure), compost or cover crops (ORG BD). Organic production systems, in comparison with other conventional production systems, can only be efficient, if the conditions in the soil do not hold back the mineralization of N and other nutrients (micro and macro elements) that need to be at the disposal of the root system during a plant's intensive growth periods (Berry et al. 2002). The results of a meta-analysis (Seufert et al. 2012) show that when good management practices are applied, organically produced oleaginous plant yields are only less than 10% smaller than those produced in conventional production systems. In our long-term study, the results of pumpkin seed yield and the yield components were compared in two consecutive years.

The aim of the study was therefore to analyse the effect of the PS (biodynamic, organic, integrated and conventional) and the type of pumpkin variety (hybrid and population variety) on the characteristics of the fruit, the mineral nitrogen in the soil in different growth stages, the agronomic efficiency of added nutrients, and their connection to seed yield. The goal was to compare the adequacy of the oil pumpkin production in different production systems.

MATERIALS AND METHODS

Long-term field trial

The oil pumpkin field trial started in May 2013 in the seventh growth season of the long-term field trial located at the University Agricultural Centre of the University of Maribor in Pivola near Hoče (46°28′ N, 15°38′ E, 282 m above sea level). The experiment was arranged in a randomized complete block design in split-plots with four replicates. The size of each experimental plot was 29.4 m^2 (7.0 m long × 4.2 m wide). The distances between the rows were 1.4 m and about 0.6 m (12 seeds per row) within the rows.

The experimental design involved two factors. The first was four different production systems (conventional - CON, integrated INT, organic - ORG, biodynamic - BD) and control plots (without plant protection or added nutrients)). The second factor involved two oil pumpkin varieties known for their production in Central Europe, namely hybrid (HY) and population variety (PV). The agricultural practices in the PS in oil pumpkins are shown in Table 1. Soil tillage, sowing and harvesting were similar among experimental plots. The production in the different PS differed in type and amount of fertilizer with the approximately same amount of nutrients, plant protection and weed control, and was performed on the same dates and in a similar manner than the adjacent fields.

Agricultural practices in specific production systems were carried out according to the legislations and standards in effect during 2013 and 2014 for organic farming (EC 834/2007 2007, ES 889/2008 2008, MKGP 2006, 2008, 2014a), biodynamic farming (Demeter International e.V. 2012), conventional farming (MKGP 2008, 2012a, 2012b, 2014a) and integrated farming production (MKGP 2010, 2013, 2014b).

Basic fertilizing with manure (ORG) and composted manure (BD) was carried out in an amount of 22 t/ha. Additional fertilizing on both plots was carried out using ground pumpkin cakes. 560 kg/ha of pumpkin cakes with 9.6% nitrogen were used. With this, 54 kg of N/ha were added to the crop. The basic fertilization was carried out 10 days before sowing the oil pumpkins, and the additional fertilizing was done at the beginning of June in the BBCH 109/501 growth stage. The fertilization was done on the basis of left over winter cereal straw (previous field crop) and the nutrient intake in the average Slovene yield of oil pumpkins which is 0,6 t/ha (SURS 2017) (84 kg N/ha, 80 kg P_2O_5/ha in 180 kg K_2O/ha).

Considering the amount of phosphorus and potassium (Table 2) and the leftovers of the winter wheat straw (0 kg N/ha, 20 kg P_2O_5/ha in 70 kg K_2O/ha), nutritional needs were covered in both years in all production systems, apart from ORG in BD in which the average for both years resulted in -12% and -18% of K_2O and P_2O_5, and a slight surplus of K_2O in CON +26%. The entire planting area was treated with a rotary harrow, after fertilization. The oil pumpkin sowing was done manually on 10th May in both years. After the sowing, a chemical treatment against weeds was applied. For the INT production system, we applied 1.6 l/ha of Successor (*petoksamid* 600 g/l) and 0.25 l/ha of Centium (*klomazon* 360 g/l), and for the CON areas, we used the same chemicals in a dose of 2.0 l/ha and 0.25 l/ha. The hoeing was done twice (after first emergence of plants from the ground and after the applied fertilizing) in ORG, BD and the control plots, and only once in the INT plot after applied fertilizing. Manual harvesting took place at the end of September in both years.

The same varieties were used in all farming systems in the study, namely the population variety 'Gleisdorfer Ölkürbis' and the F1 'GL Opal' hybrid. They were of conventional origin, without chemically treated seeds for ORG, BD systems and control plots.

Table 2: The average content of P2O5 and K2O nutrients in the soil (mg/100 g soil) in a long-term experiment at the University Agricultural Centre in 2014.

Production system	P_2O_5		K_2O	
	(mg/100 g soil)	Nutrient level	(mg/100 g soil)	Nutrient level
BD	18	C	26	C
ORG	22	C	25	C
INT	20	C	27	C
CON	23	C	26	C
Control	15	C	15	B

C = good (optimal) supply level of P_2O_5/K_2O in the soil using the AL method
B = medium supply level of P_2O_5/K_2O in the soil using the AL method.

Table 1: Important differences in agriculture practice among the four production systems and control plots.

	Production system				
	BD	**ORG**	**INT**	**CON**	**Control**
Soil tillage	Ploughing, seedbed preparation, sowing, hoeing, harvesting	Ploughing, seedbed preparation, sowing, hoeing, harvesting	Ploughing, seedbed preparation, sowing, hoeing, harvesting	Ploughing, seedbed preparation, sowing, harvesting	Ploughing, seedbed preparation, sowing, hoeing, harvesting
Weed control	Mechanical control of weeds	Mechanical control of weeds	Use of herbicides according to the Guidelines for integrated field crops (MKGP, 2013, 2014b) Mechanical control of weeds	Preventive use of herbicides	Mechanical control of weeds
Fertilization	Composted livestock manure (1.4 LU/ha) with added BD compost preparations	Well - rotted cattle manure (1.4 LU/ha)	NPK and other nitrogen mineral fertilizers used based on soil analysis and nutrient removal estimates	NPK and other nitrogen mineral fertilizers used based on good agricultural practice and nutrient removal estimates	None

*LU – Livestock unit = 500 kg of live animal weight

Soil characteristics

The University Agricultural Centre in Pivola has dystric cambisol (FAO 2006). Following the USDA Textural Classification System (2017), it belongs to the heavier clay texture class, whit 31% clay, 47% silt and 22% sand in 0-0.3 m depth. The advantage of this soil is that it provides more accessible water to the plants during extreme drought circumstances than lighter soil types.

The content of phosphorus and potassium in the soil in individual treatments was analysed using the AL method in 2014 (table 2). The soil's phosphorus and potassium supply in the C level was optimal, except for the potassium amount in the control production system (B level), where the plots have not been fertilized since the beginning of the long-term experiment in 2007.

The percentage of humus in the soil was between 2.2 and 2.5% and the pH was between 5.7 and 5.9 (in 1 M KCl).

Weather conditions

The annual mean air temperature of the area is 10.5 °C, where the mean monthly minimum occurs in January at -0.1 °C, and the average monthly maximum is in July at 21.0 °C. The average annual rainfall in the area measured from 1981 until 2010 was 893 mm (ARSO 2017).

The air temperatures in spring 2013 were low, which inhibited the growth until almost mid-June, and that period was followed by a sudden transition to extreme summer conditions. After that, the area experienced three heat waves and a summer drought, which was interrupted by heat storms. Because of the occasional rainfall on the semi-heavy and heavy soil, the summer drought did not limit the growth of the plants. The oil pumpkins compensated the slow spring growth in the summer and reached their technological maturity in the middle of September (Fig. 1) (ARSO 2017).

In May 2014, there were a lot of above average intermitted warm and cold periods. The exchange of extreme heat to normal, and even under average temperatures in the first decade of June, also continued into the whole month. The consequences of the stressful circumstances affecting the growth and development of the cucurbits manifested themselves in a lower number of fruits per plant and thus exhibiting a smaller yield of oil seed pumpkins. There was an above average quantity of rainfall and rainy days during the growth period throughout Slovenia. The soil was sometimes too wet during the harvesting of the fruits and seeds in the north-east of the country, which made the ripening harder for the plants (Fig. 2) (ARSO 2017).

