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REACTION OF SPRING BARLEY TO LONG-TERM MONOCULTURE IN DIVERSIFIED CONDITIONS OF CHEMICAL PROTECTION

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Key words: spring barley, crop rotation, monoculture, herbicides, fungicides, yielding.

Abstract

The reaction of spring barley to 38–47-year monoculture carried out in 10-year studies (2005–2014) on albic luvisol soil at the Production and Experimental Station in Bałcyny near Ostróda town in different conditions of chemical protection has been presented. The following chemical protections of a barley canopy: 0 – without protection; *H* – protection from weeds and HF – protection from weeds and diseases have been considered. The cultivation of spring barley in 6-field crop rotation: sugar beet – maize – spring barley – pea – winter rape – winter wheat was as a comparative object. In a crop rotation the average yield of spring barley grain amounted to 6.88 t ha^{-1}. The cultivation in 38–47-year monoculture has decreased its yielding on average by 20.4%. The biggest decrease of its productivity took place on the object without protection – 27.5%. The application of herbicides has limited the decrease to 17.5% while combined application of herbicides and fungicides up to 16.5%. Less decrease is the result of plant protection products higher efficacy in monoculture than in a crop rotation. Herbicides on that field has increased spring barley yield on average by 17.9%, the combined application of fungicides and herbicides by 24.8% while in the case of a crop rotation by 3.6 and 8.3% respectively. Worse yielding of spring barley in a monoculture are conditioned by thinning out density of ears per 1 m^2, and the decrease of grains weight from an ear and the weight of 1.000 grains.

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Introduction

The cultivation of wheat, rice, maize and barley dominates agricultural land in many countries of the world (FAOSTAT 2017). Appropriate succession of crops based on environmental factors in connection with the appropriate agricultural practices to a large degree ensures a successive increase in spring barley yields (ADAMIAK et. al. 2000, BLECHARCZYK et. al. 2005, GAWĘDA and KWIATKOWSKI 2013, GAWĘDA et. al. 2014). In crop rotations with a large share of cereals, particularly in monocultures, undesirable phenomena may occur, mainly stress from pests and unbalanced depletion of nutrients in soil. These phenomena, in turn, are the main reasons for reduced productivity (ADAMIAK et. al. 2000, BERZSENYI et al. 2000, JOŃCZYK and KAWALEC 2001, LEMAŃCZYK 2002, TANAKA et al. 2002, BLECHARCZYK et al. 2004, WOŹNIAK 2006, BURACZYŃSKA and CEGLAREK 2008, FERNANDEZ-GETINO et al. 2015). As far as the negative response to cultivation in a monoculture, wheat is the most sensitive (NORWOOD 2000, ADAMIAK 2007, WESOŁOWSKI et al. 2007). The high sensitivity of barley to this system of crop succession results to a large degree from its high vulnerability to diseases of the base of the blade (BLECHARCZYK et. al. 2005, KUROWSKI and ADAMIAK 2007, WANIC et. al. 2012). The negative effect of spring barley on a monoculture is also connected with its weak competitiveness towards weeds (WESOŁOWSKI et. al. 2003, BLECHARCZYK et. al. 2005, ADAMIAK 2007, FERNANDEZ-GETINO et al. 2015, ADAMIAK et. al. 2019). On the other hand, due to the importance of barley in human and animal nutrition, it cannot be expected that its share will decrease in the cultivation structure. The negative effects of its large share in crop rotations may be alleviated by introducing factors reducing undesirable succession (ADAMIAK et. al. 2000, KWIATKOWSKI 2009, WANIC et. al. 2012). In some cases, in order to compensate for the negative effect of spring barley monoculture on the yield level and weed infestation, it is sufficient to introduce intercrops or organic fertilization (YANG et. al. 2000, BLECHARCZYK et. al. 2005, TURKINGTON et. al. 2005, KWIATKOWSKI 2009). Achieving high yields requires the application of chemical protection, however, in the opinion of some authors (ADAMIAK et. al. 2000, KWIATKOWSKI 2004, STUPNICKA-RODZYNKIEWICZ et. al. 2004, GAWĘDA et. al. 2013) the comprehensive protection of spring barley only partially compensates the effects of inappropriate crop succession.

The aim of this article was to determine the impact of plant protection (herbicides and fungicides) on the yielding of spring barley grown in a 38–47-year monoculture.

Materials and Methods

The field experiment on plots concerning the crop of 10 species of plants in monoculture has been set up in autumn 1967 at the Production and Experimental Station in Bałcyny (53°36′ N, 19°51′ E, Poland) village near Ostróda town, on albic luvisol soil formed from sandy clay. Its composition was as follows: 2.8% – 3.2% of fractions with their diameters below 0.002 mm; 30.4–31.9% of fractions with their diameters range – 0.002–0.050 mm, and 64.9–66.8% of fractions with their diameters range 0.050–2.00 mm, respectively. Till 1992 the cultivation of 10 species of plants in two 5-field crop rotations was as a comparative object. During the growing season 1992/1993 the winter triticale and pea were introduced into the experiment and from that time two 6-field crop rotations were as a comparative object.

This paper presents results of a 10-year experiment (2005–2014) concerning spring barley response to a 38–47 – year monoculture. Previous results were published in ADAMIAK (2007).

Before starting the present research, the soil was characterized by the following chemical properties: pH value in 1M KCl solution was equal 5.7–6.0; C_{org} content amounted to 0.90–0.96%, and the content of available macroelements as follows: phosphorus (P) 8.9–9.6 mg; potassium (K) 15.0–20.7 mg; calcium (Ca) 48–56 mg, and magnesium (Mg) 6.4–7.0 mg in the sample of 100 g of the soil.

The following research factors in 3 replications in this experiment have been considered:

I. Crop sequence system:

A. The cultivation of spring barley in 6-field crop rotation: sugar beet – maize – spring barley – pea – winter rape – winter wheat.

B. The cultivation of spring barley in a 38–47-year monoculture.

II. Chemical protection.

0 – without protection;

H – protection from weeds;

HF – protection from weeds and diseases.

Weeds were controlled with the following herbicides: Chisel 75 WG (a.s.: thifensulfuron-(methyl) + chlorosulfuron) – 2005, 2006, 2009 and 2010; Mustang 306 SE (a.s.: florasulam + 2,4 D) – 2007, 201, 2012 and 2014; Chwastox Turbo 340 SL (a.s.: MCPA + dicamba) + Puma Uniwersal 069 EW (a.s.: phenoxaprop-P-ethyl + mefenpyr diethyl) – 2008, 2013. Fungal diseases were controlled through 1-2-fold spraying in the growing season of spring barley with the following fungicides: Amistar 250 SC (a.s.: azoxystrobin); Amistar 250 SC + Artea 330 EC (a.s.: propiconazole + cypro-

conazole); Capalo 337,5 (a.s.: phenpropimorphe + metrafenone + epoxiconazole); Fandango 200 EC (a.s.: prothioconazole + fluoxastrobin).

Spring barley was sown in the amount of 300 sprouting grains per 1 m^2 between 27 March and 14 April. Mineral fertilization was identical in both crop rotation systems and amounted to 184 kg ha^{-1} of NPK (N-70; P-31; K-83). Additionally every 3 years the monoculture of spring barley was fertilized with manure in the amount of 15 t ha^{-1} in order to equal the organic fertilization applied in the case of the sugar beet crop rotation (30 t ha^{-1}).

In the analysed 10-year experiment, the influence of the experiment factors on the yield of grains from one hectare, and on the number of ears per of 1 m^2 surface, length of an ear, the number and a weight of grains from an ear, the weight of 1.000 grains have been carried out. In order to assess relationships between the yield of grains and the aforementioned parameters as well as the number and the weight of weeds per 1 m^2, the classical methods of correlation and simple regression were used. In the analyses of variance, correlation and regression, the statistic package STATISTICA® 13 has been applied.

In stages of the biggest demand of spring barley for water, precipitations were optimal in general (Table 1). A water shortage that caused lower yielding in the case of spring barley took place in the growing seasons 2011/2012 only. The discussed growing seasons differed in thermal conditions. In comparison with the long-term average values (1981–2012),

Table 1

Sum of precipitation [mm] in the years 2004–2014 (data according to the Research Station at Bałcyny, 53°36′ N, 19°51′ E, Poland)

Growing season	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sum IX–VIII
2004/2005	41.9	77.6	27.8	39.5	50.3	21.4	29.3	22.0	68.2	35.4	83.9	39.6	537
2005/2006	17.9	19.3	31.1	82.9	15.3	26.7	3.1	24.2	93.2	83.5	27.1	141.7	566
2006/2007	105.6	34.3	107.3	60.0	110.2	14.6	27.9	26.8	79.7	60.8	176.5	81.0	885
2007/2008	65.4	48.9	50.0	9.0	30.8	33.9	47.1	33.8	48.4	27.8	47.0	103.1	545
2008/2009	17.0	104.6	40.5	29.4	16.2	14.7	68.0	3.7	89.6	133.1	82.2	25.7	625
2009/2010	15.6	58.5	40.8	29.6	13.3	14.2	23.8	9.4	105.5	73.7	87.8	99.3	572
2010/2011	45.0	11.2	110.4	39.2	29.6	20.5	8.6	33.7	41.5	56.2	171.9	83.6	651
2011/2012	38.9	29.9	9.6	46.0	87.7	24.9	21.3	44.7	42.5	107.2	11.2	25.7	490
2012/2013	41.0	57.6	48.5	15.1	34.6	21.3	14.0	22.5	46.2	45.4	163.8	37.6	548
2013/2014	69.3	15.4	23.2	34.1	44.0	11.4	55.7	26.1	34.9	72.2	20.4	35.0	442
1981–2012	56.2	51.2	46.1	42.6	30.1	23.1	30.7	29.8	62.3	72.9	81.2	70.6	597

warmer growing seasons were: 2006/2007 (the warmest one) 2007/2008, 2008/2009, 2011/2012 and 2013/2014; colder ones were 2005/2006, 2009/2010, 2010/2011 and 2012/2013 respectively (Table 2).

Table 2

Mean air temperature [ºC] in the years 2004–2014 (data according to the Research Station at Bałcyny, 53°36′ N, 19°51′ E, Poland)

Growing season	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Av. IX–VIII
2004/2005	13.0	9.2	2.4	2.3	0.6	-3.2	-1.4	7.7	12.5	14.9	18.9	16.8	7.8
2005/2006	15.3	8.3	2.8	-1.1	-8.7	-3.3	-2.5	7.8	12.5	16.0	21.0	17.3	7.1
2006/2007	15.7	10.1	5.6	4.2	2.4	-2.0	5.4	7.3	13.7	17.5	17.5	18.2	9.6
2007/2008	12.6	7.4	1.0	0.4	0.7	2.3	2.9	7.8	12.3	16.6	18.3	17.8	8.3
2008/2009	11.8	8.7	4.0	-0.1	-3.7	-1.5	1.9	9.7	12.2	14.7	18.9	18.5	7.9
2009/2010	14.7	5.9	5.2	-1.7	-8.9	-2.9	2.1	7.9	12.0	15.7	20.8	19.3	7.5
2010/2011	12.2	5.3	4.4	-6.9	-1.6	-6.1	2.0	9.7	13.6	17.5	18.0	18.1	7.2
2011/2012	14.6	8.6	3.1	2.4	-2.0	-7.5	3.5	8.4	13.9	15.2	19.0	17.9	8.1
2012/2013	14.0	7.9	4.9	-3.3	-4.5	-0.8	-4.0	6.3	15.0	17.4	17.9	17.4	7.4
2013/2014	11.5	9.3	4.9	2.3	-3.5	2.0	5.5	9.5	13.3	14.8	21.0	17.0	9.0
1981–2012	13.0	8.1	2.8	-1.0	-2.4	-1.6	1.8	7.7	13.2	15.8	18.3	17.7	7.8

Results

Spring barley was cultivated in a crop rotation in the period 2005–2014; its average yield amounted to 6.88 t ha^{-1} (Table 3). In particular years its yield varied from 6.36 (2011) to 7.36 t ha^{-1} (2007).

In the case of monoculture in the tested 10-year experiment, the average yield amounted to 5.48 t ha^{-1} of grains i.e. by 20.4% less than in the crop rotation. The biggest yield decline i.e. by 34% was stated in 2006, in the 39^{th}-year of spring barley cultivation in a monoculture. After this quite deep slump, the decreases of yields in next years have stabilized on the level 15–26%, except for 2011 (44^{th} year of the monoculture) and amounted to zero (0). In essence what is presented through the trend of yielding throughout of the years (Figure 1), along with the elongation of the cultivation time of spring barley in monoculture, the differences of its yielding in the compared crop rotation systems have decreased.

Table 3

Grain yields of spring barley [t ha^{-1}]

Year	Crop rotation				Monoculture				Monoculture in relative to crop rotation [%]				$LSD_{0.05}$**
	0*	*H**	HF*	means	O	*H*	HF	means	O	*H*	HF	means	
2005	7.10	7.02	6.99	7.04	5.72	5.88	6.03	5.88	80.6	83.8	86.3	83.5	I – 0.48; II – n.s.; I × II – n.s.
2006	6.34	6.84	7.27	6.82	4.28	4.64	4.65	4.52	67.5	67.8	64.0	66.3	I – 0.68; II – 0.32; I × II – 0.42
2007	7.26	7.21	7.62	7.36	5.10	5.57	5.95	5.54	70.3	77.2	78.1	75.3	I – 1.01; II – 0.25; I × II – 0.41
2008	6.43	6.81	6.70	6.66	4.63	6.08	6.26	5.66	72.0	89.3	93.4	85.0	I – 0.36; II – 0.14; I × II – 0.20
2009	6.66	6.46	7.40	6.84	4.92	5.10	5.59	5.20	73.9	79.0	75.5	76.0	I – 0.33; II – 0.25; I × II – n.s.
2010	6.81	7.22	7.60	7.21	4.98	5.24	6.07	5.43	73.1	72.6	79.9	75.3	I – 0.28; II – 0.31; I × II – 0.44
2011	6.30	6.25	6.54	6.36	4.85	6.95	7.34	6.38	77.0	111.2	112.2	100.3	I – 0.22; II – 0.17; I × II – 0.28
2012	6.39	6.71	6.89	6.66	4.18	5.13	5.39	4.90	65.4	76.5	78.2	73.6	I – 0.29; II – 0.21; I × II – 0.22
2013	6.60	7.24	7.86	7.13	4.77	5.85	6.29	5.64	75.7	80.8	80.0	79.1	I – 0.35; II – 0.16; I × II – 0.42
2014	6.62	6.81	6.89	6.77	4.53	6.15	6.37	5.68	68.4	90.3	92.5	83.9	I – 0.33; II – 0.15; I × II – 0.21
means	6.62	6.86	7.17	6.88	4.80	5.66	5.99	5.48	72.5	82.5	83.5	79.6	I – 0.31; II – 0.18; I × II – 0.25

*0 – without plant protection; *H* – protection from weeds; HF – protection from weeds and disease

**$LSD_{0.05}$: I – crop sequence system; II – plant protection; I × II – interaction

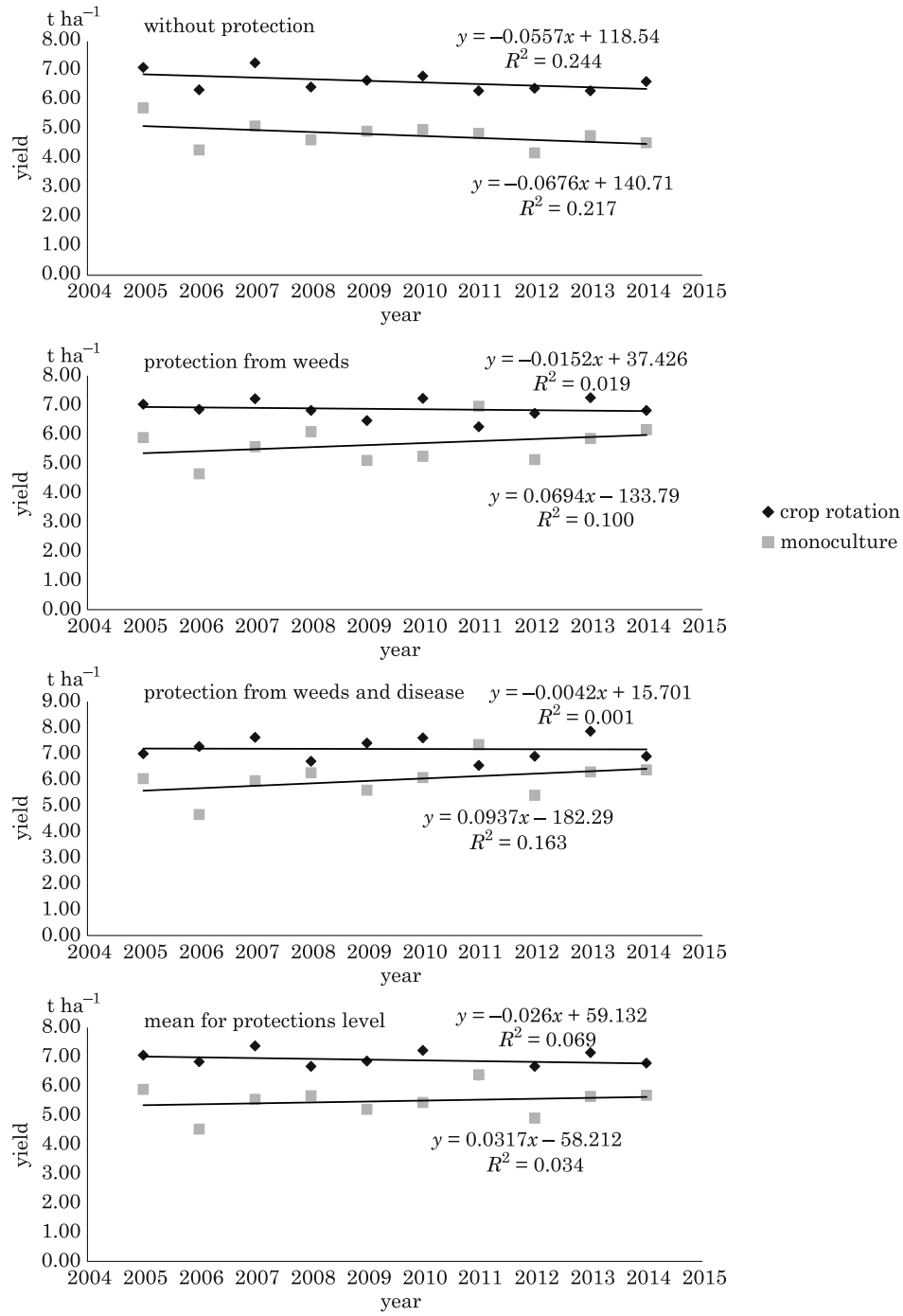

Fig. 1. Yield trend of spring barley in 2005–2014

The scale of yield reduction in monoculture depended on the control level. In the monoculture with no control, the yield of spring barley in particular years were by 19–35% lower than on the analogical crop rotation object, on average by 27.5 % less. The yielding trend presented on Figure 1 shows that continuation of spring barley cultivation through the years on that protection object in both crop sequence systems resulted to a similar decrease tendency.