Content of mineral nitrogen in the soil

Mineral nitrogen (Nmin) in the soil has been sampled according to PS in both years. The sampling was carried out at sowing (BBCH 001), at the beginning of June in growth stage BBCH 109/501 (beginning growth stage of first flower initial with elongated ovary visible on main stem) and at harvesting at the end of September (BBCH 909). The growth stage determination was done by Feller et al. (1995). The two nitrogen types NO_3^- and NH_4^+ were identified by the Sharf and Wehrmann (1979) method in the chemical laboratory at the Faculty of Agriculture and Life Sciences. The sampling for identifying the mineral nitrogen content in the soil was carried out at a depth of 0 - 0.3 and 0.3 - 0.6 m. Six samples at both depths have been obtained per sampling in four repetitions of the experiment. In order to calculate the mineral nitrogen content, specific soil mass quantity for a specific location, which were obtained at sampling by the Kopetcky method, was used (1280 kg/m^3 and 1350 kg/m^3, at a soil depth from 0 - 0.3 m and 0.3 - 0.6 m).

Yield and fruit characteristics

The harvest in both years took place when the pumpkin fruits reached their technological maturity, which means that the fruits were almost entirely yellow and had a dry stalk. The fruits of oil pumpkins were counted, weighed and

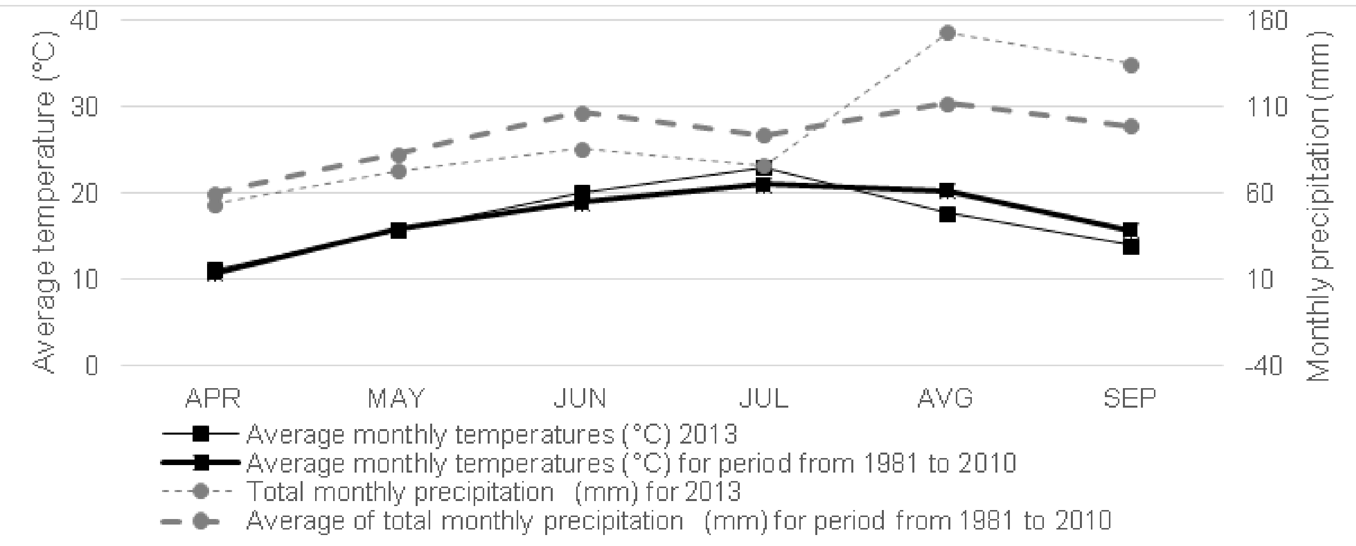

Fig. 1: Modified Walter-Gaussen climate diagram for the growing season period of pumpkins in 2013 compared to the long-term average (1981-2010) for Maribor (ARSO 2017).

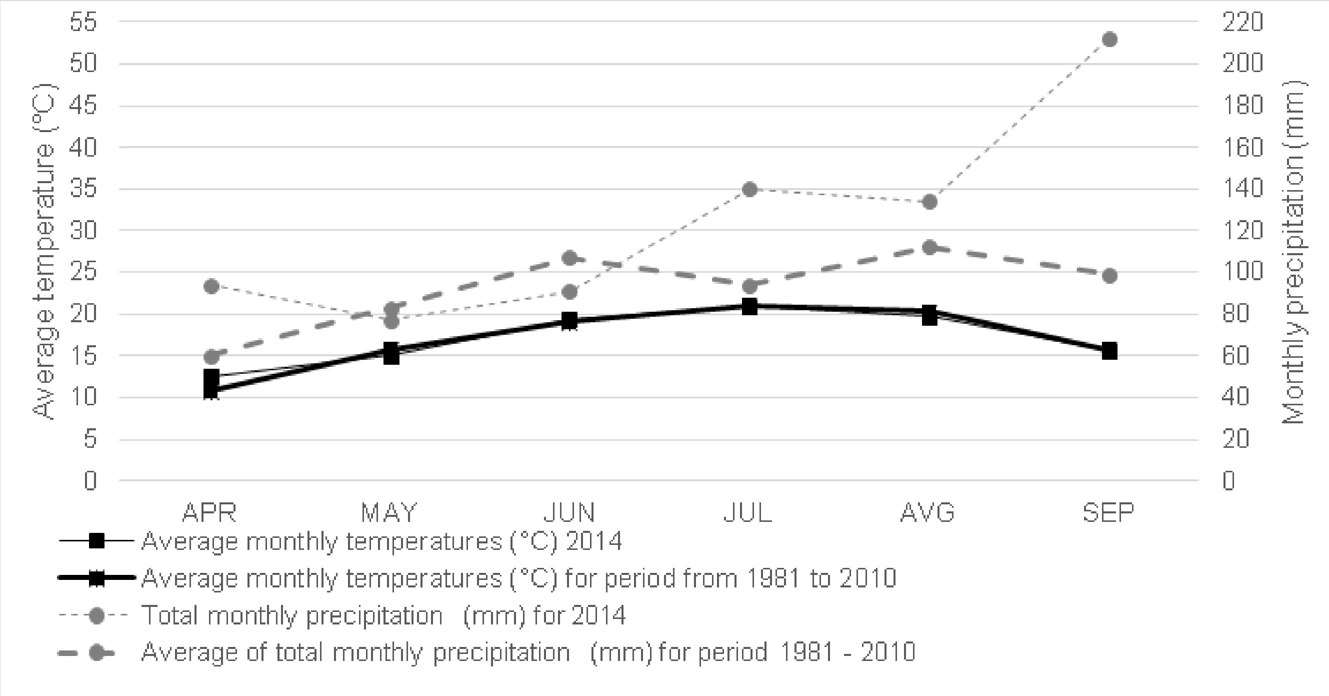

Fig. 2: Modified Walter-Gaussen climate diagram for the growing season period of pumpkins in 2014 compared to the long-term average (1981-2010) for Maribor (ARSO 2017).

put into three categories; harvestable, unripe and decayed fruits. The collected data have been calculated per ha and are thus presented in the tables. The seed yields were harvested manually and weighed for each plot. The moisture in the seeds was determined on an average sample weighing using a drying procedure for three days at 60 °C. The results of the pumpkin seed harvest have been calculated at 9% moisture in the seeds.

Agronomic efficiency of applied nutrients

Agronomic efficiency of applied nutrients (AE) is one of many important defined fertilizer efficiency parameters (Good et al. 2004). It measures the efficiency of converting applied nutrients (N, P_2O_5, K_2O) to yield of crop. However, AE, defined as crop yield increase per kg nutrient applied and presents one of the indicators of successful agricultural production (Ma at al. 2015). For calculating the agronomic efficiency for added NPK nutrients in this study, the formula used by Xu et al. (2015) has been applied;

$$AE(N, P_2O_5 \text{ or } K_2O) = (Y_{PS} - Y_{\text{Control Plot}}) / F(\text{Nutrients } N, P_2O_5 \text{ or } K_2O) \quad (1)$$

Where is Y_{PS} is the yield of oil pumpkin seeds in a specific production system (kg/ha), $Y_{\text{Control Plot}}$ is the yield of oil pumpkin seeds in control plots without fertilization (without adding any nutrients) (kg/ha) and F being N, P_2O_5 or K_2O representing the amount of fertilizer applied (kg/ha). This was statistically evaluated and done separately for each year and plot. The amounts of nutrients (N, P_2O_5, K_2O) applied in 2013 and 2014 (Table 3) were used to calculate AE.

Table 3: The average content of P_2O_5 and K_2O nutrients in the soil (mg/100 g soil) in a long-term experiment at the University Agricultural Centre in 2014.

	2013			2014		
PS	**N**	**P_2O_5**	**K_2O**	**N**	**P_2O_5**	**K_2O**
CON	89	70	139	89	70	139
INT	83	58	115	83	58	115
ORG	101	56	96	88	48	84
BD	101	56	96	88	48	84
Control	0	0	0	0	0	0

PS – Production systems, N – nitrogen, P_2O_5 – phosphorus, K_2O – potassium.

Statistical methods

Statistical analyses were performed by using the program Statgraphics Centurion (Statgraphics 2005). The impact of the investigated factors was analysed with the multifactorial analysis of variance (ANOVA). The differences between the treatments were checked using the Duncan multiple range test (α = 0.05). The results are shown as mean values of repetition with a standard error mean (± SEM).

RESULTS AND DISCUSSION

Mineral nitrogen in the soil

The content of Nmin in the soil (0 - 0.6 m) in all three growth stages of oil pumpkins (BBCH v 001,109/501 and 909) has been significantly influenced by the production system

(Table 4). The year of production, along with the production system, also significantly influenced the Nmin in spring, at the beginning of May when the pumpkins started growing (BBCH 001), and at harvesting (BBCH 909). In both growth stages, the early one because of a smaller area of the root system, and the one in autumn because of a lower activity of decayed roots, nitrate nitrogen in the soil can present a source of pollution for the groundwater, due to stronger rainfalls. The PS ($P \leq 0.001$) has a significant impact on the amount of mineral nitrogen in the zone of the oil pumpkin roots, in the BBCH 109/501 growth stages. In May, at the beginning of the pumpkin growth period (BBCH 001), the amount of Nmin in ORG production (59.6 kg/ha) was significantly higher when compared to the other PS, except for CON (51.1 kg/ha). At the beginning of June, the distinctively highest content of Nmin in the soil was in BD and the control plots (106.7 in 98.3 kg/ha, respectively) when compared to the other production systems. After the harvest, the remaining Nmin in the soil was significantly higher on the BD and ORG plots in comparison with the CON and control plots. INT only significantly differed from BD.

Table 4: Mineral nitrogen content in soil (0-0.6 m) in different production systems.

	Nmin (0 - 0.6 m) kg/ha		
Factor	BBCH 001	BBCH 109/501	BBCH 909
Production system (PS)	**	***	**
Year (Y)	***	NS	***
Interaction			
PS × Y	**	NS	NS
Production system			
BD	49.3±6.5^{bc}	106.7±7.1^{a}	107.0±22.0^{a}
ORG	59.6±5.5^{a}	63.2±9.2^{b}	95.2±12.4^{ab}
INT	44.4±3.5^{bc}	62.0±7.9^{b}	86.0±18.2^{bc}
CON	51.1±7.8^{ab}	65.5±7.8^{b}	73.2±16.2^{c}
Control	40.1±2.5^{c}	98.3±6.3^{a}	73.3±14.4^{c}
Year			
2013	38.2±2.4^{b}	75.0±7.7	45.7±3.2^{b}
2014	59.5±3.0^{a}	83.3±4.7	128.2±6.4^{a}

, * significant at the 0.01 and 0.001 probability levels, respectively; NS - non significant; a-c mean values (± SEM) followed by different letters within a column and particular factor are significantly different (Duncan, α=0.05).

The Guidelines for integrated vegetable production (MKGP 2014c) state that the recommended residual of Nmin in the soil (0 – 0.6 m) after an oil pumpkin harvest needs to be less than 80 kg/ha. The Nmin in the soil was generally higher in BD and ORG treatments, where we added composted or mature stable manure each year before sowing. Fertilisation in those treatments was also combined with double hoeing performed after the emergence of the plants and after additional fertilising with N in June. The yearly addition of organic fertilizer during optimum conditions for mineralization and suitable agricultural practices, also ensure the intensive nitrogen mineralization in autumn when the plants' need for nutrients is lower. Our results are the same as those of Bavec et al. (2015), which state that higher hoeing frequencies in sweet corn tillage sped up the process of mineralization and thus significantly increased the amount of Nmin in the soil in June, and after harvest, when compared with their control plot (Nmin values in soil). The higher Nmin quantities in the soil also significantly influenced the higher yield of sweet corn. Becker and Boehrnsen (1994) also state that hoeing speeds up the processes of transforming organic nitrogen into mineral forms. Mechanical treatment of soil influences oxidation processes in the soil and enable a better and faster access to nutrients, while improving the physical, chemical and biological conditions in the soil, especially in organic farming systems (Silgram and Shepherd 1999, Bavec and Bavec 2007). The significantly higher amounts of Nmin in the soil at the beginning of May 2014 when compared to 2013, are a result of higher air temperatures in spring, resulting in higher temperatures of the upper soil layer, which influenced a higher mineralization in the soil. De Neve and Hofman (2000) state that when supplying soil with a sufficient amount of moisture, higher temperatures significantly influence the mineralization in the soil, making it stronger and faster. After storing the harvest in 2014, the left over amounts of mineral nitrogen were significantly higher, almost three times higher, when compared to the previous year. High amounts of Nmin can also be a consequence of residual Nmin in the soil because of poor oil pumpkin growth in the summer months of 2014, resulting in a lower fruit and pumpkin seed yield.

Yield of oil seed pumpkin and fruits characteristics

Production system, variety and year had a significant impact on the number of all fruits and the number of harvested fruits (Table 5). The significantly highest number of all fruits and number of harvested fruits per hectare were in the INT (16071, 15479 respectively) production system. The number of all fruits and harvested fruits were not significantly different in BD and ORG. BD and INT were the only ones among the production system treatments, which had an above average relative number of harvested fruits in the experiment (116% in 101%). Interactions among years, production systems and varieties for analysed factors were not significantly different. A significantly higher number of all fruits and number of harvested fruits are found in the hybrid variety when compared to the population variety (for 11% and 10%) and in 2013 when compared to 2014 (29% in 30%). A smaller number of fruits in 2014 was due to the exchange of high and low temperatures during the flowering of the pumpkins in June. Wien (1997) states that in the *Cucurbita pepo* L. species, cold weather causes the male flowers to open a few days later than the female ones, which causes a decreased fertility and thus a lower number of fruits at harvest. For successful fertilization, simultaneous blooming of male and female flowers is of importance (Ivančič 2002).

The production system and year had a significant impact on the harvest fruit weight. On average, the production system and variety had a significant impact on the weight of the harvested fruits. Interaction was significant in case of PS and year (PS × Y) (Table 6). In the control plot and CON, the weight harvest fruits were significantly lower when compared

Table 5: The effect of treatments on number of all, harvested, unripe decayed oil pumpkin fruits and relative number of harvested fruits.