The application of protection from weeds (*H*) significantly reduced the negative influence of monoculture on yielding of spring barley. On that object the yield of spring barley in 2011 (the 44^{th} year of monoculture) were by 11% bigger than in the case of a crop rotation. In the remaining years they were by 10–32% lower on average by 17.5%. The limitation of losses in the yields of monoculture results from a higher increase of spring barley productivity in the monoculture than in the case of crop rotation through the influence of herbicides activity. First of all herbicides significantly have limited the competition of such weeds as: *Avena fatua, Chenopodium album, Fallopia convolvulus, Galium aparine, Polygonum lapathifolium and Veronica persica,* that numerously occurred on a canopy of the unprotected monoculture (data not shown). The result of herbicides application has increased spring barley yielding in a monoculture in the range from 3 to 43%, on average by 17.9% (i.e.by 0.86 t ha^{-1}). In the crop rotation, the yield-creative effect of herbicides varied from its negative value i.e. (-3%) up to maximally increasing level by 15%. The average increase of the yield within the 10-year assessed period amounted to not quite 4% (0.24 t ha^{-1}). Thanks to better the yield-creative effect of herbicides in the monoculture they were not able to cause increased trend of yielding in the monoculture, and minimal decreased trend in the crop rotation (Figure 1). Therefore on this object, the difference in the yields of spring barley has expanded for the worse of the monoculture but with its continuation it has decreased.

The combined application of protection from weeds and diseases (HF) has reduced the decrease of the yields of spring barley in the monoculture on average by 16.5% (Table 3). In particular years of the cultivation the yields varied from 12% of the increase over the level in the case of the crop rotation up to 36% of the decrease. So the function of that protection variant in the compensation of the negative position, which is a monoculture, has appeared practically the same as in the case of the application only herbicides. It results from the fact these fungicides in relation to the object treated solely with herbicides have increased the productivity of spring barley in both crop sequence systems in a similar scale i.e. in monoculture on average by 5.8% (i.e. by 0.33 t ha^{-1}) while in the case of the crop

rotation by 4.5% (i.e. by 0.31 t ha^{-1}). Analyzing spring barley yielding trend through the years on this object its distinct growth tendency in the case of monoculture and practically invariable one in the case of crop rotation (Figure 1) have been noticed. It means that also on this object, the difference in the yields of spring barley between crop rotation and the monoculture has decreased with the time of that experiment continuation.

The crop sequence system the most strongly diversified the number of ears per 1 m^2 and next the weight of grains from an ear and the weight of 1.000 grains (Table 4). The aforementioned parameters have achieved the least values on unprotected object of a monoculture. They were by 6–22% smaller than on the analogical object of a crop rotation. Intensification of chemical protection has decreased the differences among positions because herbicides and fungicides more favourably increased their values in the case of a monoculture than in the case of a crop rotation. The favourable action of plant protection products taking into account the best of their efficacy has appeared in the density of ears per 1 m^2, and next the weight of grains from an ear causing the decrease of differences among crop sequence systems up to 4–10% in discussed parameters. The crop sequence systems did not diversify the number of grains in an ear.

Table 4

Simple correlation factors of spring barley yields vs. weed infestation and with elements of structure and biometry, average values for 2005–2014

Specification	Crop rotation				Monoculture			
	0*	*H**	HF*	means	0	*H*	HF	means
Number of weeds per 1 m^2	n.s.	n.s.	n.s.	n.s.	-0.41	-0.60	-0.62	-0.68
Weight of weeds per 1 m^2	-0.38	n.s.	n.s.	n.s.	-0.40	-0.55	-0.66	-0.61
Number of ears per 1 m^2	n.s.	0.38	0.47	0.47	n.s.	n.s.	n.s.	n.s.
Number of grains in ear	n.s.	0.42	0.38	0.53	n.s.	n.s.	n.s.	n.s.
Weight of grains per ear [g]	0.45	n.s.	n.s.	n.s.	-0.47	0.47	n.s.	n.s.
Weight of 1.000 grains [g]	0.42	0.48	n.s.	0.46	n.s.	0.79	0.76	0.67

**O* – without plant protection; *H* – protection from weeds; HF – protection from weeds and disease

The size of the spring barley yields in monoculture on all protection levels was significantly negatively correlated with the number and the weight of weeds per 1 m^2, and additionally on the object without protection was correlated with the weight of grains from an ear. On the objects chemically protected, the yields positively correlated with the weight of 1.000 grains, and only on the object treated with herbicides were correlated with the weight of grains from an ear (Table 5). In the case of the crop rotation significantly negatively impact on spring barley yielding on the object

without protection had the weight of weeds per 1 m^2 while positive one had the weight of grains from an ear and the weight of 1.000 grains. Next in the case of objects with chemical protection the yield positively correlated with the number of ears per 1 m^2 and the number of grains in an ear, and with the weight of 1.000 grains on the object with weed control only.

Table 5

Crop yield structure and biometry of spring barley, average values for 2005–2014

Specification	Plant protection	Crop rotation	Monoculture	Relative values
		(CR)	(M)	M:CR [%]
1. Number of ears per 1 m^2	0*	709	554	78
	*H**	749	673	90
	HF*	739	707	96
	means	732	645	88
2. Ear length [mm]	0	62.6	61.4	98
	H	64.5	63.2	98
	HF	63.0	63.0	100
	means	63.4	62.5	99
3. Number of grains in ear	0	17.6	17.3	98
	H	17.9	17.9	100
	HF	17.8	17.7	99
	means	17.8	17.6	99
4. Weight of grains per ear [g]	0	0.90	0.81	90
	H	0.93	0.88	95
	HF	0.95	0.89	94
	means	0.93	0.86	92
5.Weight of 1.000 grains [g]	0	51.5	48.5	94
	H	51.7	48.2	93
	HF	53.2	50.2	94
	means	52.1	49.0	94

$LSD_{0.05}$ for:	**1**	**2**	**3**	**4**	**5**
I. Crop sequences system :	23	0.8	n.s.	0.05	0.5
II. Plant protection:	15	1.1	0.3	0.03	0.5
III. Interaction I × II:	21	n.s.	n.s.	n.s.	n.s.

*0 – without plant protection; *H* – protection from weeds; HF – protection from weeds and disease

Discussion

The decline of spring barley yielding, by 20.4% on average in the 38–47-year monoculture should not be recognized as a big one. There are, nevertheless, papers informing about similar or even greater losses of yields in a shorter time of monocultures. BLECHARCZYK et. al. (2005, 2009)

has informed about 30% decline of spring barley productivity in 42–49-year monoculture. The aforementioned studies have shown the reduction of yields in monoculture depends on apart from the time of its continuation, agrotechnological factors as well as weather course in consecutive years. Especially the variability of the latter ones causes that reductions of yields in monoculture duration have fluctuating character.

The negative response of cereals to its crop in a monoculture forces to the application of different soil sickness factors inhibiting the yield declines. From among factors compensating the faulty position, the intensification of mineral and organic fertilization were the most often (BLECHARCZYK et al. 2005, TURKINGTON et. al. 2005, MAŁECKA et. al. 2007) ones. In studies carried out by the authors of this paper researches, the chemical protection from weeds and fungal diseases served as a compensating factor. The application of herbicides thanks to the competition elimination of weeds has increased the yields of spring barley in a monoculture by 17.9% while in the case of a crop rotation by 3.6% only. As a result of that application the regress of spring barley yielding has limited from 27.5% to 17.5% on the object without protection i.e. by 10.0%. In previous researches carried out by ADAMIAK (2007) the compensating activity of herbicides has achieved 2–5%.

The application of chemical protection from pathogens has increased the yields of barley in the range 0.2–29% (YANG et al. 2000, BLECHARCZYK et. al. 2005, KUROWSKI et. al. 2007, WANIC et. al. 2012). In the presented studies fungicides in relation to the object treated with herbicides has increased the yields of spring barley in the crop rotation on average by 4.5% and in monoculture by 5.8% respectively. Their combined application with herbicides gave similar soil sickness effect as the only herbicides application because that protection variant (fungicides + herbicides) has reduced losses of spring barley yields by 11.0% compared to the object without protection. However, making a reference to only fungicides activities it should state their soil sickness effect was insignificant. They have caused the decrease of yields only by 1.0% in a monoculture. In previous studies in the carried out experiment the fungicides have slowed down losses in yields of barley in the range 2–5% (ADAMIAK 2007).

Lower yielding of spring barley in the monoculture than in the case of crop rotation has been caused by deterioration in phytometric factors, especially the density of ears and the number of grains from an ear, and a smaller weight of 1.000 grains (MAŁECKA et. al. 2007). In presented studies, especially the monoculture without protection has shown the biggest reduction of ears density and then the weight of grains from an ear and the weight of 1.000 grains.

Conclusion

1. The cultivation in a 38÷47-year monoculture has reduced the yielding spring barley by 20.4%. The application of chemical protection of a canopy significantly decreased losses of spring barley i.e. from 27.5% on an object without protection to 17.5% on the object with a protection from weeds, and to 16.5% on the object with combined protection from weeds and diseases.

2. The chemical protection had significant effect on yielding of spring barley in monoculture. In this position the application of herbicides has increased yields of spring barley by 17.9% (by 0.86 t ha^{-1}) while in the case of combined application of herbicides and fungicides by 24.8% (by 1.19 t ha^{-1}). In the case of a crop rotation the yield of increase of spring barley amounted to 3.6% and 8.3% respectively.

3. The crop sequence system diversified the yield structure parameters of spring barley. The long-term crop of spring barley in monoculture has caused the biggest decrease of ears density (by 12%), a little bit smaller of the weight of grains from an ear (by 8%) and the weight of 1.000 grains (by 6%). The biggest decreases of the aforementioned parameters took place on the object of unprotected monoculture.

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References

ADAMIAK J., ADAMIAK E., STĘPIEŃ A., URBAŃSKI B. 2000. *Response of spring barley cultivars to higher cereal proportion in crop rotations and to monoculture*. Pol. J. Natur. Sc., 5: 35–43.

ADAMIAK E. 2007. *Struktura zachwaszczenia i produktywność wybranych agrocenoz zbóż ozimych i jarych w zależności od systemu następstwa roślin i ochrony łanu* [*Weed infestation structure and productivity of chosen winter and spring crop agrocenoses depending on vegetal succession and standing corn protection*]. Rozprawy i Monografie/Dissertations and Monographs, 129, Wyd. UWM Olsztyn.

ADAMIAK J., ADAMIAK E., STĘPIEŃ A. 2018. *Response of winter wheat on long-term monoculture in diversified conditions of chemical protection*. Pol. J. Natur. Sc., 33(2): 201–213.

BERZSENYI Z., GYÓRFFY B., LAP D. 2000. *Effect of crop rotation and fertilization on maize and wheat yields and yield stability in long-term experiment*. Eur. J. Agron., 13: 225–244.

BLECHARCZYK A., MAŁECKA I., SAWINSKA Z. 2004. *Response of winter wheat to long-term direct drilling system*. Fragm. Agron., 21(2): 125–136.

BLECHARCZYK A., MAŁECKA I., PIECHOTA T., SAWINSKA Z. 2005. *Efekt nawożenia jęczmienia jarego uprawianego w monokulturze*. Acta Sci. Pol., Agricult., 4(1): 25–32.

BLECHARCZYK A., MAŁECKA I., PIECHOTA T., SAWINSKA Z. 2009. *Wpływ wieloletniego nawożenia i systemu następstwa roślin na plonowanie jęczmienia jarego oraz akumulację azotu*. Fragm. Agron., 26(4): 14–21.

BURACZYŃSKA B., CEGLAREK F. 2008. *Plonowanie pszenicy ozimej uprawianej po różnych przedplonach*. Acta Sci. Pol., Agricultura, 7(1): 27–37.

FAOSTAT. 2017, http://faostat.fao.org, access: 1.12.2018.

Fernández-Getino A.P., Santín-Montanyá M.I., Zambrana E., Andrés E.F., Tenorio J.L. 2014. *The response of barley to rainfall and temperature in different tillage and crop rotation systems in semi-arid conditions*. Ann. Appl. Biol., 166: 43–153.

Gawęda D., Kwiatkowski C. 2013. *Plonowanie jęczmienia jarego uprawianego w krótkotrwałej monokulturze w zależności od międzyplonu i sposobu odchwaszczania.* Fragm. Agron., 30(1): 27–35.

Gawęda D., Wesołowski M., Kwiatkowski C.A. 2014. *Weed infestation of spring barley (Hordeum vulgare L.) depending on the cover crop and weed control method*. Acta Agrobot., 67(1): 77–84.

Jończyk K., Kawalec A. 2001. *The preliminary estimation of usefulness of some winter wheat varieties to cultivation in different crop production systems*. Biul. IHAR, 220: 35–43.

Kurowski T., Adamiak E. 2007. *Occurrence of stem base diseases of four cereal species grown in long-term monocultures*. Pol. J. Natur. Sc., 22(4): 574–583.

Kwiatkowski C. 2009. *The consequent influence of crop rotation and six-year-long spring barley monoculture on yields and weed infestation of white mustard and oats*. Acta Agrobot., 62(2): 241–247.

Kwiatkowski C. 2004. *Wpływ międzyplonu na plonowanie i zachwaszczenie jęczmienia jarego uprawianego w monokulturze*. Ann. UMCS, sect. E, 59(2): 809–815.

Lemańczyk G. 2002. *Impact of different forecrops on health status of stem base of winter wheat cultivated on the soil of good wheat comple*x. Acta. Sci. Pol., Agricult., 1(1): 111–119.

Małecka I., Blecharczyk A., Sawinska Z. 2007. *Wpływ następstwa i uprawy roli na fizyczne właściwości gleby, porażenie przez choroby oraz plonowanie jęczmienia jarego.* Fragm. Agron., 3(95): 300–307.

Norwood C. 2000. *Dryland winter wheat as affected by previous crops*. Agron. J., 92: 121–127.

Stupnicka-Rodzynkiewicz E., Stępnik K., Lepiarczyk A. 2004. *Wpływ zmianowania, sposobu uprawy roli i herbicydów na bioróżnorodność zbiorowisk chwastów*. Acta Sci. Pol., Agricultura, 3(2): 235–245.

Tanaka D.L., Krupinsky J.M., Liebig M.A., Merrill S.D., Ries R.E., Hendrickson J.R., Johnson H.A., Hanson J.D. 2002. *Dynamic cropping systems: an adaptable approach to crop production in the great plains*. Agron. J., 94(5): 957–961.

Turkington T.K., Xi K., Tewari J.P., Lee H.K., Clayton G.W., Harker K.N. 2005. *Cultivar rotation as a strategy to reduce leaf diseases under barley monoculture*. Can. J. Plant Pathol., 27: 283–290.

Wanic M., Majchrzak B., Nowicki J., Waleryś Z., Orzech K. 2012. *Role of undersown catch crops and crop rotation in state of health of spring barley*. Acta Sci. Pol., Agricult. 11(1): 113–124.

Wesołowski M., Dąbek-Gad M., Maziarz P. 2007. *Wpływ przedplonu i herbicydu na plonowanie pszenicy ozimej*. Fragm. Agron., 24(4): 240–246.