	Number of all fruits (ha)	Number of harvested fruits (ha)	Relative number of harvested fruits (%)	Number of unripe fruits (ha)	Number of decayed fruits (ha)
Production system (PS)	***	***	/	NS	NS
Variety (V)	**	**	/	NS	NS
Year (Y)	***	***	/	NS	NS
Interaction					
PS × V	NS	NS	/	NS	NS
PS × Y	NS	NS	/	NS	NS
Y × V	NS	NS	/	NS	NS
PS × V × Y	NS	NS	/	NS	NS
Production system (PS)					
BD	13992±815[b]	13455±791[b]	101	382±90	156±68
ORG	13959±650[b]	13232±608[bc]	99	486±122	242±78
INT	16071±845[a]	15479±806[a]	116	504±130	88±40
CON	13259±571[b]	12547±623[bc]	94	393±100	319±90
Control	12653±638[b]	11835±569[c]	89	571±159	246±81
Variety					
PV	13283±470[b]	12707±484[b]	95	428±65	148±43
HY	14690±460[a]	13910±432[a]	105	506±87	272±49
Year					
2013	15744±491[a]	15031± 444[a]	113	552±96	161±44
2014	12230± 294[b]	11588± 301[b]	87	383±48	259±50
Average	13987	13310	**100**	467	211

, * significant at the 0.01 and 0.001 probability levels, respectively; NS - non significant; a-c mean values (± SEM) followed by different letters within a column and particular factor are significantly different (Duncan, α=0.05).

Table 6: The effect of treatments on weight of harvest, unripe pumpkin fruits, average fruit weight of harvest and relative weight of harvested fruits.

	Number of all fruits (ha)	Number of harvested fruits (ha)	Relative number of harvested fruits (%)	Number of unripe fruits (ha)	Number of decayed fruits (ha)
Production system (PS)	***	***	/	NS	NS
Variety (V)	**	**	/	NS	NS
Year (Y)	***	***	/	NS	NS
Interaction					
PS × V	NS	NS	/	NS	NS
PS × Y	NS	NS	/	NS	NS
Y × V	NS	NS	/	NS	NS
PS × V × Y	NS	NS	/	NS	NS
Production system (PS)					
BD	13992±815[b]	13455±791[b]	101	382±90	156±68
ORG	13959±650[b]	13232±608[bc]	99	486±122	242±78
INT	16071±845[a]	15479±806[a]	116	504±130	88±40
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Variety					
PV	13283±470[b]	12707±484[b]	95	428±65	148±43
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Year					
2013	15744±491[a]	15031± 444[a]	113	552±96	161±44
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Average	13987	13310	**100**	467	211

, * significant at the 0.01 and 0.001 probability levels, respectively; NS - non significant; a-c mean values (± SEM) followed by different letters within a column and particular factor are significantly different (Duncan, α=0.05).

to INT and BD, but BD and CON not being significantly different. The same holds true for the average weight of harvested fruits, with the difference of BD and CON being significantly different. In 2013, the harvest fruit weight was significantly higher by a third, in comparison to 2014.

Although the average weight of a mature fruit in the population variety is significantly higher by 14% when compared to the hybrid, other fruit characteristics such as number of harvested fruits (Table 5) and higher percentage of seed harvest (Table 7) have influenced the final significantly higher yield of the hybrid variety per hectare (Table 7). The seed yield of pumpkins (given at 9% moisture in seed) and the average seed weight per fruit, varied among PS, varieties and years (Table 7). The interaction between the studied factors is not statistically significant in any of the observed characteristics. The INT production system had a significantly higher pumpkin seed yield (922 kg/ha) and a higher average weight of pumpkin seed per fruit (57.6 g) when compared to all the other production systems apart from BD (804 kg/ha, 57.1 g). The lowest pumpkin seed yield occurred on CON and control plots (666 kg/ha in 630 kg/ha). The average seed yield in 2013 and 2014 in our study was a third higher on average than the average across Slovenia (SURS 2017). Turinek (2011) also conducted a three-year long-term study with the same design and did not find any significant influence of production system and year of production on the pumpkin seed yield, whereas we found a significant influence of those factors on the seed yield. Regardless of this, the comparison between the value and the relative yield of pumpkin seeds (in %) in the production systems in both experiments show comparable results. Both studies show that the seed yield in INT and BD are above the average of the whole experiment (2009-2011; 124%, 107%, 2013-2014; 123%, 108%). A significant influence of the PS and the growth of the yield in both organic PS, are the result of several years of good farming practices carried out in those plots. Martini et al. (2004) and Seufert et al. (2012) state in their research, that soil fertility in organic farming establishes itself after three or more years of applying an organic farming practice, when compared to conventional farming practices. CON, ORG and the control plots had a significantly lower average weight of pumpkin seeds than INT. The higher average weight of the pumpkin fruits (kg) (Table 6), trend of higher number and weight of harvest fruits (Table 5 and 6) and average weight of seeds/fruit (Table 7) in BD, when compared to ORG, can be explained through the significantly higher amount of available Nmin in the soil during the BBCH 109/501 growth stage (Table 4). Bavec and Bavec (2007) claim that in oil pumpkins, during the BBCH 501 stage, the supply of nitrogen significantly influences the amount of pumpkin seed yield. The significantly higher or equal yield of seed/ha and seed/fruit in BD and ORG when compared to CON, are the result of adequate supply of organic production systems with organic fertilizers. Providing oxidizing conditions in the soil using the appropriate mechanical measures, which are essential for the mineralization of nutrients from stable manure during crop growth from May to Avgust, ensures a comparable or even better supply of mineral nitrogen in organic than in conventional systems. Seuferta et al. (2012) state that nitrogen is mostly a limiting factor for yield formation in organic production systems. In organic production systems, where there is enough available nitrogen in the soil for creating the necessary conditions for a fertile soil, the yield is not lower than in conventional systems.

When comparing the productivity of the new hybrid ('GL Opal') and the older population variety ('Gleisdorfer Ölkürbis'), a significantly higher yield is observed in the hybrid variety (21%). In comparison with the whole experiment, it is 11% higher. The average weight of pumpkin seeds per fruit (55.4 g) is also significantly higher in the hybrid when compared with the population variety. Most new varieties have increased their resistance to illnesses and pests and improved their use of nutrients in the soil, which has been achieved by using improved methods of plant breeding, which significantly influences the yield formation (Rochester and Constable 2015). The weather conditions in both years influenced the pumpkin seed yield equally significantly ($P \leq 0.001$) than the other included factors (PS, V). Unfavourable conditions for the growth and development of the pumpkins in 2014 have been the above average amount of rainfall in the summer, and many temperature fluctuations in May and June (Figure 2) which alternated in a few day intervals during the days and nights. The unfavourable growth conditions have also significantly influenced the lower average weight of pumpkin seeds per fruit (50.2 g), when compared to the more favourable 2013 (55.1 g). The oil pumpkin is a very low temperature sensitive plant when in its initial growth stage (Bavec and Bavec 2007) and it is also sensitive to temperature fluctuations which negatively impacts the blooming timing of the female and male flowers and thus the number of pumpkins and their seed yield (Wien 1997, Ivančič 2002).

Agronomic efficiency

The AE of the nutrients added in the form of mineral or organic N, P_2O_5, and K_2O (depending on the individual PS) differ significantly among PS, and in P_2O_5 and K_2O among varieties (Table 8). The year did not have a significant influence on AE in any of the studied nutrients. Based on the analysis of the results, it can be concluded that all three nutrients have a better AE in using N in INT, and a better usage of P and K in INT and BD when compared to CON. Organic production systems of oil pumpkins are in the middle between BD and CON when it comes to AE. Turinek (2011) showed in the first years of his long-term experiment in the same location, that he received a higher AE of nutrients in oil pumpkins in different PS when compared to our results. The trends among the PS are the same, although the differences among the PS were not significant in 2008, 2009 and 2010. The results show that along with fertilizing and adding nutrients, agrotechnical measures of aerating and loosening the soil during vegetation are necessary in order to ensure conditions that are beneficial for the growth and development of the roots. That enables AE, which is shown in the presented study in INT, BD and ORG production systems. AE is influenced by a lot of factors, such as nutrient content in the soil of control and other plots, types and amounts of fertilizers, agrotechnical measures and climate conditions (Chuan et al. 2013). Nitrogen and phosphorus have the highest average AE amount among the nutrients, where the pumpkin yield increases by 2 kg per 1

Table 7: The effects of treatments on yield of oil pumpkin seeds with 9% of moisture (kg/ha) and average weight of seeds/harvested fruits (g).