Wesołowski M., Kwiatkowski C., Stępień A. 2003. *Wpływ mieszanek międzyodmianowych jęczmienia jarego na zachwaszczenie tej rośliny w uprawie monokulturowej i płodozmianie.* Acta Agroph., 1(4): 795–802.

Woźniak A. 2006. *Effect of forecrops on the yield and quality of winter wheat grain*. Acta Sci. Pol., Agricultura, 5(2): 99–106.

Yang J.P., Sieling K., Hanus H. 2000. *Effects of fungicide on grain of barley grown in different cropping systems*. J. Agron. Crop Sci., 185: 153–162.

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EFFECT OF THREE BEDDING MATERIALS ON THE MICROCLIMATE CONDITIONS, COWS BEHAVIOR AND MILK YIELD

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Key words: cold stress, deep litter, sawdust, compost manure, cow comfort.

Abstract

This work aim was to study the influence of low temperatures on the behavior, productivity and heat production of cows with different types of bedding material (deep litter, sawdust, compost manure) keeping conditions. During keeping at low temperatures on deep litter, the highest average daily temperature was observed (-11.8°C) and the lowest humidity was 84.4% as compared to the sawdust and compost manure litter. There were observed higher temperature values of the room and resting place under the lying cow when keeping on a deep litter. The total energy expenditure for heat production in cows with keeping on deep litter was by 2.95 and 2.43 MJ lower, as compared to keeping on sawdust and compost manure bedding. With temperature decrease there was observed a tendency to increase the duration of rest in a lying position with all variants of litter. The highest value was with keeping on deep litter – 846 minutes per day. At the same time, the duration of food consumption was slightly decreased. Productivity of cows, when keeping on sawdust litter, declined by 9.11% (2.38 kg), with compost manure bedding – by 8.36% (2.45 kg), and with deep litter – by 5.31% (1.36 kg).

Introduction

Among weather factors, affecting the functioning of dairy cattle, the most impact is done by environment temperature (GANTNER et al. 2011, SCHÜLLER et al. 2014). KNIZKOVA et al. (2002) have found out that thermo--neutral temperature for dairy cattle is in the range from -5 to 25°C. WEST (2003) states that the temperature change in the range from -0.5 to + 25°C

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does not affect the cows productivity. During thermo-neutral temperature, the body spends a minimum amount of energy to maintain life or balance with the environment. The amount of lost heat is equal to the amount of produced heat. As the distance from the optimal temperature goes higher or lower, the energy exchange and the level of heat production increases, which leads to inappropriate consumption of feed and relevant reduction in feed conversion. The effect of cold on the body was mostly investigated on meat or lactating animals in conditions of year-round grazing (GRANKE et al. 2011, WEBSTER et al. 2008). However, the low temperature has a negative effect on the cows body also when kept inside (HERBUT 2013, BORSHCH et al. 2017a). Cold weather has an impact on the cattle physiological characteristics and behavior (BERGEN et al. 2001, KENNEDY et al. 2005). The sympathetic nervous system causes three basic physiological responses to cold stress: increase in heat production metabolism, pulse rate, and mobilization of free fatty acids for metabolism (BROUCEK et al. 1991). Behavioral reactions to low temperature can be divided into two categories: search for a warm comfort rest place to reduce the temperature factor influence and change in the duration of the basic behavior acts (HOUSEAL and OLSON 1995, REDBO et al. 2001). When cows are kept inside at low temperatures, the lying position rest and food eating duration increases (FISHER et al. 2003). Cold stress significantly affects animals body during keeping the whole year on pastures. Thus, the research results of TUCKER et al. (2007) and WEBSTER et al. (2008), conducted in New Zealand under grazing conditions, indicate significant changes in behavior and productivity during a periodof cold load. Lying and grazing duration decreased in comparison with the thermo-neutral period. Low temperature, combined with wind and precipitation, increases the cortisol level in cows blood, which is a stress marker (WEBSTER et al. 2008). Animals begin to seek shelter in the form of a tree, or sheds, and are reluctant to lie on wet and dirty land, which leads to loss of productivity (SCHUTZ et al. 2010). Accordingly, the effect of these conditions leads to the cows increased metabolic activity to provide heat to maintain their body temperature (AMES 1987). As a result, there is an increased need for energy for the basic metabolism (main exchange), and accordingly the amount of energy for other processes, such as milk production and sexual activity, decreases (BROUCEK et al. 1991).

The bedding material has special place in ensuring comfort of dairy cows (VAN GASTELEN et al. 2011, MITEV et al. 2012, BORSHCH et al. 2017b, JONES et al. 2017). The most common litter material in Ukraine is the straw of grain crops. In addition, depending on the geological and industrial characteristics of certain regions, sand, sawdust, peat and compost manure are also used. Each of these litter variants has advantages and

disadvantages, connected with storing, depositing, removing, recycling and ensuring the closest natural conditions for comfort and well-being of cows at high and low temperatures (HULSEN 2006, ECKELKAMP et al. 2016, PILATTI and VIEIRA 2017).

Our research purpose was to study the influence of different bedding material types during the cold-thermal period on the behavior, productivity and heat production of cows.

Material and Methods

Climate

The research was conducted at various temperature periods in the central part of Ukraine (49°34'56" North latitude, 30°38'10"East longitude; 49°48'45" North latitude, 30°38'56' East longitude; 48°57'47" North latitude, 30°7'57" East longitude) during January 2018. The first period (12 days) was thermo-neutral with average daily temperature + 2.8°C, and the second cold-thermal period (10 days) with temperature -17.9°C with strong wind and daily precipitation in the form of snow. The main weather indicators in different periods of research are given in the Table 1. The Ukraine territory is located in a moderate belt. The continental climate is characterized by four distinct seasons of the year. The winter weather changes average daily temperature from +4 to -18°C. This range is due to frequent changes in air mass types. Tropical air masses provide warm and dry weather in winter, and the arctic ones – long low temperatures, sometimes with significant precipitation in the form of snow.

Table 1

The main weather indicators in different periods of research

Indicators	Thermo-neutral period	Cold-thermal period
Air temperature [°C]	2.8	-17.9
Relative air humidity [%]	54.8	94.3
Wind speed [km h^{-1}]	18.4	59.8
Average daily precipitation amount [mm]	28	429

Stall bedding types and barns

To conduct our research, there were selected three farms with cows, kept on different types of bedding material: deep straw litter, sawdust and compost manure. All the farms had lightweight types talls and each of

barns are divided into 4 sections. In a deep litter stall there were kept 409 cows. The room parameters were (L x W x H): 100 x 60 x10.5 m. Animals rest in a separated zone from the feeding passage. The straw delivery into recreation area is done daily at 8 kg rate per head. Manure removal is performed three times a year. A stall with sawdust litter holds 422 cows. Room parameters are: 150 x 40 x 10 m. Animals rest in boxes equipped with rubber mattresses. The stall with compost manure litter holds 393 cows. Parameters of the room are 150 x 40 x 10.5 m. The solid manure fraction is disinfected and dried in an aerobic bioreactor for 24 hours (dry matter content 36–41%). Rest of cows takes place in the boxes. Compost manure brought into the boxes everyday (layer 2–3 sm). Removal of manure from passages is carried out by a scraper. The farms apply same type year-round feeding of cows with complete mixed fodders. The feeding level is quite high: animals consume 21.5–22.4 kg of dry matter per day, the energy value of the consumed feed is 211–223 MJ.

Animals

The Holstein breed cows were used as the research material. The dynamics of productivity, dry matter consumption and behavior in different temperature periods were studied on all the livestock. Energy expenditure for heat production was studied in cows after 60–70 days of lactation (n = 25).

Behaviour

Cow's behavior was determined using internal surveillance cameras. In farm with deep straw litter installed 12 IP cameras (2 MP). In farms with sawdust and compost manure litters installed 16 Hikvision cameras (Full HD). Filming in all barns takes place around the clock. Placing cameras in the barns allows you to record a recreation area, feeding passage and drinking bowls area and also cows moving. At first processed obtained data for each of the four sections. After it determined average indicators on a farm. The daily behavior of cows was studied during 2 consecutive days in the thermo-neutral period and in the period of low temperature. Every 10 minutes, in experimental groups, there was recorded the number of cows, which during the observation consumed food, were resting by standing or lying, were moving and drinking water. Data undertook by us from video cameras two last days before temperature decrease and two first days after temperature decrease.

Thermal conditions

The air temperature and relative humidity in the barns were determined by a combined digital environment meter Velleman, model DVM401 (Belgium). The wind speed inside the barn was determined by handheld pocket digital anemometer AZ, model AZ-8919 (Taiwan). The average daily precipitation was determined by the Kyiv Center for Hydrometeorology. The cows skin surface temperature was determined in two places: on rumen and in the region of the last inter costal space by using a remote infrared thermometer Thermo Spot Plus (Germany). The temperature at the resting place as well as under the lying cow was determined by the thermometer A36PF-D43 (USA). Costs of energy for heat production were calculated according to the methods of KADZERE et al. (2002).

Calculation of and wind chill temperature index and cold stress index

The wind chill temperature index (WCT) was calculated according to TUCKER et al. (2007). This index helps to evaluate the effect of low air temperature in combination with the wind speed on the cold stress of animals:

$$WCT = 13.12 + 0.6215 \cdot T_{air} \cdot 13.17 \cdot V^{0.16} + 0.3965 \cdot T_{air} \cdot V^{0.16}$$

where:

WCT – wind chill temperature [°C]

T_{air} – air temperature [°C]

V – wind speed [km h^{-1}].

The cold-stress index (CSI), which indicates the level of animals stress resistance to sharp wind speed and precipitation, was determined by the DONNELLY (1984) method:

$$CSI = [11.7 + (3.1 \cdot WS^{0.5})] \cdot (40\text{-}T) + 481 + R$$

where:

CSI – cold-stress index [$MJ/m^2/h$]

WS – mean daily wind speed [m s^{-1}]

T – is the mean daily temperature [°C]

$$R = 418 \cdot (1\text{-}e^{-0,04 \cdot rain})$$

where:

rain is the total daily rainfall in millimeters

e – natural logarithm = 2.718.

Statistical analysis

The obtained data were statistically processed using STATISTICA (Version 11.0, 2012) software. The Student's *t*-test was used to estimate the statistical significance of the obtained values. Data were considered significant at $P < 0.05$, $P < 0.01$, $P < 0.001$.

Results and Discussion

The cattle organism is under the constant influence of combined action of meteorological factors: temperature, humidity, atmospheric pressure, air speed, precipitation. At the same time, one of them may be overwhelming, and other factors increase or weaken its effect on the organism of animals. The manifestation of meteorological phenomena within a day can widely vary and affect their health, behavior and productivity (BROUCEK et al. 1991, KADZERE et al. 2002, ANGRECKA and HERBUT 2015).

The results of our research indicate that the decrease in air temperature in combination with wind gusts and atmospheric precipitation significantly influenced the indoor microclimate (Table 2). Due to daily straw and excrement accumulation and the permanent microbiological processes in the bedding, the room air temperature with the deep litter was somewhat higher than when using of other types of bedding material (SOMMER 2001).

Table 2

Indoor microclimate indicators under different weather conditions

Indicators	Thermo-neutral period			Cold-thermal period		
	deep litter	sawdust	compost manure	deep litter	sawdust	compost manure
Air temperature [°C]	6.7±0.22	5.7±0.31*	6.4±0.38	-11.8±0.14	-14.4±0.29***	-13.9±0.16***
Relative air humidity [%]	55.3±2.56	56.3±3.32	56.9±1.71	84.4±2.37	85.7±2.74	85.9±2.19
Wind speed [km h^{-1}]	1.58±0.05	1.72±0.08	1.65±0.04	2.66±0.11	2.66±0.18	2.62±0.12

Note: as compared with deep litter bedding material *$P < 0.05$; ***$P < 0.001$.

Thus the average daily temperature during keeping on deep litter decreased by 18.5°C, as compared with the thermos-neutral period, and amounted –11.8°C. The most significant decline was observed in the keeping technology with sawdust as litter material – by 19.9°C, with an average temperature of -14.4°C, which is by 1.5 and 2.6°C lower than when keeping on compost manure and deep litter. Indoor keeping of cows on all types of litter material, had the air humidity increase during the period of temperature load by 29.1–29.5%. The highest average daily air humidity was in rooms with compost manure bedding – 85.9%. With the deep litter and sawdust technology, these values were 84.4% and 85.7% respectively. This is explained by the fact that the daily adding of straw, which has a hygroscopic quality of 450%, contributes to a decrease in the room humidity.

Sawdust as bedding material has also high hygroscopic quality – 490% (NORRING et al. 2008). In addition, wind speed also increased during the cold thermal period by 0.94 to 1.08 km/h in comparison of thermos-neutral period.

By the wind chill temperature (WCT) and cold-stress (CSI) indices there was estimated the influence of low temperatures in combination with air flow speed and precipitation on the cold stress. In the cold period, with all variants of litter, there was observed WCT and CSI decrease (Table 3).

Table 3

The indoor bio-climatic indices values in temperature load period

Indicators	Bedding material		
	deep litter	sawdust	compost manure
Wind-Cold index [°C]	-17.06±0.27	-18.72±0.26***	-18.17±0.31***
Cold-stress index [MJ/m^2/h]	1671.46±73.74	1713.88±81.42	1700.51±67.87

Note: as compared with deep litter bedding material ***$P < 0.001$.

The WCT value shows the effect of low temperatures, combined with the air flow speed in the room, on productivity, comfort of keeping conditions and behavior of cows. According to our research, it was found that the lowest average daily value of WCT in the period of low-temperature load was in the keeping technology, using sawdust as a litter -18.72°C. At the same time in a room with compost manure litter this value was somewhat higher and amounted -18.17°C. The highest value was observed in keeping animals on deep litter – (-17.06°C).

Knowledge of CSI allows to consider how wind speed and precipitation, which are the main factors of the real temperature sensing, affect the productivity and behavior of cows. The highest average daily value of CSI was observed with keeping on the sawdust litter – 1713.88 MJ/m^2/h. With keeping on compost manure and deep straw bedding, these indicators were somewhat lower – 1700.51 and 1671.46 MJ/m^2/h, respectively.

The environmental temperature is the biggest influence on the thermal state of animals, changing the course of vital processes. The thermoregulatory mechanisms allow animals to adapt to different temperature fluctuations of the environment and to transiently tolerate significant temperature deviations from the usual for them values. However, the body heat regulation values are not limitless and the violation of the thermal equilibrium changes the physiological state, resistance to diseases and animal productivity. Functional body disorders are possible due to the action of both very high and very low air temperature. High productivity is closely linked to high heat production (BERMAN et al. 1985). In the indu-

strial conditions, dairy cows experience lack of heat in the winter and transition seasons of the year. Decrease of the indoor air temperature dramatically increases the body heat release (LOBECK et al. 2011, ITO et al. 2014). Animals are trying to reduce the heat release, thus the pulse slows down, breathing deepens, and food consumption increases. Due to excessive and prolonged air temperature decrease the animals over cooling occurs, and this helps to provoke chills and other diseases. Daily yield at average daily temperature below -10°C can be reduced by 12–14%, and at temperatures below -20°C the loss of productivity is even greater.

The greatest impact on the duration of animal rest, along with the bed characteristics (solid or elastic coating, the presence or absence of litter, clean or contaminated, dry or wet) and the type of litter, has the heat capacity of the bedding material (CALAMARI et al. 2009, ECKELKAMP et al. 2014). When the animal is lying, one third of its body surface contacts the floor, so the floor should be quite warm. Loss of heat through the floor is 12–20% of the total heat loss of the room and depends on the floor thermo--physical characteristics and the bedding material. Feed energy loss by animal for creation and use of heat energy, to warm the bed, instead of turning it into milk is rather irrational. Low temperature cause the increase of physiological heat expenditures in cows. When these costs exceed 100 kcal h^{-1}, then for 12 hours (average rest period in a lying position), they are equivalent to a caloric value of 2 kg of milk (KIBLER and BRODY 1951).

In our studies, the resting places temperature was not much different from the indoor air temperature (Table 4). The highest temperature of resting places during the cold period for cows on deep litter was – (-11.4°C), which is by 2.1 and 1.9°C higher than for keeping on the sawdust and compost manure bedding. The temperature of the resting place under the lying

Table 4

Temperature indices of rest places and energy expenditure for heat production in different weather conditions (n = 25)

Indicators	Thermo-neutral period			Cold-thermal period		
	deep litter	sawdust	compost manure	deep litter	sawdust	compost manure
Rest place temperature [°C]	5,8±0,15	5,1±0,20**	5.3±0.12*	-11.4±0.39	-13.5±0.84*	-13.3±0.57**
Rest place temperature under lying cow [°C]	26.5±0.42	23.8±0.78**	24.2±0.58**	24.5±1.03	19.9±1.17**	20.5±1.24*
∑ energy for heat production [MJ]	45.34±1.03	45.73±1.12	45.59±1.38	54.08±0.88	57.03±1.23*	56.51±1.39

Note: as compared with deep litter bedding material *$P < 0.05$; **$P < 0.01$

cow during the cold period was also the highest in deep litter – 24.5°C, which is by 4.0 and 4.6°C higher than for keeping on the compost manure and sawdust bedding.

Significant decrease in the average daily temperature for all keeping options led to increase in energy expenditure on heat production. The energy expended on evaporation, radiation and convection is directly related to the ambient temperature and has a significant impact on the cows behavior and productivity. In experimental cows with keeping on deep litter the energy consumption for heat production was increased by 8.74 MJ, as compared with the thermo-neutral period. The heat production on the compost manure and sawdust bedding increased by 10.78 and 11.44 MJ, respectively. The highest average daily energy expenditure was observed with keeping on sawdust bedding – 57.03 MJ. This is explained by the fact that, along with good hygroscopic and adsorption properties of sawdust at low temperatures, it is less heat accumulating in comparison with other variants of the bedding material.

A similar tendency was observed for all types of bedding material in the cold period: the duration of rest in the lying position increased by 47–53 min in comparison with the thermo-neutral period (Table 5).

Table 5

Duration of main daily behavior reactions under different weather conditions, min (n = 25)

Indicators	Thermo-neutral period			Cold-thermal period		
	deep litter	sawdust	compost manure	deep litter	sawdust	compost manure
Lying	793±8.93	761±8.72*	774±10.63	846±6.11	808±12.94*	821±8.24*
Fodder consuming	274±3.91	259±2.63**	286±3.35*	265±4.19	252±5.32**	274±3.53*
Moving	56±0.16	48±0.21***	58±0.27***	39±0.12	32±0.19***	42±0.26***
Standing	181±2.24	212±3.49***	162±2.57***	174±3.51	187±2.26***	159±3.18***
Water drinking	39±0.21	31±0.54***	36±0.17***	25±0.14	27±0.32***	32±0.22***

Note: as compared with deep litter bedding material $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

At the same time, the increase in the duration of rest in the lying position was accompanied by a reduction of time for walking, standing and water drinking. Duration of feed consumption during the low temperature period has somewhat decreased. Our data do not coincide with the data of MULLER et al. (1996) and FISHER et al. (2003) who report, that at low temperatures the lying time of cows has decreased. The results of our studies coincide with the data of ADAMS et al. (1986), which show that at low tempera-

tures there is some reduce in feed activity and duration of eating. The cows rest in lying position together with the feed consumption are the main ethological indicators, which values indicate both positive and negative signs of keeping technologies and use of different bedding material types. The highest duration of cows resting during the cold period was with keeping on deep litter – 846 minutes, which is by 23 and 38 min more than for keeping on compost manure and sawdust, respectively. Under keeping with the optimal daily delivery of straw, which is extremely effective heat--insulating material, and it also massages and dries the animal's skin, provides the most comfortable conditions (LOMBARD et al. 2010).

Our studies do not coincide completely with the studies of OFNER--SCHRÖCK et al. (2015), which found that in the thermo-neutral period with keeping cows on the compost manure bedding, the duration of rest in a lying position was prevailing similar indicators for other types of bedding material.

Typically, low temperature periods are accompanied by cow productivity decrease by 5–20% (KADZERE et al. 2002). It is established that at low temperatures the feed conversion is reduced. Our studies confirm this evidence, because in all variants of litter material in experimental cows there was a decrease in productivity (Table 6).

Table 6

Cow productivity and feed conversion under different weather conditions

Indicators	Thermo-neutral period			Cold-thermal period		
	deep litter	sawdust	compost manure	deep litter	sawdust	compost manure
Yield [kg]	25.63±0.51	26.12±0.84	27.34±0.46	24.27±0.73	23.74±1.08	24.89±0.32
Dry matter consumption [kg]	21.43±0.23	21.77±0.36	22.43±0.31**	21.38±0.25	21.63±0.38	22.36±0.43*
Feed conversion, kg of dry matter [kg of milk]	1.19	1.20	1.22	1.13	1.08	1.11

Note: as compared with deep litter bedding material *$P < 0.05$; **$P < 0.01$

Thus, the greatest reduction in cow productivity was under the technology of keeping with on sawdust litter – by 9.11% or 2.38 kg. The keeping on compost manure litter during the cold period, the average decline in productivity was 8.96% or 2.45 kg. At the same time, the highest productivity stability during low-temperature load was showed by the cows keeping technology on deep litter. Under this variant of the litter, the productivity dropped only by 5.31% or 1.36 kg. As for the dry matter consumption during the cold period there was no significant decrease of this indicator

for different variants of the litter material – by 0.05–0.14 kg. This is due to the fact that low temperatures led to decrease in the duration of feed consumption by cows. Decrease in productivity and consumption of dry matter, along with an increase in the cost of heat production, resulted in feed conversion decrease by 0.06 kg of dry matter kg^{-1} of milk with keeping on deep litter and by 0.11 and 0.12 kg of dry matter kg^{-1} of milk with keeping on compost manure and sawdust.

Conclusions

The air temperature decrease became a significant stress factor, which significantly influenced the duration of basic behavioral reactions and productivity of cows, as compared to the thermo-neutral period. The keeping technology on deep straw bedding due to the higher thermal insulation and hygroscopic properties and constant microbiological processes in it showed the best indicators of microclimate. At the same time, due to a smaller decrease of the indoors air temperature, there were the smallest losses of energy for heat production and productivity decrease of cows, as compared to keeping on sawdust and compost manure litter.

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References

Adams D.C., Nelsen T.C., Reynolds W.L., Knapp B.W. 1986. *Winter grazing activity and forage intake of range cows in the northern great plains*. Journal of Animal Science, 62: 1240–1246.

Ames D.R., Insley L.W. 1975. *Wind-chill effect for cattle and sheep*. Journal of Animal Science, 40: 161–165.

Angrecka S., Herbut P. 2015. *Conditions for cold stress development in dairy cattle kept in free stall barn during severe frosts*. Czech Journal of Animal Science, 60(2): 81–87.

Bergen R.D., Kennedy A.D., Christopherson R.J. 2001. *Effects of intermittent cold exposure varying in intensity on core body temperature and resting heat production of beef cattle*. Canadian Journal of Animal Science, 81: 459–465.

Berman A., Folman Y.M., Kaim M., Mamen Z., Herz D., Wolfenson A., Graber Y. 1985. *Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a tropical climate*. Journal of Dairy Science, 68: 488–495.

Borshch O.O., Borshch O.V., Donchenko T.A., Kosior L.T., Pirova L.V. 2017a. *Influence of low temperatures on behavior, productivity and bioenergy parameters of dairy cows kept in cubicle stalls and deep litter system*. Ukrainian Journal of Ecology, 7(3): 73–77.

Borshch O.O., Borshch O.V., Kosior L.T., Pirova L.V., Lastovska I.O. 2017b. *Influence of various litter materials and premises characteristics on the comfort and behavior of cows*. Ukrainian Journal of Ecology, 7(4): 529–535.

Broucek J., Letkovicova M., Kovalcuj K. 1991. *Estimation of cold stress effect on dairy cows*. International Journal of Biometeorology, 35: 29–32.

Calamari L., Calegari F., Stefanini L. 2009. *Effect of different free stall surfaces on behavioural, productive and metabolic parameters in dairy cows*. Applied Animal Behavioural Science, 120: 9–17.

Donnelly J.R. 1984. *The productivity of breeding ewes grazing on lucerne or grass and clover pastures on the tablelands of southern Australia*. III. *Lamb mortality and weaning percentage*. Australian Journal of Agricultural Research, 35(5): 709–721.

Eckelkamp E.A., Gravatte C.N., Coombs C.O., Bewley J.M. 2014. *Case study: characterization of lying behavior in dairy cows transitioning from a freestall barn with pasture access to a compost bedded pack barn without pasture access*. Professional Animal Scientist, 30(1): 109–113.

Eckelkamp E.A., Taraba J.L., Akers K.A., Harmon R.J., Bewley J.M. 2016. *Sand bedded freestall and compost bedded pack effects on cow hygiene, locomotion, and mastitis indicators*. Livestock Science, 190: 48–57.

Fisher A.D., Stewart M., Verkerk G.A., Morrow C.J., Matthews L.R. 2003. *The effects of surface type on lying behaviour and stress responses of dairy cows during periodic weather--induced removal from pasture*. Applied Animal Behaviour Science, 81: 1–11.

Gantner V., Mijić P., Kuterovac K., Solić D., Gantner R. 2011. *Temperature-humidity index values and their significance on the daily production of dairy cattle*. Mljekarstvo, 61(1): 56–63.

Graunke K.L., Schuster T., Lidfors L.M. 2011. *Influence of weather on the behaviour of outdoor-wintered beef cattle in Scandinavia*. Livestock Science, 136: 247–255.

Herbut P. 2013. *Temperature, humidity and air movement variations inside a free-stall barn during heavy frost*. Ann. Animal Science, 13(3): 587–596.

Houseal G.A., Olson B.E. 1995. *Cattle use of microclimates on a northern latitude winter range*. Canadian Journal of Animal Science, 75: 501–507.

Hulsen J. 2006. *Cow signals. A practical guide for dairy farm management*, p. 95.

Ito K., Chapinal N., Weary D.M., Von Keyserlingk M.A. 2014. *Associations between herd-level factors and lying behavior of freestall-housed dairy cows*. Journal of Dairy Science, 97(4): 2081–2089.

Jones B.W., Stone A.E., Wood C.L., Mcquerry K.J., Clark J.D., Ray D.L., Bewley J.M. 2017. *Characterization of milk yield, lying and rumination behavior, gait, cleanliness, and lesions between 2 different freestall bases*. Professional Animal Scientist, 33(1): 140–149.

Kadzere C.T., Murphy M.R., Silanikove N., Maltz E. 2002. *Heat stress in lactating dairy cows: a review*. Livestock Production Science, 77: 59–91.

Kennedy A.D., Bergen R.D., Christopherson R.J., Glover N.D., Small J.A. 2005. *Effect of once daily 5-h or 10-h cold-exposures on body temperature and resting heat production of beef cattle*. Canadian Journal of Animal Science, 85: 177–183.

Kibler H.H., Brody S. 1951. *Environmental physiology with special reference to domestic animals. XVIII. Influence of increasing temperature on heat production and cardiorespiratory activities in Brown Swiss and Brahman cows and heifers*. Univ. Missouri Agric. Exp. Stat. Res. Bull., 475.

Knizkova I., Kunc P., Koubkova M., Flusser J., Dolezal O. 2002. *Evaluation of naturally ventilated dairy barn management by a thermographic method*. Livestock Production Science, 77: 349–353.

Lobeck K.M., Endres M.I., Shane E.M., Godden S.M., Fetrow J. 2011. *Animal welfare in cross--ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest*. Journal of Dairy Science, 94 (11): 5469–5479.

Lombard J.E., Tucker C.B., Von Keyserlingk M.A., Kopral C.A., Weary D.M. 2010. *Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms*. Journal of Dairy Science, 93: 4668–76.

Mitev J., Varlyakov I., Miteva T., Vasilev N., Gergovska J., Uzunova K., Dimova V. 2012. *Preferences of freestall housed dairy cows to different bedding materials*. Veteriner Fakultesi Dergisi (Istanbul), 38(2): 135–140.

MULLER C.J.C., BOTHA J.A., SMITH W.A. 1996. *Effect of confinement area on production, physiological parameters and behaviour of Friesian cows during winter in a temperate climate.* South African Journal of Animal Science, 26: 1–5.

NORRING M., MANNINEN E., DE PASSILLE A.M., RUSHEN J., MUNKSGAARD L., SALONIEMI H. 2008. *Effects of sand and straw bedding on the lying behavior, cleanliness, and hoof and hock injuries of dairy cows.* Journal of Dairy Science, 91:570–576.

OFNER-SCHRÖCK E., ZÄHNER M., HUBER G., GULDIMANN K., GUGGENBERGER T., GASTEINER J. 2015. *Compost bedded pack barnss for dairy cows aspects of animal welfare.* Journal of Animal Science, 5: 124–131.

PILATTI J.A., VIEIRA F.M. 2017. *Environment, behavior and welfare aspects of dairy cows reared in compost bedded pack barns system*. Journal of Animal Behavior and Biometeorology, 5: 97–105.

REDBO I., EHRLEMARK A., REDBO-TORSTENSSON P. 2001. *Behavioural responses to climatic demands of dairy heifers housed outdoors*. Canadian Journal of Animal Science, 81: 9–15.

SCHÜLLER K.L., BURFEIND O., HEUWIESER W. 2014. *Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices.* Theriogenology, 81(8):1050–1057.

SCHUTZ K.E., CLARK K.V., COX N.R., MATTHEWS L.R., TUCKER C.B. 2010. *Responses to short-term exposure to simulated rain and wind by dairy cattle: time budgets, shelter use, body temperature and feed intake.* Animal Welfare, 19: 375–383.

SOMMER S.G. 2001. *Effect of composting on nutrient loss and nitrogen availability of cattle deep litter.* European Journal of Agronomy, 14:123–133.

TUCKER C.B., ROGERS A.R., VERKERK G.A., KENDALL P.E., WEBSTER J.R., MATTHEWS L.R. 2007. *Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter*. Applied Animal Behaviour Science, 105: 1–13.

VAN GASTELEN S., WESTERLAAN B., HOUWERS D.J., VAN EERDENBURG F.J. 2011. *A study on cow comfort and risk for lameness and mastitis in relation to different types of bedding materials*. Journal of Dairy Science, 94(10): 2878–2888.

WEBSTER J.R., STEWART M., ROGERS A.R., VERKERK G.A. 2008. *Assessment of welfare from physiological and behavioural responses of New Zealand dairy cows exposed to cold and wet conditions*. Animal Welfare, 17: 19–26.

WEST J.W. 2003. *Effects of heat-stress on production in dairy cattle*. Journal of Dairy Science, 86: 2131–2144.

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RESPONSE OF TWO STRAINS OF BROILER CHICKENS TO OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) EXTRACT IN THE TROPICS

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Key words: broiler chickens, *Pleurotus ostreatus*, prebiotics, growth indices, gut health.

Abstract

This study was aimed to determine the influence of *Pleurotus ostreatus* on response of broiler chicken. One hundred and eighty day-old broiler chicks (90 each of Cobb and Marshall strain) were administered extract of *Pleurotus ostreatus* at 0, 2000 and 4000 mg/litre water. Data were collected on performance, carcass attributes, faecal oocyte per gram (OPG) and caecum microbial (CM) population. At day 28, strain had effect ($p < 0.05$) on feed: gain (F:G) with better value observed in Cobb. At day 56, Cobb had higher ($p < 0.05$) final weight (FW), total weight gain (TWG) and daily weight gain (DWG) with lower F:G. Highest ($p < 0.05$) FW and lower F:G were observed in birds administered 2000 and 4000 mg l^{-1}. Interaction had effect ($p < 0.05$) on FW, TWG, DWG and F:G with best value observed in Cobb administered 4000 mg l^{-1}. Cobb recorded higher ($p < 0.05$) dressed weight (DW%), breast% and thigh%. Increasing *Pleurotus* level resulted in highest DW% and breast in both strains. OPG and CM was lowest at 4000 mg l^{-1} in both strains. The study therefore concluded that for optimal performance, *Pleurotus* can be administered at 4000 mg l^{-1}.

Introduction

Conventionally, control and treatment of poultry diseases is based on the use of synthetic products but the increasing trend of antimicrobial resistance calls for the need of having alternative approaches to manage-

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ment of poultry diseases. Antimicrobial resistance, cost and availability of the conventional medicines and residues in animal products and the environment are the major challenges in the poultry industry in developing countries. As the consequences of these problems, people in rural communities have continued to use indigenous plants as a convenient alternative for control and treatment of diseases (BAKARI et al. 2012, EKUNSEITAN et al. 2016a,b). In most cases, plants are used for treatment in their crude forms without known or determined concentration, established dosages and known side effects to treated subjects.

Mushroom have been reported to have immune enhancing and stress-reducing properties (DALLOUL and LILLEHOJ, 2006, BORCHERS et al. 2008) which is a necessary requirement for perfect biological processes. Oyster mushroom (*Pleurotus ostreatus*) is one of the commonly cultivated mushroom spices and its antiviral and anticancer properties ascertained (BOBEK and GALBAVY 2001). Additionally, several medicinal mushrooms have demonstrated powerful antioxidant activities (MINARECI et al. 2011, LIU et al. 2013) likewise antibacterial potentials (EKUNSEITAN et al. 2018) and, as a consequence, have potential application as natural growth and health enhancing agent. Mushroom (Shiitake: *Lentinus edodes*) extract contains substrates capable of increasing the population of important bacteria (bifidobacteria and lactobacilli) (GUO et al. 2004)

It has been reported that the microbial communities in the gastrointestinal track of poultry are influenced by a number of factors including stocking density, diet, feeding practices, housing conditions, age of birds and pathogens (TOROK et al. 2007). Also it is very well established that bacterial communities change radically between the different anatomical segments of the digestive tract. The use of synthetic drugs has been implicated in the decimation of important microbes e.g in the gut of birds which are necessary for proper biological functions. Increase in pathogenic microbial substances due to gap in the intestinal immune system may lead to antagonistic reactions to nutrients and tolerance to pathogens. Intestinal microbial profile can be modified and affected by the diet, phytobiotics and changes in dietary composition (YEGANI and KORVER 2008). Therefore, the maintenance of health of intestinal ecosystem is highly essential for optimal health and growth of animals.