	Seed yield of pumpkins with 9% moisture (kg/ha)	Relative seed yield (%)	Average weight of seeds/harvested fruits (g)
Production system (PS)	***		*
Variety (V)	***		*
Year (Y)	***		*
Interaction			
PS × V	NS		NS
PS × Y	NS		NS
Y × V	NS		NS
PS × V × Y	NS		NS
Production system (PS)			
BD	804±73[ab]	108	57.1±3.2[ab]
ORG	716±74[bc]	96	49.1±3.5[b]
INT	922±64[a]	123	57.6±2.5[a]
CON	666±51[c]	89	49.5±2.2[b]
Control	630±56[c]	84	49.5±2.8[b]
Variety			
PV	669±37[b]	90	49.9±1.9[b]
HY	826±45[a]	111	55.4±1.8[a]
Year			
2013	882±49[a]	118	55.1±2.3[a]
2014	613±20[b]	82	50.2±1.2[b]
Average	747	**100**	52.6

*, *** significant at the 0.05 and 0.001 probability levels, respectively; NS - non significant; a-c mean values (± SEM) followed by different letters within a column and particular factor are significantly different (Duncan, α=0.05).

kg of added N or P_2O_5, K_2O is about half less effective. The two to five times lower AE of nutrients in our experiment, when compared to other studies conducted on oil pumpkins and studies conducted on other plants (Turinek 2011, Chuan et al. 2013, Fixen et al. 2015, Ren et al. 2015), is probably due to the supply of particular nutrients in the soil of the control plot. The control plot is relatively well supplied with phosphorus, potassium (Table 2) and mineral nitrogen (Table 3) throughout the entire vegetation growth period of the oil pumpkins. The high amounts of Nmin in the soil are due to the good mineralization of plant residue, which stays on the soil after harvesting.

When analysing the effects of variety on the usage of macro nutrients in oil pumpkins, the results show higher amounts of AE in P_2O_5 and K_2O in hybrid when compared to population variety. There are no differences among the varieties when it comes to nitrogen utilization, although one would expect that due to plant breeding, the new varieties would be more efficient in utilizing the nitrogen. Our results are similar to those obtained by Rochester and Constable (2015), who found that newer population varieties and hybrids can be expected to have a better micro and macro nutrient utilization, which results in better quality and quantity of the yield. In the future, plant breeding will be even more focused on improving the utilization of all nutrients.

Table 8: Agronomic efficiency of added N, P2O2 and K2O nutrients depending on production system, variety and year.

	AE (N)	AE (P_2O_5)	AE (K_2O)
Production system (PS)	***	*	*
Variety (V)	NS	*	*
Year (Y)	NS	NS	NS
Interaction			
PS × V	**	***	***
PS × Y	NS	NS	NS
Y × V	NS	NS	NS
PS × V × Y	NS	NS	NS
Production system (PS)			
BD	1.8[b]	2.7[ab]	1.6[a]
ORG	0.9[bc]	0.7[bc]	0.4[ab]
INT	3.5[a]	3.2[a]	1.6[a]
CON	0.4[c]	0.3[c]	0.2[b]
Control	0.0[c]	0.0[c]	0.0[b]
Variety			
PV	1.2	0.4[b]	0.2[b]
HY	1.4	2.3[a]	1.2[a]
Year			
2013	1.5	2.0	1.1
2014	1.2	0.7	0.4

*,**, *** significant at the 0.05, 0.01 and 0.001 probability levels, respectively; NS - non significant; a-c mean values (± SEM) followed by different letters within a column and particular factor are significantly different (Duncan, α=0.05).

CONCLUSIONS

The article presents the results of producing oil pumpkins in the seventh and eighth year of a long-term field trial with four different production systems at the University Agricultural Centre Pohorski Dvor of the University of Maribor. We can conclude that the BD (biodynamic) and ORG (organic) production systems result in higher soil Nmin contents, because of a more efficient mineralisation than in CON (conventional) and INT (integrated) production systems. The AE response of oil pumpkins, considering the amount of added nutrients in biodynamic and organic production systems, is better or equal when compared to a conventional production system. Similarly, that also holds true for the hybrid variety. By using an appropriate system in all cultivation treatments, we can expect equal or significantly higher parameters of fruit characteristics and higher seed yields in ORG and BD production systems when compared to the other production systems (INT, CON). The use of the hybrid instead of the population variety can increase the yield significantly and express a more stable yield even in years when the soil and climate conditions are less favourable.

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Vpliv pridelovalnih sistemov in sort oljnih buč (*Cucurbita pepo* L. group *Pepo*) na pridelek ter agronomsko učinkovitost hranil

IZVLEČEK

V letih 2013 in 2014 smo na trajnostnem poskusu, ki poteka na lokaciji Univerzitetnega kmetijskega centra Pohorski dvor že od leta 2007, z različnimi pridelovalnimi sistemi (konvencionalni, integrirani, ekološki, biodinamični) izvedli poljski poskus. Namen raziskave je bil preučiti vplive različnih pridelovalnih sistemov, sort (hibridna in populacijska sorta) ter leta na pridelavo oljnih buč. Tehnike pridelave so bile izvedene v skladu z veljavno zakonodajo in standardi za posamezni pridelovalni sistem. V času setve, pred dognojevanjem v začetku junija in ob spravilu pridelka smo analizirali količino mineralnega dušika v tleh. Vrednotili smo število in maso dozorelih, nedozorelih in propadlih plodov buč, pridelek bučnic ter izračunali agronomsko učinkovitost dodanih hranil. Sklepamo, da imajo leto, pridelovalni sistem in sorta statistično značilen vpliv na oblikovanje pridelka in pridelek bučnic. Na količino mineralnega dušika v tleh v maju in septembru vplivata pridelovalni sistem in leto, v juniju le pridelovalni sistem. Hibridna sorta oblikuje večji pridelek bučnic v vseh pridelovalnih sistemih, tudi v neugodnih rastnih razmerah posameznega leta. Agronomska učinkovitost dodanih hranil v biodinamičnem in ekološkem pridelovalnem sistemu je, v primerjavi s konvencionalnim, večja ali enaka, podobno velja za hibrid v primerjavi s starejšo sorto. Ob primerljivi oskrbi oljnih buč s hranili in kakovostno izvedbo pridelovalnih ukrepov v povezavi z novejšo sorto, lahko tudi v biodinamičnem in ekološkem načinu kmetovanja pričakujemo primerljive pridelke buč in bučnic v primerjavi z manj trajnostno naravnanimi sistemi pridelave, ki v naravi mnogokrat povzročajo škodo.

Ključne besede: pridelovalni sistemi, sorta/hibrid, oljna buča, mineralni dušik, agronomska učinkovitost, pridelek.

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DE GRUYTER OPEN

Growth performance, haematology and serum biochemistry of West African dwarf sheep fed cassava peel - oil palm leaf meal based diets in a hot humid tropics

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ABSTRACT

The growth performance, haematological and serum biochemical characteristics of thirty-six West African Dwarf (WAD) sheep of about 10 – 12 months of age and averaged 8.53kg in weight were sourced from the College flock. Four dietary treatments designated as T1, T2, T3 and T4 were formulated to contain 0%, 10%, 20% and 30% oil palm leaf meal (OPLM) respectively were randomly assigned to the animals. The experimental animals were divided into four groups of nine animals each, with each group replicated thrice with three animals per replicate. Each group was allotted to one of the diets in a completely randomized design. The animals were weighed at the beginning of the trial and weekly subsequently and data for growth performance were generated. Blood samples were obtained from one animal in each replicate, and data generated were analyzed statistically. Average daily feed intake, total dry matter intake and average daily weight gain were significantly (P<0.05) influenced by the dietary treatment, with animals on T4 group having higher and better values. Feed conversion ratio (FCR) also differed significantly (P<0.05) with sheep on T3 and T4 (11.82 and 11.49 respectively) having the best FCR. The haematology showed that the packed cell volume (PCV), red blood cell (RBC), and mean cell haemoglobin concentration (MCV) were significantly (P < 0.05) improved at 10%, 20% and 30% inclusion levels of OPLM, respectively. Sheep in treatment groups had improved (P < 0.05) white blood cell (WBC) count than those on the control group. Serum biochemistry results showed that total protein, albumin and globulin were significantly influenced (P < 0.05) and tended to increase with increasing levels of test ingredient. Sheep in treatment groups had higher (P < 0.05) urea values than those on control. Creatinine values at 20% and 30% inclusion differed significantly (P < 0.05) with the control value. Cholesterol was significantly influenced (P < 0.05) and followed an irregular trend across the treatment groups. The results showed that inclusion of OPLM had a beneficial effect on the general performance of the WAD sheep. Therefore, 30% OPLM supplementation was recommended for optimum performance in WAD sheep.