Although researches have been conducted on the influence of intensive growth of mushrooms with various cellulosic substrates which, after fruiting remains as a source of protein that can be used to feed livestock strategies. A possible strategy in reducing the presence of pathogenic microbe shedding in final products from poultry is the possibility of using or ascertain its antibacterial potentials (EKUNSEITAN et al. 2018) of *Pleurotus ost-*

reatus in decreasing their populations in the gut. However, documented information on the use of *Pleurotus* spp. as regards to its effect on broiler performance and carcass characteristics is limited in the tropics where the growth of bacteria is highly favoured. This research work was directed towards investigating the influence of *Pleurotus* spp. on growth performance indices and carcass characteristics when orally administered to broiler chickens at different levels of concentration.

Materials and Method

The experiment was carried out at the Poultry unit of the Teaching and Research Farms, Federal University of Agriculture, Alabata, Abeokuta, Ogun state, Nigeria. The Farm lies within latitude 7 10 N. Longitude 3 2 E and altitude 76 mm (*Google maps* 2018).

A 1 kg of fresh oyster mushroom was soaked with 2litres of ethanol at a ratio of 1:2 and was left for 3 days (72 hours) for extraction. After 72 hours, the extract was sieved out using a muslin cloth. The extract was clarified by filtration through celite on water pump and then concentrated in vacuo using a rotation evaporator.

One hundred and eighty day old broiler chicks of two strains (90 each of Cobb and Marshall Strain) were used for this experiment. Birds were raised under intensive management system. Variation in this research was based on two (2) factors; strain type (Cobb and Marshall) and levels of oral administration of *Pleurotus ostreatus* mushroom extract: 0 for control, 2000 and 4000 mg l^{-1} of water. Each strain was allotted to the three levels of administration of *Pleurotus ostreatus* containing 30 birds per treatment and further divided into replicates of 10 birds each. The extract of *Pleurotus ostreatus* was administered via water once in a week at the stated levels. Vaccination was given to all treatment while medication (antibiotics and coccidiostat) was administered only to the control group.

The birds were given starter diet up to four weeks of age (crude protein: 22%, metabolizable energy [kcal kg^{-1}]: 3000, fibre [%]: 3.5, fat [%]: 6.00) and 29–56days (crude protein: 19%, metabolizable energy [kcal kg^{-1}]: 2900, fibre [%]: 3.5, fat [%]:4.00). Fresh feed and water was provided on a daily basis. Data on performance parameters were taken on weekly basis.

At the 56^{th} day, two birds from each replicate with weight closest to the mean of the group was selected. Selected birds were starved for 12 hours. The birds were slaughtered via neck slit and properly bled, plucked and eviscerated. The cut-up parts and Organs were weighed using a sensitive scale and were expressed as percentages of the live weight.

Caecum was removed at the 56th day from slaughtered birds and placed in a sample tube. Estimation of total bacteria count in the gut sample while bacteria identification was carried out according to COWAN and STEEL method (2004) using morphological and biochemical tests.

Triplicate fresh feaces (5 g) were collected randomly from each replicate at 56 days of age using swab sticks Oocyst count was determined out using McMaster egg-counting technique (HAUG et al. 2006).

Data obtained was arranged in a 2 x 3 factorial layout and analyzed using the General Linear Model of SAS. Differences amongst groups were determined using Duncan's multiple-range test while statistical significance were based on $P < 0.05$.

Results

The effects of strain and oral administration of *Pleurotus ostreatus* (oyster mushroom) extract on the growth performance of broiler chickens at starter phase is shown in Table 1. All parameters considered were not

Table 1

Main effects of strain and oral administration of *Pleurotus ostreatus* (oyster mushroom) extract on growth performance of broiler chickens at starter phase (0–4weeks)

Strain					*Pleurotus ostreatus*					
Parameter	Marshall	Cobb	SEM	P-Va-lue	control	2000 mg l^{-1}	4000 mg l^{-1}	SEM	P-Value	S x PE
Initial weight [g/bird]	36.45	38.36	0.08	0.300	37.39	37.44	37.39	0.44	0.937	NS
Final weight [g/bird]	795.68	824.69	10.38	0.085	806.48	810.19	813.89	14.94	0.839	NS
Total weight gain [g/bird]	759.28	786.33	11.31	0.105	769.10	772.75	776.50	14.77	0.840	NS
Daily weight gain [g/day]	108.46	112.33	1.62	0.105	109.87	110.39	110.93	2.11	0.840	NS
Total feed intake [g/bird]	1334.48	1325.70	4.50	0.536	1326.92	1326.88	1336.47	4.44	0.484	NS
Daily feed intake [g/day]	47.66	47.35	0.16	0.536	47.39	47.39	47.73	0.16	0.484	NS
Feed to gain	1.76^{a}	1.69^{b}	0.02	0.042	1.73	1.72	1.72	0.03	0.897	NS

$^{a, b}$ – Means in the same rows by factor with different superscripts differ significantly ($P < 0.05$)
SEM – standard error of means
S x PE – strain and *Pleurotus ostreatus* interaction
NS – not significant

influenced ($p > 0.05$) by strain except feed: gain. The feed to gain (FCR) was lower and thus, better in the Cobb strain (1.69). It was observed that final weight (816.67 g/bird), total weight gain (778.53 g/bird) and daily weight gain (111.19 g/bird) values were numerically higher in the Cobb strain. The effect of oral administration of *Pleurotus ostreatus* (oyster mushroom) on growth performance of broiler chickens at starter phase revealed no significant ($p > 0.05$) effect. However, the final weight, total weight gain and daily weight gain were numerically higher in the 4000 mg l^{-1} dosed group. The interactive effect between strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) had no effect ($P < 0.05$) on growth performance indices of broiler chickens at starter phase.

Table 2

Main effects of strain and oral administration of *pleurotus ostreatus (*Oyster mushroom) extract on growth performance of broiler chickens at finisher phase

Strain					*Pleurotus ostreatus*					
Parameter	Marshall	Cobb	SEM	P-Value	control	2000 mg l^{-1}	4000 mg l^{-1}	SEM	P-Value	S x PE
Initial weight [g/bird]	795.68	824.69	10.98	0.094	806.48	810.19	813.89	15.45	0.931	NS
Final weight [g/bird]	2278.09^{b}	2549.38^{a}	27.51	<.0001	2352.78^{b}	2443.98^{a}	2444.44^{a}	6.39	0.046	S
Total weight gain [g/bird]	1482.41^{b}	1724.69^{a}	30.33	<.0001	1546.30	1633.80	1635.56	60.62	0.124	S
Daily weight gain [g/day]	52.94^{b}	61.59^{a}	1.08	<.0001	55.22	58.35	58.23	2.16	0.124	S
Total feed intake [g/bird]	4283.49	4282.72	13.99	0.972	4275.00	4308.56	4265.74	17.72	0.276	NS
Daily feed intake [g/day]	152.98	152.95	0.49	0.972	152.68	153.88	152.35	0.63	0.276	NS
Feed to gain	2.89^{a}	2.49^{b}	0.05	<.0001	2.78^{a}	2.65^{b}	2.65^{b}	0.10	0.040	S
Mortality [%]	0.11	0.00	0.06	0.337	0.00	0.17	0.00	0.06	0.397	NS

$^{a, b}$ – Means in the same rows by factor with different superscripts differ significantly ($P < 0.05$)
SEM – standard error of means
S x PE – strain and *Pleurotus ostreatus* interaction
NS – not significant

Table 2 shows the effects of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on the growth performance of broiler chickens at finisher phase. Final weight, total weight gain, daily weight gain and feed conversion ratio were significantly ($P < 0.05$) affected by strain type. The final weight (2549.38 g/bird), total weight gain (1724.69 g/bird) and daily weight gain (61.59 g/bird) values were higher in Cobb strain. The feed to gain (FCR) was higher in the Marshall strain (2.89). Total feed intake value was slightly higher in the Marshall strain (4283.49 g/bird).

The oral administration of *Pleurotus ostreatus (*oyster mushroom) extract had no significant effect ($P > 0.05$) on the growth parameters of broiler chickens measured except final weight and feed: gain. The observed final weight was highest (2444.44 g/bird) in birds administered 4000 mg l^{-1} administration level and lowest in the control groups. However, numerically feed to gain (FCR) value observed was similar and best in 2000 mg l^{-1} and 4000 mg l^{-1} groups but highest (2.78) in the control group.

The interactive effect between strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on growth performance indices of broiler chickens at finisher phase is presented in Table 3. The interactive

Table 3

Interactive effect of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on growth performance parameters of broiler chickens at finisher phase

Strain	Marshall			Cobb				
Pleurotus ostreatus	control	2000 mg l^{-1}	4000 mg l^{-1}	control	2000 mg l^{-1}	4000 mg l^{-1}	SEM	P-Value
Parameter								
Initial weight [g/bird]	781.48	785.19	820.37	831.48	835.19	807.41	18.39	0.219
Final weight [g/bird]	2238.89^{d}	2325.00^{d}	2270.37^{d}	2466.67^{c}	2562.96^{b}	2618.52^{a}	37.70	0.017
Total weight gain [g/bird]	1457.41^{e}	1539.81^{d}	1450.00^{e}	1635.19^{c}	1727.78^{b}	1811.11^{a}	38.42	0.035
Daily weight gain [g/day]	52.05^{d}	54.99^{c}	51.79^{d}	58.39^{bc}	61.71^{ab}	64.68^{a}	1.04	0.040
Total feed intake [g/bird]	4261.11	4337.50	4251.85	4288.89	4279.63	4279.63	20.67	0.219
Daily feed intake [g/day]	152.18	154.91	151.85	153.18	152.84	152.84	0.74	0.219
Feed to gain	2.94^{a}	2.82^{b}	2.94^{a}	2.62^{b}	2.48^{c}	2.37^{d}	0.08	0.029
Mortality [%]	0.00	0.33	0.00	0.00	0.00	0.00	0.06	0.397

$^{a, b, c, d, e}$ – Means in the same rows by factor with different superscripts differ significantly ($P < 0.05$)
SEM – standard error of means

effect had influence ($P < 0.05$) on final weight, total weight, daily weight gain and feed to gain. Final weight, total weight gain and daily weight values increased in Cobb strain with increasing level of administration of *Pleurotus ostreatus* with the highest weight observed at 4000 mg l^{-1}. Feed to gain ratio decreased with increasing *Pleurotus ostreatus* administration in Cobb with lowest and best feed to gain ratio observed in cobb strain on 4000 mg l^{-1}.

Table 4 shows the effects of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on carcass characteristics of broiler chickens. Live weight, dressed weight, kidney: live weight, liver: live weight breast, thigh, head and the following organs; liver, kidney, gizzard, and spleen were significantly ($P < 0.05$) affected by strain. The dressed weight observed was greater in the Cobb strain (73.36 g). The Cobb strain also

Table 4

Main effects of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on carcass characteristics of broiler chickens

Parameter	Strain				*Pleurotus ostreatus*					
	Marshall	Cobb	SEM	P-Value	control	2000 mg l^{-1}	4000 mg l^{-1}	SEM	P-Value	S x PE
Live weight [g]	2255.56^b	2555.56^a	31.07	<.0001	2350.00	2425.00	2441.67	75.62	0.245	NS
Dressed weight [%]	68.02^b	73.36^a	0.61	<.0001	70.84	70.56	70.68	1.36	0.967	S
Back [%]	13.87	13.79	0.23	0.807	13.64	13.85	13.99	0.27	0.713	NS
Breast [%]	19.53^b	24.61^a	0.65	<.0001	22.19	22.28	22.74	1.36	0.865	S
Thigh [%]	10.59^b	11.41^a	0.24	0.044	10.40	10.83	11.87	0.34	0.500	NS
Drumstick [%]	9.96	10.04	0.23	0.805	9.96	9.70	10.34	0.28	0.356	NS
Wings [%]	7.77	7.83	0.14	0.727	7.52	7.90	7.98	0.15	0.138	NS
Neck [%]	5.77	5.31	0.22	0.193	5.77	5.56	5.28	0.28	0.501	NS
Liver [%]	2.10^a	1.72^b	0.09	0.015	1.86	1.89	1.80	0.14	0.785	S
Kidney [%]	0.52^a	0.37^b	0.04	0.044	0.45	0.42	0.41	0.06	0.836	NS
Gizzard [%]	1.67^a	1.43^b	0.07	0.043	1.52	1.62	1.51	0.10	0.69	NS
Heart [%]	0.5	0.43	0.02	0.064	0.49	0.46	0.44	0.03	0.494	NS
Abdominal fat [%]	2.26	1.81	0.17	0.089	1.73	2.18	2.19	0.22	0.244	NS
Spleen [%]	0.12^a	0.07^b	0.01	0.0004	0.11	0.09	0.09	0.01	0.296	S

$^{a, b}$ – Means in the same rows by factor with different superscripts differ significantly ($P < 0.05$)
All parameters are expressed as a percentage of the live-weight
SEM – standard error of means

recorded greater breast, thigh and drumstick weights (24.61g, 11.41g and 10.04 g respectively). Back, shank, wings, neck and liver: spleen weights' values observed were comparable between both strains.The oral administration of *Pleurotus ostreatus* (oyster mushroom) extract at different levels had no significant effect ($P > 0.05$) on all carcass characteristics of broiler chickens across all treatment groups. Although not significant, observed live weight improved with increasing administration levels with the highest value (2441.67 g) recorded in 4000 mg l^{-1}. Breast weight values were comparable in control and 2000 mg l^{-1} administration level.

Table 5

Interactive effect of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on carcass characteristics of broiler chickens

Strain	*Marshall*			*Cobb*				
Pleurotus ostreatus	control	2000 mg l^{-1}	4000 mg l^{-1}	control	2000 mg l^{-1}	4000 mg l^{-1}	SEM	P-Value
parameter								
Live weight [g]	2283.33	2300.00	2183.33	2516.67	2566.67	2583.33	51.99	0.872
Dressed weight [%]	67.69^{c}	68.97^{c}	67.39^{c}	72.38^{b}	73.42^{a}	74.29^{a}	0.97	0.0320
Back [%]	14.11	13.94	13.57	14.05	13.59	13.71	0.38	0.691
Breast [%]	18.87^{c}	19.12^{c}	20.61^{b}	22.87^{b}	25.69^{a}	25.67^{a}	1.04	0.011
Thigh [%]	10.63	10.41	10.74	11.32	11.03	11.87	0.40	0.708
Drumstick [%]	9.97	9.85	10.05	10.83	9.44	9.87	0.35	0.220
Wings [%]	7.79	8.05	7.46	7.91	8.01	7.58	0.21	0.736
Neck [%]	5.84	5.54	5.93	5.03	5.28	5.62	0.39	0.953
Liver [%]	2.21^{a}	2.06^{ab}	2.05^{ab}	1.89^{ab}	1.57^{b}	1.68^{ab}	0.15	0.0392
Kidney [%]	0.48	0.57	0.52	0.37	0.36	0.37	0.06	0.879
Gizzard [%]	1.80	1.55	1.66	1.48	1.43	1.38	0.12	0.522
Heart [%]	0.49	0.45	0.57	0.44	0.43	0.42	0.03	0.336
Abdominal fat [%]	2.42	2.59	1.76	1.79	1.94	1.69	0.28	0.486
Spleen [%]	0.12^{a}	0.10^{a}	0.10^{a}	0.09^{b}	0.07^{b}	0.07^{b}	0.01	0.00813

$^{a, b, c, d}$ – Means in the same rows by factor with different superscripts differ significantly ($P < 0.05$)
All parameters are expressed as a percentage of the live weight
SEM – standard error of means

Table 5 shows the interactive effect of strain and oral administration of *Pleurotus ostreatus* (Oyster mushroom) on carcass characteristics of broiler chickens. The interactive effect had no influence ($P > 0.05$) on all parameters considered. Increasing level of *Pleurotus ostreatus* administration

resulted in higher live weight and dressing percentage in both strains. Increase in breast [%] was observed in Cobb strain statistically similar values in 2000 mg l^{-1} and 4000 mg l^{-1} groups. Cobb strain had the lowest values for spleen in the 2000 mg l^{-1} and 4000 mg l^{-1} groups.

Table 6

Main effect of stains and varying levels of *Pleurotus ostreatus* on Oocyst Per G of Broiler birds

Parameter	Strains				*Pleurotus ostreatus*				
	Marshall	Cobb	S.E.M	P-value	0	2000	4000	SEM	P-value
OPG [10^6cfu ml^{-1}]	83.33	50.00	24.49	0.25	100.00^a	87.50^b	12.50^c	21.94	0.007

^{a,b,c} – Means with different superscripts along the same row are significantly different ($p \leq 0.05$)
SEM – standard error of means

Main effect of strain and varying levels of *Pleurotus ostreatus* on OPG is presented in Table 6. The strain types had no significant ($p > 0.05$) effect on the OPG of the excreta of the birds at week 8. However, Cobb strain recorded lower OPG value ($p > 0.05$) compared to the Marshall strain. Varying levels of administration *Pleurotus ostreatus* had significant effect ($p < 0.05$) on OPG of birds. A continuous reduction in OPG value was observed as the level of administration increased with the lowest value

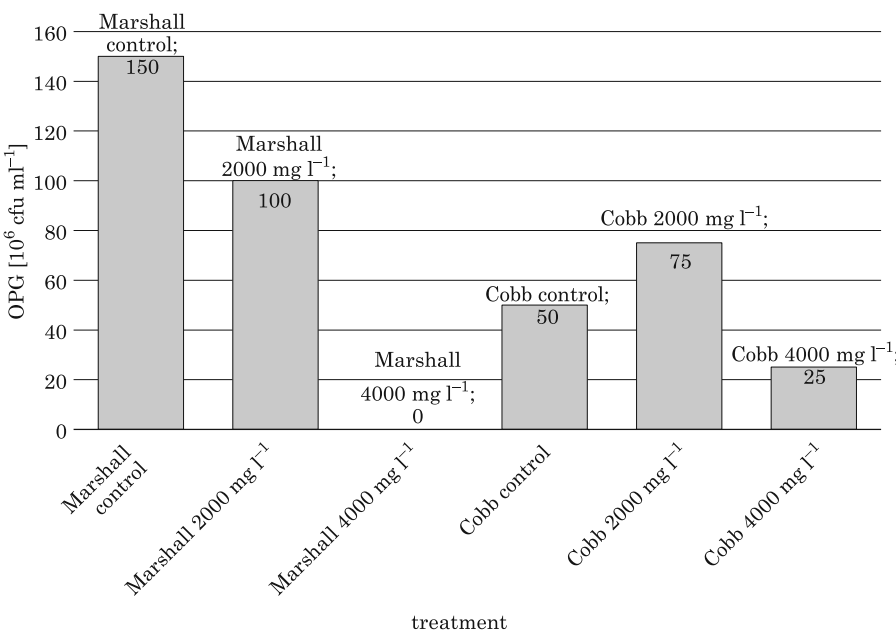

Fig. 1. Interactive effect of strains and varying levels of *Pleurotus ostreatus* on OPG of broiler chickens

recorded in birds administered 4000 mg l^{-1} *Pleurotus ostreatus* while the highest was recorded in the control group.

Figure 1 shows the Interactive effect of strains and varying levels of *Pleurotus ostreatus* on OPG of broiler chickens. Gradual decrease was observed as level of administration of Pleurotus ostreatus increased in Marshall Strain. Lowest OPG values was recorded in Marshall and Cobb strain administered 4000 mg l^{-1} *Pleurotus ostreatus.*

Effects of strains and varying levels of *Pleurotus ostreatus* on Microbiota population caecum of broiler chickens (week 8) is presented in Table 7. The strain type had no significant ($p > 0.05$) effect on the microbiota population. *Pleurotus ostreatus* was observed to significantly ($p < 0.05$) affect the microbiota population of the caecum. Highest ($p < 0.05$) microbiota population in caecum was recorded in birds administered 0 mg l^{-1} and 2000 mg l^{-1} *Pleurotus ostreatus*. Interactive effect of Strain and varying levels of *Pleurotus ostreatus* on microbiota population of the caecum of broiler chickens revealed significant ($p < 0.05$) differences in the values of microbiota population recorded. The lowest population was observed in 4000 mg l^{-1} in both strain.

Table 7

Effects of strain and varying levels of *Pleurotus ostreatus* on microbiota population of the caecum of broiler chickens (week 8)

Parameters	Strains				*Pleurotus ostreatus*				
	Marshall	Cobb	S.E.M	P-value	0	2000	4000	S.E.M	P-value
Caecum [10^6cfu ml^{-1}]	0.90	1.22	0.27	0.23	1.43^a	1.43^a	0.33^b	0.19	0.01
Strain	Marshall				Cobb				
Pleurotus ostreatus	0	2000	4000	0	2000	4000	S.E.M	P-value	–
Caecum [10^6cfu ml^{-1}]	1.10^b	1.40^b	0.20^c	1.75^a	1.45^b	0.45^c	0.21	0.006	–

a,b,c – Means with different superscripts along the same row are significantly different ($p \leq 0.05$)
cfu – colony-forming unit

Discussion

Strain affected feed to gain significantly with the Cobb strain consuming lower feed quantity daily and in total to gain more weight daily and in total consequently reaching higher final weight of and also lower and better feed to gain value within the pair at the end of the starter phase. At the end of the starter phase, administration of *Pleurotus* extract nume-

rically improved the performance of the birds as the bird in the 2000 and 4000 mg l^{-1} group recorded higher daily and total weight gain to attain greater final weights and recording numerically similar and better feed to gain values than the control group with the 2000 mg l^{-1} group consuming the least quantity of feed among the treatment groups. These outcomes synchronize with the reports of (KABIR et al. 2004, SAMLI et al. 2007) that feeding of probiotics options to broiler birds cumulates to improvement in growth performance and feed efficiency and also the reports of (NAHANSHON et al. 1992) that the means of action of probiotics in poultry include improving feed intake and digestion.

Strain effect was noticeable in the performance of the birds at the finisher phase particularly in Cobb strain which demonstrated better feed utilization resulting in substantially higher final weight, daily and total weight gain while consuming lesser quantity of feed than the Marshall. This is a reflection of considerable individual variations which is a consequence of type and feed ingested, species and breed (KOKOSZYNSKI et al. 2017) as genetic variations in broiler lines affects the rates of development of intestinal tracts and likewise the digestion potentials. These differences accounts for the distinctions observed in some performance indices of the two strains.

The oral administration of *Pleurotus ostreatus* extract positively impacted the final weight and feed to gain values of the birds at the finisher phase. Observable increase in the final weight, total weight gain and daily weight values as well as the similar but better feed to gain values of the birds in both 2000 and 4000 mg l^{-1} group can be associated with improvement in the growth performance of birds administered *Pleurotus ostreatus* extract. These results indicates improvement in growth performance and feed efficiency of broiler chickens prompted by the direct action on improving feed intake and digestion analogous to probiotic mode of action postulated by NAHANSHON et al. (1992). This mode of activity has been posited (TOGHYANI et al. 2012) to be the decimation of gut microbial load which results in improvement in animal's utilization of nutrients from diets fed resulting in feed efficiency and enhancement of growth.

At the finisher phase, the final weight, total weight gain, daily weight gain and feed to gain of the birds were influenced by the interaction of strain and oral administration of *Pleurotus* extract with no particular trend observed in the combination of Marshall Strain and *Pleurotus ostreatus* extract across all treatment groups. The interaction of *Pleurotus ostreatus* extract and Cobb strain displayed a noticeable development as seen in the increasingly changing final weight, total and daily weight gain values and better values of feed to gain with declining total and daily feed

intake from the control group to the 4000 mg l^{-1} group. These trends indicate a better performance outcome of birds administered *Pleurotus ostreatus* extract at both 2000 and 4000 mg l^{-1} treatment groups compared to the control group. These outcomes support the theory of (AWAD et al. 2009) that the efficacy may be potentiated by several approaches: the selection of more efficient strains, combination of probiotics and synergistically acting components of mushrooms (EKUNSEITAN et al. 2017). These differences accounts for the distinctions observed in some performance indices of the two strains.

Strain effect was evident on some carcass traits such as Live weight and dressed weight percentages, cut-up parts and organs. The Cobb strain indicated lower organ relative to live weight. Cut-up parts which are economically important components of carcass were of higher percentages in the Cobb than the Marshall. Similar observation was put forward by SANDERCOCK et al. (2009) of strain direct effect on carcass attributes. The oral administration of *Pleurotus ostreatus* extract had no influence any of the carcass characteristics considered. Although cut-up parts increased numerically as level of administration increased. The interactive relationship of strain and *Pleurotus ostreatus* extract brought about improvement in the dressed weight percentage, breast percentage of body weight as well as lesser organ weights with increase in administration of *Pleurotus ostreatus* extract. The non-significant effect observed in organ percentages indicate no health issue, denoting no overload on the functions of these organs and therefore pose a threat to health of animals. The 2000 and 4000 mg l^{-1} groups of both strains had better breast weight compared to their control groups. This portrays better carcass production when *Pleurotus ostreatus* extract is administered as the breast is the meatiest part of chicken however, the Cobb strain recorded the higher breast percentages between both strains.

The reduction in OPG observed with increase in *Pleurotus ostreatus* possibly indicate that the prophylactic efficacy of *Pleurotus ostreatus* in reducing *Eimeria* oocysts with increased inclusion level. This is possible by binding and removing pathogens from the intestinal tract resulting thereby in better intestinal tract integrity and stimulation of animal's immune system (YEGANI and KORVER, 2008, TOGHYANI et al. 2012) as *Eimeria* multiplication in the intestinal tract causes hemorrhagic tissue damage results in mortality, disruption of digestive processes, depressed weight gain, and vulnerability to other disease causing organisms (MC DOUGALD and FITZ-COY 2008). Since there exist a direct relationship between oocysts concentration and lesion development, a reduction in oocyst counts will help in curtailing the incidence of severe intestine

lesions or eroding of absorption sites resulting in better improvement in broiler growth performance. This positive observation may suggest that parasite reproductive status has been compromised by the administration of *Pleurotus ostreatus*, leading to the possible reduction in the production of trypsin and bile in the duodenum responsible for activation and release of its sporozoites (JORDAN et al. 2011).

Microbes in the GIT is subdivided into pathogenic or beneficial groups, pathogenic bacteria causes systemic infections, intestinal putrefaction, and toxin formation. The identified microbes in the study were harmful bacterial and their population was greatly reduced in both strain at the highest level of administration. *Pleurotus ostreatus* administration demonstrated similar ability as probiotics (JEURISSEN et al. 2002) in offering protection from gut pathogenic organisms. This is also supported by study conducted by EKUNSEITAN et al (2018) which demonstrated the ability of *Pleurotus ostreatus* in inhibiting *in vitro* growth of enteric pathogens. And as widely emphasized a good gut health leads to positive production attributes. This will also help in reducing the shedding of microbes in flesh of meat during processing

The use of *Pleurotus ostreatus* can serve as an option in replacing conventional and growth promoting drugs as indicated by its ability improving feed to gain thereby resulting in better growth in Cobb strain up to 400 mg l^{-1} and 2000 mg l^{-1} in Marshall. Likewise, *Pleurotus ostreatus* should be administered at 4000 mg l^{-1} for reduction of caecal pathogenic population and bacterial organisms in both Marshall and Cobb broiler chicken strains.

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References

AWAD W.A., GHAREEB K., ABDEL-RAHEEM S., BÖHM J. 2009. *Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens*. Poult. Sci., 88(1): 49–56.

BAKARI G.G., MAX R.A., MDEGELA R.H., PHIRI E.C., MTAMBO M.M. 2012. *Effect of crude extracts from Commiphora swynnertonii (Burtt) against selected microbes of animal health importance.* J. Med. Plants Res., 6(9): 1795–1799.

BOBEK P., GALBAVY S., 2001. *Effects of plevran (beta-glucan from plevrotus ostreatus) on the antioxidant status of the organism amd on dimethylhydrazine-indixed precancerous lesion in rat colon*. Br. J. Biomed. Sci., 58: 164–168.

BORCHERS A.T., KRISHNAMORTHY A., KEEN C.L., MEYERS F.J., GERSHWIN M.E. 2008. *The Immvnobiology of mushrooms*. Exp. Bio. Med., 233(3): 259–276.

COWAN S.T., STEEL K.J. 2004. *Cowan and Steel's manual for the identification of medical bacteria.* Cambridge University Press, Cambridge.

DALLOUL R.A., LILLEHOJ H.A. 2006. *Poultry coccidiosis. Recent Advancements in control measures and vaccine development*. Expert Rev. Vaccine, 5: 143–163.

EKUNSEITAN D.A., AYOOLA A.A., ADEGUN W.O. 2018. *Antibacterial activity of ethanolic extract of Pleurotus ostreatus on selected pathogenic organisms*. [In:] *Exploring science and technology. Innovations for sustainable livestock development*. Proceedings of 43rd Annual Conference of the Nigerian Society for Animal Production. March 18th – 22nd 2018, FUT Owerri. Eds. I.C. Okoli, I.P. Ogbuewu, O.O. Emenalom, B.O. Esonu. Published by NSAP, pp. 974–976.

EKUNSEITAN D.A., EKUNSEITAN O.F., ODUTAYO O.J., ADEYEMI P.T. 2017. *Pleurotus ostreatus. Its effect on Carcass, serum metabolites and meat lipoprotein content of broiler chickens*. *Pertanika Journal of Tropical Agricultural Science,* 40(4): 629–638.

EKUNSEITAN D.A., YUSUF, A.O., OLAYINKA O.A., AYOOLA A.A., ADEGBENJO A.A. 2016a. *Comparative study of two plants (Lagenaria breviflora and Petiveria alliacea) and their phytobiotic potential in poultry health*. Nig. J. Anim. Prod., 43(1): 289–298.

EKUNSEITAN D.A., ADEYEMI M.A., ABIOLA S.S., OLUWATOSIN O.O., SOGUNLE O.M., FABUSORO E. 2016b. *'Perception of ethno-veterinary practices in selected villages in Ogun state.* Nig. J. Anim. Sci., 18(1):108–127.

Google maps. (2018). Federal University of Agriculture. Retrieved from https://earth.google.com, access: 21.02.2018.

GUO F.C, WILLIAMS B.A, KWAKKEL R.P, LI H.S, LI X.P, LUO J.Y, LI W.K., VERSTEGEN M.W.A. (2004b). *Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens.* Poult. Sci., 83: 175–182

HAUG A., WILLIAMS R.B., LARSEN S. 2006. *Counting coccidial oocysts in chicken faeces. A comparative study of a standard McMaster technique and a new rapid method*. Vet. Parasit., 136(3–4), pp. 233–242.

JEURISSEN S.H., F. LEWIS, J.D. VAN DER KLIS, Z. MROZ, J.M. REBEL, A.A. TER HUURNE. 2002. *Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality*. Curr. Issues Intest. Microbiol., 3: 1–14.

JORDAN A., CALDWELL D.J., KLEIN J., COPPEDGE J., POHL S., FITZ-COY S., LEE J.T. 2011. *Eimeria tenella oocyst shedding and output in cecal or fecal contents following experimental challenge in broilers.* Poult. Sci., 90(5): 990–995.

KABIR S.M.L., RAHMAN M.M., RAHMAN M.B., RAHMAN M.M., AHMED S.U. 2004. *The dynamics of probiotics on growth performance and immune response in broilers.* Int. J. Poult. Sci., 3(5): 361–364.

KOKOSZYŃSKI D., BERNACKI Z., SALEH M., STĘCZNY K., BINKOWSKA M. 2017. *Body conformation and internal organs characteristics of different commercial broiler lines.* Rev. Bras. de Ciên. Avícola, 19(1), 47–52.

LIU J., JIA L., KAN J., JIN CH. 2013. *In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom* (*Agaricus bisporus*). Food Chem. Toxicol, 51: 310–316.

MCDOUGALD L.R., FITZ-COY S. 2008. *Coccidiosis in diseases of poultry*. 12th ed. Y. M. Saif. Blackwell Publishing, Ames, IA. pp 1068–1085.

MINARECI E., EROGONUL B., KAYALAR H., KALYONCU F. 2011. *Chemical compositions and antioxidant activities of five endemic asperula TAXA.* Arch. Bio. Sci. Belgrade, 63(3): 537–543.

MOUNTZOURIS K.C., TSIRTSIKOS P., KALAMARA E., NITSCH S., SCHATZMAYR G., FEGEROS K. 2007. *Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities*. Poult. Sci., 86(2): 309–317.

NAHANSHON S.N., NAKAUE H.S., LLIROSH L.W. 1992. *Effects of direct fed microbials on nutrient retention and parameters of laying pullets*. Poult. Sci., 71: 111.

SAMLI H.E., SENKOYLU N., KOC F., KANTER M., AGMA, A. 2007. *Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota.* Arch. of Anim. Nutri., 61(1): 42–49.

SANDERCOCK D.A., NUTE G.R., HOCKING P.M. 2009. *Quantifying the effects of genetic selection and genetic variation for body size, carcass composition, and meat quality in the domestic fowl (Gallus domesticus).* Poult. Sci., 88: 923–931.

SAS (Statistical Analysis System) Institute Inc. 2010. SAS/STAT® 9.22 User's Guide. Cary, NC: SAS Institute Inc. Cary, North Carolina.

TOGHYANI M., TOHIDI M., GHEISARI A., TABEIDIAN A., TOGHYANI M. 2012. *Evaluation of oyster mushroom (Pleurotus ostreatus) as a biological growth promoter on performance, humoral immunity, and blood characteristics of broiler chicks*. J. Poult. Sci., 49(3): 183–190.

TOROK V.A., OPHEL-KELLER K., HUGHES R.J. FORDER R., ALI, M., MACALPINE R., 2007. *Environment and age: impact on poultry gut microflora*. Proceedings of the 19th Australian Poultry Science Symposium, Sydney, New South Wales, Australia. 12–14 February, pp. 149–152.

YEGANI M., KORVER D.R. 2008. *Factors affecting intestinal health in poultry*. Poult. Sci., 87: 2052–2063.

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THE EFFECT OF HYDROTHERMALLY PROCESSED SOYBEAN AND RAPESEED PRODUCTS ON NUTRIENT DIGESTIBILITY IN GROWING-FINISHING PIGS

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Key words: extruded rapeseed cake, soybean, pigs, digestibility, calcium, phosphorus, balance.