Keywords: Sheep, supplemental diets, proximate composition, oil palm leaf meal, cassava peel, blood parameters

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INTRODUCTION

Sheep is an important source of animal protein and contributes immensely to the diversification of livestock production, thus making it an integral part of the tropical agricultural system. In Nigeria, sheep are found in all agro-ecological zones of the country. They are used for meat, milk, hair (wool), skin and as a financial reverse for the rural and peri-urban population as well as playing sociocultural roles in customs and traditions of many Nigerian societies especially in a region dominated by Muslims. Nigeria is known to have the largest sheep population in Africa (33.9 million), which constitutes about 3.1% of the 1078.2 million sheep in the world (FAOSTAT 2008), and this population may be higher today thus making sheep a major contributor to animal protein. There are four main indigenous breeds of sheep in Nigeria; West African Dwarf (WAD), Balami, Ouda (Uda as the case may be) and Yankasa sheep.

The WAD sheep is a trypanotolerant small ruminant meat breed distributed widely within the rain forest and derived Savanna zones of the humid Tropics (Jiwuba *et al.* 2016a). The ewes are characteristically good mothers with proven records on multiple births, good milking ability and ability to be suckled soon after lambing. They are however less fancied than their caprine counterparts for reasons bothering more on religion and meat quality; but nevertheless, contribute substantially to the annual domestic meat consumption (Jiwuba *et al.* 2016a). The production system for this breed is mostly extensive in the south eastern Nigeria and has been left in the hands of peasant/subsistent farmers and rural families who mostly rear 1-18 of these animals per household. During the dry season, the natural feeds (roughages) available for the animal become highly fibrous and deficient in most essential nutrients required for increased rumen microbial fermentation and improved performance of the animal (Jiwuba *et al.* 2016b); thus the need for a dry season supplementation. The use of cassava peel and oil palm leaf meal could be considered in this respect as a cheap, local and available dry season supplement.

The agricultural wastes of importance in this study are cassava peels and oil palm leaves which are in abundance and hold inestimable values in ruminant feeding. Cassava is the major staple food in Nigeria, thereby enhancing the availability of the peels which are grossly underutilized and are hitherto discarded as agricultural waste (Jiwuba and Ezenwaka 2016). Oil palm leaves are obtained from pruning old midrib or harvesting palm fruit, while some people intentionally harvest fresh frond for direct feeding for their small ruminants. Cassava peel has been implicated with low protein content; hence the need to fortify it with higher protein resource. Oil palm leaf meal has a relatively high crude protein content of 16.24% (Ukanwoko *et al.* 2013) needed to maintain the ruminal environment. The leaves have also been reported to have antioxidant and so many other health benefits (Yin *et al.* 2013; Mohamed 2014). Recent efforts to optimize feed utilization of agricultural waste have been focused on different processing methods. Therefore, the processing of the cassava leaves and the oil palm leaves into meal in this study is centered toward enhancing their utilization. The study was therefore designed, to determine the growth performance, haematology and serum biochemistry of West African Dwarf sheep fed cassava peel-oil palm leaf meal supplement.

MATERIALS AND METHODS

Location of the experiment: The experiment was carried out at the sheep and goat unit, Federal College of Agriculture, Ishiagu, Ivo L.G.A., Ebonyi state, Nigeria. The College is located at about three kilometers (3km) away from Ishiagu main town. The College is situated at latitude 5.56^{0}N and longitude 7.31^{0}E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.50^{0}C and relative humidity of about 80%.

Sources and processing of experimental material: The cassava peels used in this study were sourced from the garri (a popular creamy-white West African food with a slightly fermented flavour and a slightly sour taste made from fermented, gelatinized fresh cassava tubers) processing unit, Federal College of Agriculture, Ishiagu, Ebonyi State. The peels were subsequently sundried dried (to ensure proper removal of HCN which abound in cassava and its by-products) to about 10% moisture before milling and used in the formulation of the experimental diets. Fresh green oil palm (*Elaeis guineensis*) leaves were also harvested within the College from the oil palm plantation unit. The oil palm leaves were shade-dried in batches, milled and also used at different levels in the formulation of the experimental diets.

Experimental diets: Experimental diets designated as T1, T2, T3 and T4 were formulated from cassava peel, brewers dried grain, wheat offal, oil palm leaf meal, bone meal, molasses and common salt. Diet T1 served as a positive control and contained 0% of oil palm leaf meal. Diets T2, T3 and T4 contain 10%, 20% and 30% levels of oil palm leaf meal (OPLM) respectively as illustrated in Table 1.

Management of experimental animals: Thirty-six WAD sheep of about 10 – 12 months of age and averaged 8.53kg in weight were sourced from the College flock. The experimental animals were divided into four groups of nine animals each, with each group replicated thrice with three animals per replicate. Each group was allotted to one of the diets in a completely randomized design. Care was taken to balance the weight of these animals among the groups. Each animal received the designated treatment diet in the morning for 87 days. Each animal was housed in a compartment measuring 10 × 15 m in an open-sided sheep house, and feed offered was based on 3.5% body weight per day; the animals in addition, were fed 2kg wilted *Panicum maximum* later in the day. Regular access to fresh drinking water was made available. Feed offered and refused were recorded on a daily basis. Initial weights of the animals were taken at the beginning of the trial and weekly subsequently. The experimental animals were acclimatized for 21 days before the commencement of the study in accordance with the permission and stipulated guidelines of the Federal College of Agriculture, Ishiagu (FCAI) Animal Ethics Committee. During the acclimatization period, the animals were dewormed and sprayed against external parasites. They were dewormed using kepromec (Ivermectin) subcutaneously and given

Table 1: composition of the experimental diets

Ingredients (%)	T1	T2	T3	T4
Cassava peel meal	55.00	55.00	55.00	55.00
Wheat offal	10.00	10.00	10.00	10.00
Brewers dried grain	30.00	20.00	10.00	0.00
Oil palm leaf meal	0.00	10.00	20.00	30.00
Molasses	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Common salt	1.00	1.00	1.00	1.00
Total	**100.00**	**100.00**	**100.00**	**100.00**

acaricide bath using Roys' Amitraz 20 at the rate of 1ml in 2 litre water prior to the experiment.

Blood profile studies: Blood samples (10 ml) were drawn from the animals on the last day (87) of the study. The sheep were bled through the jugular vein. The samples were separated into two lots and used for biochemical and haematological determinations as described by Dacie and Lewis (1991). An initial 5ml was collected from each sample in a labelled sterile universal bottle containing 1.0 mg/ml ethylene diamine tetraacetic acid (EDTA) and used for haematological analysis. Another 5ml was collected over the anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), MCV and mean cell haemoglobin concentrations (MCHC) were calculated.

Chemical Analysis: Proximate analysis of different experimental diets and that of the test ingredients were carried out at the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Animal Nutrition Laboratory, according to the methods of AOAC (2000).

Statistical Analysis: The data obtained 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. Differences between treatment means were separated by Duncan New Multiple Range Test as outlined by Obi (1990).