Abstract

The aim of this study was to determine nutrient digestibility, nitrogen retention, and calcium and phosphorus balance in growing-finishing pigs fed control complete diets containing soybean meal and soybean oil, and experimental complete diets where soybean meal and soybean oil were replaced with toasted full-fat soybeans, cold-pressed rapeseed cake and extruded rapeseed cake.

The experiment was performed on 28 crossbred pigs (Polish Large White x Polish Landrace sows) x (Hampshire x Pietrain boars) with average body weight of 55–60 kg. The animals were divided into 4 groups of 7 animals each, based on the percentage content of the following components in complete diets: soybean meal, toasted full-fat soybeans, cold-pressed rapeseed cake with increased oil content, extruded rapeseed cake with increased oil content.

The processes of extruding rapeseed cake and toasting soybeans had no significant effect on protein digestibility or nitrogen balance. The digestibility of calcium and phosphorus decreased insignificantly in pigs fed diets with rapeseed cake, compared with pigs fed diets with soybean meal and toasted soybeans. Extrusion of rapeseed cake increased the digestibility of ether extract.

Introduction

Soybean meal, which is one of the main sources of protein in animal feeds, has a high content of protein with an optimal amino acid composi-

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tion (JERZAK et al. 2012, STEIN et al. 2016). Soybean meal is characterized by a high content of essential amino acids, a low content of crude fiber and antinutritional factors and, consequently, high nutrient digestibility (BIEL 2011, KIM et al. 2012). Each year, Poland imports more than 2.0 million tons of soybean meal, but significant fluctuations in soybean prices and widespread concern regarding the use of transgenic plants and genetically modified soybeans in animal feed prompt the search for alternative, local sources of protein. In Poland, the by-products of rapeseed processing offer a viable option (FIEDOROWICZ and SOBOTKA 2013, HANCZAKOWSKA and ŚWIĄTKIEWICZ 2014, JUST and ŚMIGLAK-KRAJEWSKA 2015).

Rapeseed is one of the major industrial crops grown in Poland (KACZMAREK et al. 2016). Double-low varieties of rapeseed with a reduced content of glucosinolates and erucic acid, yellow-seeded varieties with low fiber content and the use of rapeseed as a biocomponent in the biofuel sector have increased the role and significance of rapeseed-based feeds (BRZÓSKA et al. 2010, STEIN et al. 2016). Rapeseed meal and oil cake, the by-products of rapeseed production, are used in the production of animal feeds (KALEMBASA and ADAMIAK 2010).

The chemical composition and nutritional value of rapeseed-based feeds are influenced by the production technology. Crude protein content ranges from 29% in rapeseed oil cake to 38% in rapeseed meal (BRZÓSKA et al. 2010, EKLUND et al. 2015). Rapeseed-based feeds are characterized by similar biological value of protein, and according to many authors (OCHODZKI et al. 1995, PASTUSZEWSKA et al. 1997, LIPIŃSKI 1998, STEIN et al. 2016), the biological value of rapeseed protein is comparable to that of soybean meal.

The ingredients used in the production of feeds for monogastric animals are hydrothermally processed to improve nutrient digestibility and reduce the content of antinutritional factors, including glucosinolates (EKLUND et al. 2015). Hydrothermal processing lowers the excretion of the undigested feed fraction, which delivers significant environmental benefits (SMULIKOWSKA and VAN NGUYEN 2003, LIPIŃSKI 2003, KORNIEWICZ et al. 2007b).

The aim of this study was to determine nutrient digestibility, nitrogen retention, and calcium and phosphorus balance in growing-finishing pigs fed control complete diets containing soybean meal and soybean oil, and experimental complete diets where soybean meal and soybean oil were replaced with toasted full-fat soybeans, cold-pressed rapeseed cake and extruded rapeseed cake.

Materials and Methods

Digestibility and balance trials were performed on 28 crossbred pigs (Polish Large White x Polish Landrace sows) x (Hampshire x Pietrain boars) with average body weight of 55–60 kg. The animals were divided into 4 groups of 7 animals each, based on the percentage content of the following components in complete diets: soybean meal, toasted full-fat soybeans, cold-pressed rapeseed cake with increased oil content, extruded rapeseed cake with increased oil content:

– group I – control, fed diets where soybean meal was the main protein source and soybean oil was the main energy source;

– group II – experimental, fed diets where toasted full-fat soybeans were the main source of protein and energy;

– group III – experimental, fed diets where cold-pressed rapeseed cake with increased oil content (21%) was the main source of protein and energy;

– group IV – experimental, fed diets where extruded rapeseed cake with increased oil content (21%) was the main source of protein and energy.

In each group, complete diets in mash form were offered and the amount of feed administered individually to pigs was recorded in successive stages of the fattening period. Pigs were feeding *ad libitum* from live weight 22 kg to 55–60 kg, then in digestibility trial 2 kg of feed daily, and in the end of experiment on 90 day *ad libitum*. Average daily gains, feed intake and feed consumption per kg of body weight gain were monitored on an individual basis on days 26, 60 and 90. During the digestibility and balance trial, the animals were fed complete diets in mash form. Chemical analyses of the ingredients of complete diets were performed by standard methods (AOAC 2005).

The results of the above analyses were used to determine the content of nutrients and minerals in complete diets. The energy value of diets was calculated based on own analyses of feed components, and nutrient digestibility coefficients and formulas given in the Polish edition of Pig Nutrient Requirements (2015) and CVB (2004). The percentage composition and nutritional value of diets are shown in Table 1. The glucosinolate content of rapeseeds and rapeseed cakes was determined by the glucose release method (Ciska and Waszczuk 1995). The results of chemical analyses were used to calculate nutrient digestibility coefficients, nitrogen balance and mineral balance (Jamroz 2004).

Pigs were placed in individual metabolism cages, and were fed completed diets at 2 kg daily. The feed was consumed in its entirety. A three-day adaptation period was followed by a 4-day collection period when feed

Table 1

Composition and nutritional value of experimental pig diets

Specification	Unit	Group			
		I soybean meal + soybean oil	II toasted full-fat soybeans	III rapesed cake	IV extruded rapeseed cake
Wheat	%	40.000	40.000	40.000	40.000
Barley	%	43.775	42.495	39.355	39.345
Soybean meal	%	11.500	4.000	4.000	4.000
Toasted full-fat soybeans	%	–	10.500	–	–
Cold-pressed rapeseed cake	%	–	–	14.000	–
Extruded rapeseed cake	%	–	–	–	14.000
Soybean oil	%	1.750	–	–	–
Limestone	%	0.730	0.730	0.750	0.750
Monocalcium phosphate	%	0.530	0.550	0.200	0.200
Acidifier (Lonacid Max)	%	0.300	0.300	0.300	0.300
Salt	%	0.450	0.450	0.450	0.450
L-lysine HCl	%	0.320	0.330	0.340	0.350
L-threonine	%	0.070	0.070	0.050	0.050
DL-methionine	%	0.050	0.050	0.030	0.030
L-tryptophan	%	0.010	0.010	0.010	0.010
Natuphos 5000G	%	0.010	0.010	0.010	0.010
Rovabio Excel	%	0.005	0.005	0.005	0.005
0.5% Grower Premix	%	0.500	0.500	0.500	0.500
Metabolizable energy	MJ	13.25	13.25	13.20	13.25
Crude protein	%	16.4	16.4	16.4	16.4
Crude fiber	%	3.1	3.3	4.1	4.1
Ether extract	%	3.2	3.5	4.2	4.2
Lysine	%	0.93	0.93	0.93	0.93
Methionine	%	0.29	0.29	0.29	0.29
Met+Cys	%	0.60	0.61	0.64	0.63
Threonine	%	0.59	0.59	0.59	0.59
Tryptophan	%	0.20	0.20	0.20	0.20
Calcium (Ca)	%	0.60	0.60	0.60	0.60
Available phosphorus (P av)	%	0.30	0.30	0.30	0.30
Sodium (Na)	%	0.19	0.19	0.19	0.19
Glucosinolates	mM kg^{-1}	–	–	2.37	1.96

Premix composition: 24.6% Ca, 2 000 000 IU vitamin A, 400 000 IU vitamin D_3, 14 000 mg vitamin E, 12 727 mg DL α-tocopherol, 300 mg vitamin K_3, 300 mg vitamin B_1, 800 mg vitamin B_2, 600 mg vitamin B_6, 5 mg vitamin B_{12}, 400 mg folic acid, 2 000 mg pantothenic acid, 4 000 mg niacin, 20 mg biotin, 60 000 mg choline chloride, 8 000 mg Mn, 20 000 mg Zn, 20 000 mg Fe, 4000 mg Cu, 120 mg Co, 240 mg J, 60 mg Se, 30 000 mg Herbiplant CS

intake and excretion of urine and feces were recorded daily. Urine was collected in plastic containers placed under the cages. Each day, 10 ml of 10% sulfuric acid was added to the containers to reduce ammonia emission. Feces were collected on a plastic mesh under the slatted floor of the pens. For four consecutive days, urine and feces were collected at the same time, and weighed. Samples accounting for approximately 20% of daily urine and feces collections were placed in jars sealed with cork stoppers (urine) and plastic bags (feces). The samples were stored in a refrigerator at a temperature of 3–4 °C. Urine and feces collected over four days were thoroughly mixed, and 1 kg feces and 1 l urine samples were taken. Wet feces samples were assayed for the content of dry matter and nitrogen. Dried fecal samples were assayed for the content of ether extract, crude fiber, crude ash, calcium and phosphorus. The content of nitrogen, calcium and phosphorus was determined in urine samples. All analyses were performed using standard procedures (AOAC 2005).

The results, including body weight gains, feed intake, feed conversion, nutrient digestibility and nitrogen balance, were processed statistically by one-way analysis of variance (ANOVA). The significance of differences between groups was estimated by Duncan's multiple range test using STATGRAPHICS v. 5.0 software. The results were expressed as arithmetic means and standard deviations.

Results and Discussion

Chemical composition of rapeseed cake and extruded rapeseed cake

The proximate chemical composition of rapeseed cake and extruded rapeseed cake is presented in Table 2. Extrusion contributed to an increase in dry matter content, from 92.16% to 94.31%. The analyzed rapeseed cake contained approximately 29% of crude protein (CP), 21% of ether extract (EE), 10% of crude fiber (CF), 5.7% of starch and 5.4% of crude ash. An analysis of detergent fiber revealed that neutral detergent fiber (NDF) and acid detergent fiber (ADF) accounted for approximately 20% and 16% of total dietary fiber, respectively. Extrusion had no significant effect on the mineral content of rapeseed cake.

Toasted full-fat soybeans (GMO) were finely ground in a mill to obtain a homogeneous product. Low moisture content (up to 9.5%) prevented mould growth. The major nutrients were crude protein (35%) and ether extract (21%). Crude fiber content was 5%, including 9.7% of NDF and 6%

Table 2

Nutrient content of soybean and rapeseed based feed materials

Specification	Unit	Soybean meal	Toasted full-fat soybeans	Rapeseed cake	Extruded rapeseed cake
Dry matter	%	89.98	90.68	92.16	94.31
Organic matter	%	84.08	85.98	86.81	88.77
Crude protein	%	46.40	35.32	28.57	29.39
Crude fiber	%	3.20	4.91	9.75	10.06
Ether extract	%	1.70	20.39	21.20	21.70
Crude ash	%	5.90	4.70	5.35	5.54
Starch	%	2.66	3.47	5.73	5.73
Sugars	%	8.22	6.81	8.26	8.31
N-free extractives	%	32.78	25.36	27.29	27.62
ADF *	%	5.10	6.00	15.85	16.85
NDF **	%	9.30	9.70	19.53	21.31
Calcium	%	0.29	0.22	0.67	0.67
Phosphorus	%	0.65	0.48	0.94	0.94
Sodium	%	0.03	0.01	0.01	0.01

*ADF – acid detergent fiber, **NDF – neutral detergent fiber

of ADF. Starch content was only 3.5%, sugar content was 6.8%, and ash content – 4.7%. The concentrations of mineral nutrients were similar as in soybean meal (Table 2).

Growth performance

The average daily gain of control group pigs fed diets containing soybean meal reached 942 g (Table 3). The average daily gain of group II pigs

Table 3

Growth performance of pigs

Specification	Group			
	I soybean meal + soybean oil	II toasted full-fat soybeans	III rapeseed cake	IV extruded rapeseed cake
Body weight [kg] – initial – final	 22.1 107.0	 21.9 111.6	 22.1 102.7	 22.3 107.6
Days of feeding trial	90	90	90	90
Average daily gain [g]	942±16^{b}	998±16^{c}	895±17^{a}	947±21^{b}
Daily feed intake [kg/animal]	2.44	2.51	2.45	2.47
Gain: Feed ratio [kg kg^{-1}]	2.59±0.03^{a}	2.52±0.03^{a}	2.74±0.04^{b}	2.61±0.04^{a}

a, *b* – $p \leq 0.05$
A, *B* – $p \leq 0.01$

fed diets where soybean meal and soybean oil were replaced with toasted full-fat soybeans was 5.9% higher. The average daily gain of group IV pigs receiving extruded rapeseed cake with increased oil content was comparable with that determined in the control group. Group III pigs fed diets containing cold-pressed rapeseed cake were characterized by lower average daily gain and higher feed intake per kg body weight gain (by 5%) compared with control group animals and group IV pigs receiving extruded rapeseed cake.

Nutrient digestibility and nitrogen balance

The results presented in Table 4 show that feed ingredients had no effect on dry matter digestibility, which was similar in all groups (83.3–83.9%). No significant differences in the digestibility coefficients of organic matter were found between the groups, either (84.9–85.7%). The digestibility of CP from diets based on soybean products and diets containing 14% of rapeseed cake was comparable (83.7–84.2%). The processes of toasting soybeans and extruding rapeseed cake had no significant influence on the digestibility of CP contained in complete diets. Ether extract digestibility was relatively high in all groups. In the control group, where soybean meal-based diets were supplemented with soybean oil, EE digestibility

Table 4

Apparent digestibility coefficients of nutrients [%]

Specification	Group			
	I soybean meal + soybean oil	II toasted full-fat soybeans	III rapeseed cake	IV extruded rapeseed cake
Dry matter	83.3 ± 1.71	83.9 ± 1.40	83.4 ± 0.83	83.4 ± 1.90
Organic matter	84.9 ± 1.80	85.7 ± 1.12	85.2 ± 0.90	85.0 ± 1.88
Crude protein	83.7 ± 2.14	84.2 ± 1.18	83.8 ± 1.21	84.0 ± 3.31
Ether extract	72.4^{a} ± 5.89	74.5^{A} ± 3.33	68.6^{B} ± 2.49	78.9^{Ab} ± 2.03
Crude fiber	25.7 ± 3.27	27.4 ± 1.75	25.4 ± 4.14	25.7 ± 3.61
Crude ash	53.0 ± 2.75	53.6 ± 2.34	53.1 ± 3.52	53.9 ± 3.37
N-free extractives	89.1 ± 1.71	89.6 ± 1.27	90.2 ± 1.34	90.0 ± 1.84

a, *b* – p≤0.05
A, *B* – p≤0.01

reached 72.4%. The digestibility coefficient of EE was somewhat higher in group II fed diets with toasted full-fat soybeans (74.5%). Pigs receiving diets with cold-pressed rapeseed cake with increased oil content were characterized by the lowest fat digestibility (68.6%).

The extrusion of rapeseed cake had a beneficial influence on fat utilization – the digestibility coefficient of EE was highest in group IV (78.9%). The difference in EE digestibility between group IV and group III fed diets containing cold-pressed rapeseed cake was statistically highly significant ($P \leq 0.01$). The extrusion of rapeseed cake with increased oil content contributed to a significant increase in EE digestibility, relative to the control group fed soybean meal-based diets supplemented with soybean oil ($P \leq 0.05$).

Crude fiber digestibility was at a similar level (25.7–25.4 %) in the control group and groups III and IV (fed diets containing rapeseed cake). An insignificantly higher digestibility coefficient of CF was noted in group II (fed toasted full-fat soybeans). The extrusion of rapeseed cake had no effect on CF digestibility. The digestibility coefficients of nitrogen-free extractives and crude ash were highly similar in all groups.

Similar values of nutrient digestibility coefficients were reported by KORNIEWICZ et al. (1999) in pigs fed diets containing 18% of rapeseed cake or 11% of soybean meal. In the cited study, the digestibility coefficients of dry matter and organic matter reached 83.5% and 85.3%, respectively, whereas CP digestibility was lower (77.6%) than in our experiment (83.7%) where pigs were fed diets containing 14% of rapeseed cake. The above authors demonstrated that the digestibility of all nutrients was higher in control group animals receiving 11% of soybean meal than in pigs fed 18% of rapeseed cake.