RESULTS

The proximate composition of the experimental diets, cassava peel meal (CPM) and OPLM are presented in Table 2. The performance of WAD sheep fed the cassava peel-oil palm leaf meal based diets is shown in Table 3. Total weight gain, average daily weight gain, (ADWG), total feed intake, average daily feed intake (ADFI), total dry matter intake (TDMI) and feed conversion were significantly different ($P<0.05$) while final body weight gain was similar ($P>0.05$) across the treatment groups. The haematological characteristics of WAD sheep fed cassava peel-oil palm leaf meal based diets are presented in Table 4. Packed cell volume (PCV), red blood cell (RBC), mean cell haemoglobin concentration (MCHC) and white blood cell (WBC) were significant ($P<0.05$) while haemoglobin (Hb), mean cell haemoglobin (MCH) and mean cell volume (MCV) were similar ($P>0.05$) across the treatments. The serum biochemical characteristics of WAD sheep fed cassava peel-oil palm leaf meal based diets are presented in Table 5. All the serum biochemical indices evaluated are significantly ($p<0.05$) influenced by the dietary treatment.

DISCUSSION

The dry matter (DM), crude protein (CP), crude fibre (CF), ash, ether extract (EE) and nitrogen free extract (NFE) for the experimental diets did not follow a particular trend, but all comparable with the control diet. This may be attributed to the similarities of Brewers dried grain (BDG) and oil palm leaf meal in terms of CF, and CP. The dry matter values of the experimental diets are in agreement with the reported values by Ekeocha and Fakolade (2012). The CPM values however are comparable with the values reported by Ifut (1988) and Ukanwoko (2007) for same agricultural by product. The proximate constituents of the OPLM are in agreement with the values obtained by Wong and Zahari (1992) and Esonu *et al.* (2008). However, the differences in the proximate composition values could be attributed to the stage of development when the leaves were harvested, location, season, soil type and soil fertility, the level of dryness of the leaves and processing method used; a view collaborated by Jiwuba *et al.* (2016c).

There is an improvement in the body weight gain across the treatment groups, with T2 having the lowest body weight gain and T4 having the highest body weight gain. The lower body weight gain of T2 animals compared to other treatments maybe attributed to the lower initial body weight of the T2 animals. The highest body weight observed in T4 animals may-be attributed to the higher feed and dry matter intake observed in this treatment group. Hence dry matter intake is an important factor in the utilization of feed by ruminants (Jiwuba *et al.* 2016b). In earlier reports (Rout *et al.* 2009; Chong *et al.* 2012) palm oil leaves have been reported to be high in many biologically active compounds like flavonoids, which can be of great significance to increase palatability which further can increase intake. This in agreement with the earlier report by Schlolant (1983) who opined that the quantity of feed consumed is largely dependent on the palatability of the diet. The higher and better total feed intake and ADFI observed in T4 may be attributed to

Table 2: Proximate composition of the experimental diets, cassava peel meal and oil palm leaf meal.

Compositions	T1	T2	T3	T4	CPM	OPLM
Dry matter	90.25	90.45	90.21	91.73	93.20	89.11
Crude protein	10.48	11.55	13.03	13.79	5.10	17.23
Crude fibre	26.09	24.83	23.08	24.12	16.11	18.37
Ash	9.35	8.70	15.05	10.32	22.56	9.01
Ether extract	0.45	0.86	0.64	1.22	3.94	3.91
Nitrogen free extract	43.88	44.51	38.41	42.28	45.49	40.59

OPLM=Oil palm leaf meal; CPM= Cassava peel meal

Table 3: Growth performance of WAD sheep fed cassava peel-oil palm leaf meal based diets.

Parameters	T1	T2	T3	T4	SEM
Initial body weight (kg)	8.53	7.50	9.67	8.41	0.62
Final body weight (kg)	12.77	11.00	14.33	13.63	0.64
Total weight gain (kg)	4.24^{ab}	3.50^{b}	4.66^{ab}	5.22^{a}	0.19
Average daily weight gain (g/d)	48.74^{c}	40.23^{d}	53.56^{b}	60.00^{a}	3.23
Total feed intake (kg)	53.70^{b}	50.00^{c}	55.07^{b}	58.74^{a}	3.62
Average daily feed intake (g/d)	617.24^{ab}	574.71^{b}	632.99^{ab}	675.17^{a}	11.28
Total dry matter intake	48.46^{b}	44.23^{c}	49.70^{b}	53.87^{a}	3.11
Feed conversion ratio	12.66^{ab}	14.29^{a}	11.82^{b}	11.49^{b}	0.81

$^{a-c}$ Means within the same row with different superscripts are significantly different (P < 0.05)

Table 4: Haematological indices of WAD sheep fed cassava peel-oil palm leaf meal based diets.

Parameters	T1	T2	T3	T4	SEM
Packed cell volume (%)	29.71^{b}	32.25^{a}	33.30^{a}	31.29^{a}	0.19
Haemoglobin (g/dl)	9.35	10.40	10.60	10.21	0.15
Red blood cell ($x10^{12}$/L)	12.08^{b}	12.53^{a}	12.60^{a}	12.99^{a}	0.06
Mean cell haemoglobin conc. (%)	36.50^{a}	34.25^{b}	33.68^{b}	36.27^{a}	0.23
Mean cell haemoglobin (pg)	10.35	9.50	10.26	10.44	0.21
Mean cell volume (fl)	34.80	32.70	32.85	33.81	0.20
White blood cell (x 10^{9}/L)	15.0^{c}	16.82^{b}	16.55^{a}	16.76^{a}	0.16

$^{a-b}$ Means within the same row with different superscripts are significantly different (P < 0.05)

Table 5: Serum chemistry indices of WAD sheep fed cassava peel-oil palm leaf meal based diets.

Parameters	T1	T2	T3	T4	SEM
Total protein (g/dl)	6.79^{b}	6.58^{b}	7.56^{a}	7.83^{a}	0.17
Albumin (g/dl)	2.91^{c}	3.21^{b}	4.11^{a}	4.04^{a}	0.09
Globulin (g/dl)	3.72^{b}	4.42^{a}	4.70^{a}	4.99^{a}	0.11
Urea (mg/dl)	12.68^{c}	14.37^{b}	16.32^{a}	17.02^{a}	0.31
Creatinine (mg/dl)	1.61^{b}	1.75^{b}	1.89^{a}	1.87^{a}	0.03
Cholesterol (mg/dl)	54.97^{c}	60.87^{a}	59.13^{b}	56.37^{bc}	0.21

$^{a-c}$ Means within the same row with different superscripts are significantly different (P < 0.05)

improved palatability of the diet. Palatability which is the degree of acceptability of a diet is a function of the diet and post-ingestive feedback. However, the lowest total feed intake and ADFI observed in T2 may be attributed to lower dry matter composition of the diet. Even though there was no statistical (p>0.05) difference between the T3 and T4, the latter had the least FCR indicating a better feed conversion ratio (FCR). The superior feed efficiency of diets T3 and T4 over the other diets is a reflection of the observed higher feed utilization and indeed higher growth rates of the sheep fed the respective diets. Factors which influence FCR among other include breed and sex of animals as well as nutrition and environment.

Jiwuba *et al.* (2016d) reported that PCV is generally used as an index of toxicity and its value is been influenced by breeds, age and sex of the animal. The PCV obtained in the present study (29.71 to 33.30%) is however compared with 28.00 - 34% and 27.3 - 32.3% for West African Dwarf sheep fed graded levels of *Gmelina arborea* leaves and cassava peel concentrates and West African Dwarf sheep fed Panicum-cassava peels supplemented with or without *Leucaena*-based multinutrient blocks reported by Aye and Tawose (2016) and Aye (2013). The values obtained in this study also fell within the normal physiological range of 24 – 45% and 27-45% reported for apparently healthy sheep by RAR (2009) and Kramar (2000) respectively and 27.75 – 36.67% for WAD ram reported by Oyeyemi and Ajani (2014). This thus indicated stability in the physiological status and possible absence of toxic factors affecting blood homeostasis in the experiments animals. The significant improvement in PCV values for the animals on the test diets is also in agreement with the findings of Ukanwoko *et al.* (2013) who fed oil palm leaf meal – cassava peel based diets to WAD goats. The haemoglobin values though (P>0.05) similar across the groups are within the normal physiological of 8-16g/dl and 9-15g/dl as reported by RAR (2009) and Kramar (2000) respectively for sheep and 9.13 – 12.27g/dl for WAD ram as reported by Oyeyemi and Ajani (2014). Increase in the Hb values is generally linked with the greater ability to the resistance of disease and the low level is an indication of disease infection and poor nutrition. The animals on the treatment diets (T2, T3 and T4) recorded higher values in RBC than the sheep on the control diets. The RBC values obtained in this study are within the normal range of 10.82–16.11($X10^{12}$/L) reported by Oyeyemi and Ajani (2014) for WAD rams and 9-15 ($X10^6$μl or $X10^6 mm^3$) reported by Jain (1993) and Kramar (2000) for sheep. The normal physiological range of RBC reported in this study for the WAD rams is an indication of the absence of anemia, normal oxygen circulation to the tissues and carbon dioxide to the lungs. Mean corpuscular haemoglobin concentration (MCHC) is normally a further measurement that can assist in the diagnosis of abnormalities affecting red cell counts. MCHC is very important in the diagnosis of anaemia and also serve as a useful index of the capacity of the bone marrow to produce red blood cells (Etim *et al.* 2013). The within normal physiological range of 33-38% reported by Oyeyemi and Ajani (2014) for WAD ram and 31-38g/dl reported by RAR (2009) for apparently healthy sheep gave a clear indication of the absence of anaemia among the experimental groups. The white blood cell function to fight infections, defend the body through phagocytosis against invasion by foreign organisms and to produce or transport antibodies in immune response. The decrease in WBC is an indication of fall in the production of defensive mechanism to combat infection (Ehebaet et al. 2008). The normal physiological values of WBC obtained in this study may suggest well developed immune system of the experimental animals or absence of foreign bodies or parasites in the circulatory system of experimental animals. This perhaps highlights the ethno-veterinary properties of oil palm leaves (*Elaeis guineensis*) as reported by Yin et al. (2013) and Mohamed (2014). Also, the significant variations in the WBC counts of sheep on treatment diets could be attributed in part to improved digestibility and utilization of nutrients in the diets (Jiwuba et al. 2017).

The total protein did not follow a regular pattern but however fell within the range of 6.0–7.9 g/dl reported for apparently healthy sheep (Fielder 2016). The higher total protein observed in T3 and T4 may-be attributed to the higher dietary protein of the respective diets as evidenced in the proximate composition. This is in agreement with the findings of Jiwuba et al. (2016e) who observed a positive relationship between serum protein and dietary protein. This further demonstrates better utilization of the dietary proteins by experimental animals especially those in T3 and T4, thereby aiding total protein availability. The range of values 2.91-4.11g/dl reported in this study is above the normal physiological of 2.40-3.30g/dl reported by Kaneko (1989) for apparently healthy sheep. Albumin functions chiefly in the regulation of colloidal osmotic pressure of the blood, assist in the movement of fatty acids, hormones, bilirubin, cations and drugs in the blood. However, the high albumin level (above normal range) observed in T3 and T4 may-be attributed to the higher dietary protein of the respective treatments. The range of 3.72-4.99g/dl for serum globulin reported in this present study fell within the normal of 3.50-5.70 g/dl according to Fielder (2016). This is an indication of proper functioning of the liver and high immunity response of the experimental animals. Hence, Rastogi (2008) reported that globulins function to carry lipid fraction of proteins and in the transportation of antibodies for generating an immune response. The serum urea in this study nevertheless, fell within the normal range of 8-20g/dl reported by Fielder (2016). The normal range of values reported in this study indicated therefore that the dietary protein was well utilized since urea is used as an index of renal function. The creatinine was highest (1.89mg/dl/) in T3 and lowest (1.61mg/dl) T1 and nevertheless fell within the normal range of 1.2 - 1.9 mg/dl for apparently healthy sheep as reported by Kaneko (1989) and Fielder (2016); thus suggesting that there was no wasting or catabolism of muscle and that the animals did not survive at the expense of body reserve. Hence, Prvulovic et al. (2012) noted that creatinine level is directly correlated with muscle mass and kidney function in animals. Cholesterol is the precursor of cholesterol ester, bile acids and steroid hormones. Cholesterol is implicated in vascular disease and is of diagnostic importance in hypothyroidism. The serum cholesterol in this study nevertheless, fell within the normal range of 52-76mg/dl reported by Fielder (2016). Serum Cholesterol has been associated with the quality and quantity of fat in the diet (Esonu *et al.* 2001). High serum cholesterol has for long been implicated in the etiology of arteriosclerosis and other heart diseases (Mc Donald *et al.* 1995; Ramos *et al.* 2003).

CONCLUSION

The results of the study revealed that varying levels of oil palm leaf meal in a cassava peel meal based diet for West Africa Dwarf (WAD) sheep is safe and enhanced feed and dry matter intake, body weight gain, blood homeostasis and health status of the animals. Oil palm leaf meal can, therefore, be included up to 30% without adverse effect on the performance of West Africa Dwarf (WAD) sheep.

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Vpliv krmljenja pritlikave zahodnoafriške pasme ovac vzrejenih v vlažnih tropskih razmerah z zmesjo ostankov lupljenja gomoljev kasave in listov oljne palme na rastnost in krvno sliko

IZVLEČEK

Rastnost, hematološke in biokemijske serumske lastnosti so bile proučevane pri 36. ovcah pritlikave zahodno afriške pasme, pri starosti 10 do 12 mesecev in poprečni telesni masi 8,53 kg. Živali so izvirale iz črede v lasti visoke šole. Štirje različni obroki so bili pokladani živalim T1, T2, T3 in T4, ki so vsebovali 0%, 10%, 20% in 30% zmesjo ostankov lupljenja gomoljev kasave in listov oljne palme (OPLM) v obroku ovac.

Proučevane živali so bile ločene v štiri skupine, vsaka je imela po devet živali, in znotraj te so bile deljene v tri ponovitve, v vsaki so bile po tri živali. Vsaka skupina živali je dobila specifičen obrok po naključnem oblikovanju poskusa. Živali so bile individualno tehtane tedensko. Vzorci krvi so bili odvzeti eni živali iz proučevane skupine. Poprečna konzumacija krme, celokupna konzumacija suhe snovi in velikost dnevnega prirasta so bili pod značilnim vplivom obroka. Ovce skupine T4 so se značilno boljše v proučevanih lastnostih in so se značilno ($P < 0,05$) razlikovale od ostalih skupin. Statistično značilno najboljšo konverzijo krme so imele ovce skupine T3 in T4 ($P < 0,05$). Vsebnost rdečih krvnih celic ločenih od plazme (PCV), rdeče krvne celice in koncentracija hemoglobina (MCV) je bila značilno ($P < 0,05$) izboljšana v obrokih z 10%, 20% in 30% OPLM. Prav tako so imele proučevane skupine v krvi značilno večjo vsebnost levkocitov ($P < 0,05$) kot kontrola. Vsebnost skupnih proteinov, albumina in globulina je bila značilno ($P < 0,05$) pod vplivom naraščajočih vrednosti dodatka OPLM v krmi ovac. Ovse krmljene z dodatkom OPLM so imeli značilno ($P < 0,05$) večjo vsebnost uree v primerjavi s kontrolno skupino. Prav tako je pri vključitvi 20% in 30% OPLM v obroku ovac imelo značilen ($P < 0,05$) vliv na vsebnost kreatina v primerjavi s kontrolo. Vsebnosti holesterola so bile pod značilnim ($P < 0,05$) vplivom OPLM, vendar se ni pokazal določen vzorec na osnovi katerega bi lahko naredili zaključke. Rezultati poskusa so pokazali, da ima OPLM dobrodejen na večino rastnih lastnosti pritlikave pasme zahodnoafriških ovac. Priporočljiva je uporaba 30% dodatka OPLM v obroku ovac za doseganje optimalnih rastnih lastnosti.

Ključne besede: ovca, krmni dodatki, kasavini olupki, palmini listi, krvni parametri