LIPIŃSKI et al. (1994) found that rapeseed meal and rapeseed cake decreased organic matter digestibility in growing-finishing pigs, but had no effect on the digestibility coefficients of other nutrients. In a study by KUŚNIEREK et al. (2005), the extrusion of rapeseed meal at a temperature of 140°C and 160°C decreased the total and ileal digestibility of protein and amino acids. The total digestibility of CP was 86.9% in soybean meal-based diets and 78.2% in rapeseed meal-based diets. The increase in temperature from 140°C to 160°C led to a considerably decrease in protein digestibility, from 76.18% to 67.54%. KEADY and O'DOHERTY (2000) investigated the effect of extrusion on the nutritive value of rapeseed meal as a protein supplement for growing-finishing pigs and found that the extrusion process had no influence on the digestibility coefficients of nitrogen and NDF. PASTUSZEWSKA and OCHTABIŃSKA (1998) evaluated the digestibility and biological value of protein contained in rapeseed and *Brassica*

rapa cake and meal. The protein digestibility of rapeseed cake and meal was 84.9% and 80.1%, respectively. An analysis of CF digestibility revealed even greater differences between rapeseed cake and meal (52.1% vs. 29.3%). KALDMÄE et al. (2010) compared the nutritional value of heat-treated and cold-pressed rapeseed cake and found that heat treatment improved CP digestibility (70.4% vs. 68.4%, $P \leq 0.05$).

PASTUSZEWSKA and RAJ (2003) demonstrated that CP digestibility is largely determined by technological processes which lead to the formation of indigestible complexes that resist enzyme action in the gastrointestinal tract of monogastric animals, and increase the amount of neutral detergent insoluble crude protein (ND-ICP), i.e. the amount of protein bound in the NDF fraction. According to SMULIKOWSKA and VAN NGUYEN (2003), part of rapeseed CP is strongly bound to dietary fiber fractions, which decreases its digestibility to 77–79%. BURACZEWSKA et al. (1998) determined the content of NDF, including protein and essential amino acids, in rapeseed cake and meal heated for 0, 20, 40, 60 and 80 minutes at a temperature of 130°C. In the cited study, prolonged heating increased the NDF content of rapeseed cake (from 21% to 34%) and rapeseed meal (26% to 38%). At the same time, protein concentration in the NDF fraction of rapeseed cake and meal increased from 10.5% to 24.0% and from 15.7% to 28.2%, respectively. The ileal digestibility of CP and amino acids determined in growing pigs decreased with increasing NDF content. The authors concluded that the NDF content of rapeseed meal exceeding 30% points to overheating in the processes of deoiling and toasting, and to a relatively low nutritional value of rapeseed meal, as confirmed by low ileal digestibility of CP (approx. 60%).

The results of the present study and the findings of other authors indicate that in finishing pigs over 50 kg of body weight, the utilization of protein contained in cold-pressed rapeseed cake with low glucosinolate content and soybean meal is equally efficient. In the current study, the extrusion of rapeseed cake with increased oil content did not improve protein utilization efficiency.

Nitrogen balance

Nitrogen intake was identical in all pigs, therefore nitrogen balance could be compared across dietary treatments (Table 5). Daily fecal nitrogen excretion was similar in all groups, at 8.3–8.5 g N. Relative to nitrogen intake, nitrogen excretion in feces accounted for 16% on average. Urinary nitrogen excretion was much higher, at approximately 40% of nitrogen intake. Control group pigs excreted 20.7 g of nitrogen in urine. Urinary

nitrogen excretion was at a similar level (20.9 g N) in group IV pigs fed diets with extruded rapeseed cake. Nitrogen excretion in urine was lowest in group II (19.5 g N), and the difference between group II and group III (fed cold-pressed rapeseed cake) was statistically significant ($P \leq 0.01$).

Table 5

Nitrogen balance

Specification	Group			
	I soybean meal + soybean oil	II toasted full-fat soybeans	III rapeseed cake	IV extruded rapeseed cake
N intake [g]	52.5	52.5	52.5	52.5
N excretion [g]:				
Fecal	8.5 ± 1.11	8.3 ± 0.71	8.4 ± 0.48	8.3 ± 0.63
urinary	20.7^{AB} ± 1.38	19.5^{A} ± 1.25	21.4^{B} ± 1.27	20.9^{AB} ± 0.64
N retention [g]	23.3^{AB} ± 1.43	24.6^{A} ± 1.22	22.6^{B} ± 1.21	23.3^{AB} ± 0.62
N retained as % of N intake	44.6^{ABab} ± 2.81	46.9^{Aa} ± 2.34	43.1^{B} ± 2.28	44.1^{b} ± 1.30
N retained as % of N digested	52.9 ± 2.90	55.8 ± 2.68	51.4 ± 2.72	52.7 ± 1.25

a, *b* – $p \leq 0.05$
A, *B* – $p \leq 0.01$

Nitrogen retention was highest in group II, at 24.6 g, i.e. 46.9% of nitrogen intake. Nitrogen retention in the control group and group IV (fed extruded rapeseed cake) was identical (23.3 g N). In group III pigs fed diets with cold-pressed rapeseed cake, nitrogen retention was lower (22.6 g N) than in the remaining groups. A highly significant difference ($P \leq 0.01$) was found between group III and group II (fed toasted full-fat soybeans).

Similar results were reported by McDonnell et al. (2010) who examined the effects of replacing soybean meal with rapeseed meal in diets for growing-finishing pigs. Urinary nitrogen excretion and nitrogen retention decreased with increasing levels of rapeseed meal in the ration. Lipiński et al. (1994) demonstrated that pigs fed rapeseed meal were characterized by lower nitrogen retention and utilization. In another study, Lipiński et al. (1997) found that rapeseed products (meal, cake, seeds) improved nitrogen retention and utilization efficiency.

Calcium and phosphorus balance

In the digestibility and balance trial, dietary calcium intake was 12 g and dietary phosphorus intake was 10 g; the Ca:P ratio was 1.2:1.0. The results presented in Table 6 show that the analyzed alternative sources of protein and energy had no significant effect on calcium balance. In all groups, pigs excreted from 7.10 to 7.24 g Ca in feces, which accounted for 59–60% of calcium intake. Urinary calcium excretion was low, at 0.25–0.33 g, i.e. 2% of calcium intake. Calcium retention was 4.44–4.61 g, i.e. 37.0–38.4% of calcium intake. The differences between groups were statistically not significant, but calcium retention and digestion was somewhat lower in groups III and IV fed diets with rapeseed cake.

Table 6

Calcium and phosphorus balance

Specification	Group			
	I soybean meal + soybean oil	II toasted full-fat soybeans	III rapeseed cake	IV extruded rapeseed cake
Calcium balance				
Ca intake [g]	12.00	12.00	12.00	12.00
Ca excretion [g]:				
Fecal	7.10 ± 0.33	7.10 ± 0.46	7.24 ± 0.28	7.23 ± 0.29
Urinary	0.32^{a} ± 0.08	0.29^{ab} ± 0.05	0.25^{b} ± 0.03	0.33^{a} ± 0.05
Ca retention [g]	4.58 ± 0.33	4.61 ± 0.49	4.51 ± 0.25	4.44 ± 0.32
Ca retained as % of Ca intake	38.1 ± 2.77	38.4 ± 4.06	37.6 ± 2.10	37.0 ± 2.64
Apparent total tract digestibility [%]	40.8 ± 2.76	40.8 ± 3.82	39.6 ± 2.33	39.8 ± 2.43
Phosphorus balance				
P intake [g]	10.00	10.00	10.00	10.00
P excretion [g]:				
Fecal	5.53 ± 0.25	5.57 ± 0.44	5.72 ± 0.20	5.76 ± 0.50
Urinary	0.09^{a} ± 0.03	0.12^{ab} ± 0.07	0.16^{b} ± 0.04	0.15^{b} ± 0.02
P retention [g]	4.37 ± 0.24	4.30 ± 0.42	4.12 ± 0.22	4.10 ± 0.49
P retained as % of P intake	43.7 ± 2.37	43.0 ± 4.16	41.2 ± 2.21	41.0 ± 4.90
Apparent total tract digestibility [%]	44.6 ± 2.45	44.3 ± 4.44	42.8 ± 1.98	42.4 ± 5.00

a, *b* – $p \leq 0.05$
A, *B* – $p \leq 0.01$

In all dietary treatments, phosphorus balance was more efficient than calcium balance. Pigs excreted from 5.53 to 5.76 g P in feces, which accounted for 55–57% of phosphorus intake. Urinary phosphorus excretion was low, at 0.09–0.16 g, i.e. 0.9–1.6% of phosphorus intake. Phosphorus retention was 4.10–4.37 g, i.e. 41.0–43.7% of phosphorus intake. A minor decrease in phosphorus retention and digestion was noted in pigs fed diets containing rapeseed cake (groups III and IV), but the observed differences were statistically not significant.

Similar utilization efficiency of calcium and phosphorus was reported by other authors (FANDREJEWSKI 1997, ORDA et al. 1998, USYDUS et al. 2006, KORNIEWICZ 2007a, KORNIEWICZ 2007b).

Conclusions

It can be concluded that the processes of extruding rapeseed cake and toasting soybeans had no significant effect on crude protein digestibility or nitrogen balance. Rapeseed cake with increased oil content and extruded rapeseed cake had no adverse influence on the balance of phosphorus and calcium from complete diets. Extrusion of rapeseed cake increased the digestibility of ether extract. The nutrient digestibility coefficients determined in pigs indicate that extruded rapeseed cake with increased oil content can be a viable alternative to soybean meal in pig nutrition.

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References

AOAC. 2005. *Official methods of analysis of the association of official analytical chemists.* 16th Edition, Arlington, Virginia, USA.

BIEL W. 2011. *Skład chemiczny i jakość białka konwencjonalnej i genetycznie zmodyfikowanej poekstrakcyjnej śruty sojowej.* Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech., 20: 17–24.

BRZÓSKA F., ŚLIWIŃSKI B., MICHALIK-RUTKOWSKA O. 2010. *Pasze rzepakowe – miejsce w bilansie białkowym kraju oraz wartość pokarmowa.* Cz. 1. Wiad. Zoot., 48: 11–18.

BURACZEWSKA L., GDALA J., WASILEWKO J., BURACZEWSKI S. 1998. *Zawartość białka związanego z frakcją włókna (NDF) a strawność jelitowa u świń białka i aminokwasów pasz rzepakowych traktowanych termicznie.* Rośl. Oleiste, XIX(1): 175–186.

CISKA E., WASZCZUK K. 1995. *Metody oznaczania glukozynolanów (GLS). A. Oznaczanie sumarycznej zawartości glukozinolanów metodą glukozową.* Konf. Nauk. Glukozynolany rzepaku i warzyw krzyżowych (występowanie, właściwości i metody analiz). Olsztyn, pp. 65–71.

CVB 2004. Veevoedertable 2004. Centraal Veevoeder bureau, Lelystad, Holland.

EKLUND M., SAUER N., SCHÖNE F., MESSERSCHMIDT U., ROSENFELDER P., HTOO J.K., MOSENTHIN R. 2015. *Effect of processing of rapeseed under defined conditions in a pilot plant on chemical*

composition and standardized ileal amino acid digestibility in rapeseed meal for pigs. J. Anim. Sci., 93(6): 2813–2825.

Fandrejewski H. 1997. *Możliwości ograniczenia wydalania fosforu przez świnie. Żywieniowe metody ograniczania wydalania do środowiska azotu, fosforu i innych pierwiastków przez zwierzęta gospodarskie*. Materiały z konferencji naukowej, Balice 17–18.11.1997, pp. 45–64.

Fiedorowicz E., Sobotka W. 2013. *Poekstrakcyjna śruta sojowa a alternatywne źródła białka roślinnego dla trzody chlewnej*. Przegl. Hod., 81(4):14–17.

Hanczakowska E., Świątkiewicz M. 2014. *Legume seeds and rapeseed press cake as replacers of soybean meal in feed for fattening pigs*. Ann. Anim. Sci., 14(4): 921–934.

Jamroz D. 2004. *Żywienie zwierząt i paszoznawstwo*. Wyd. Nauk. PWN, Warszawa.

Jerzak M.A., Czerwińska-Kayzer D., Florek J., Śmiglak-Krajewska M. 2012. *Determinanty produkcji roślin strączkowych jako alternatywnego źródła białka w ramach nowego obszaru polityki rolnej w Polsce*. Rocz. Nauk Roln., 7: 113–120.

Just M., Śmiglak-Krajewska M. 2015. *Extreme price risk on the market of rapeseeds and processed rapeseed products in Poland*. Roczniki Naukowe Stowarzyszenia Ekonomistów Rolnictwa i Agrobiznesu, 17(5): 91–98.

Kaldmäe H., Leming R., Kass M., Lember A., Tölp S., Kärt O. 2010. *Chemical composition and nutritional value of heat-treated and cold-pressed rapeseed cake*. Vet Zootech., 49(71): 55–60.

Kaczmarek P., Korniewicz D., Lipiński K., Mazur M. 2016. *Chemical composition of rapeseed products and their use in pig nutrition*. Pol. J. Natur. Sc., 31(4): 545–562.

Kalembasa S., Adamiak E.A. 2010. *Określenie składu chemicznego makuchu rzepakowego*. Acta Agroph., 15(2): 323–332.

Keady U., O'Doherty J.V. 2000. *The effect of extrusion on the nutritive value of rapeseed meal for growing and finishing pigs*. Irish J. Agric. and Food Research, 39: 419–431.

Kim J.C., Mullan B.P., Pluske J.R. 2012. *Prediction of apparent, standardized, and true ileal digestible total and reactive lysine contents in heat-damaged soybean meal samples*. J. Anim. Sci., 90(suppl._4): 137–139.

Korniewicz A., Ziółkowski T., Korniewicz D., Czarnik-Matusewicz H., Paleczek B. 1999. *Efektywność preparatu „Energex" w mieszankach dla tuczników z udziałem śruty rzepakowej lub makuchu rzepakowego*. Biul. Nauk. Przem. Pasz., ¼: 23–36.

Korniewicz D., Dobrzański Z., Chojnacka K., Korniewicz A., Kołacz R. 2007a. *Effect of dietary yeast enriched with Cu, Fe and Mn on digestibility of main nutrients and absorption of minerals by growing pigs*. Am. J. Agric. and Biol. Sci., 2(4): 267–275.

Korniewicz D., Różański H., Dobrzański Z., Kaczmarek P., Korniewicz A. 2007b. *Effect of Herbiplant CS preparation on basic nutrient digestibility and mineral balance and absorption in fatteners*. Ann. Anim. Sci., 7(2): 259–271.

Kuśnierek W., Potkański A., Kuśnierek S. 2005. *Strawność całkowita i jelitowa u świń białka i aminokwasów poekstrakcyjnej śruty rzepakowej przed i po ekstruzji w temperaturach 140 i 160°C*. Rośl. Oleiste, XXVI(2): 537–548.

Lipiński K. 2003. *Wartość odżywcza i przydatność paszowa żółtonasiennego rzepiku (Brassica rapa L.) odmiany Parkland w żywieniu świń*. Rozprawy i Monografie 83. Uniwersytet Warmińsko-Mazurski w Olsztynie.

Lipiński K., Tywończuk J., Kozikowski W., Biro D. 1998. *A comparative study on the protein quality of cake and meal from double low rapeseed*. Polnohospodarstvo, 44(12): 914–921.

Lipiński K., Tywonczuk J., Flis M., Sobotka W. 1997. *The effect of low-glucosinolate rapeseed meal, cake and seeds on nutrient digestibility and nitrogen and energy utilization in growing pigs*. Acta Acad. Agricult. Techn. Olst. Zoot. 46: 169–180.

Lipiński K., Tywonczuk J., Lewicki C., Rapczycka I., Sobotka W. 1994. *Zastosowanie* śruty *poekstrakcyjnej, wytłoków i nasion rzepaku odmiany Jantar 00 w* żywieniu *tuczników*. Acta Acad. Agricult. Techn. Olst. Zoot., 40: 133–144.

McDonnell P., O'Shea C., Figat S., O'Doherty J.V. 2010. *Influence of incrementally substituting dietary soya bean meal for rapeseed meal on nutrient digestibility, nitrogen excretion, growth performance and ammonia emissions from growing-finishing pigs*. Arch. Anim. Nutr., 64(5): 412–424.

Pig nutrient requirements. Nutrient content of the feedstuffs. 2015. Wyd. The Institute of Animal Physiology and Nutrition, PAN Jabłonna – Warszawa.

Ochodzki P., Rakowska M., Rek-Ciepły B., Bjergegaard Ch., Søensen H. 1995. *1. Studies on enzymatic fractionation, chemical composition and biological effect of dietary fibre in rape seed (Brassica napus L.). 2. Influence of rape seed dietary fibre on digestibility of protein and organic matter using unprocessed and heated full fat rape seed and isolated dietary fibre fractions added to rat diets*. J. Anim. Feed Sci., 4(1): 139–151.

Orda J., Fuchs B., Wiliczkiewicz A., Preś J. 1998. *Wpływ dodatku fosforanu sodu, wapnia i fitazy mikrobiologicznej na stopień wykorzystania wybranych składników mineralnych w dawkach pokarmowych tuczników*. Prace Nauk. AE Wroc., 792: 376–385.

Pastuszewska B., Buraczewska L., Ochtabińska A. 1997. *Rozpuszczalność białka śruty i wytłoków rzepakowych jako wskaźnik jego wartości odżywczej*. Rośl. Oleiste, XVIII(2): 545–551.

Pastuszewska B., Ochtabińska A. 1998. *Wartość pokarmowa wytłoku i śruty poekstrakcyjnej z żółtonasiennego rzepiku jarego (Brassica rapa) i brązowonasiennego rzepaku ozimego (Brassica napus) oznaczona w testach na szczurach*. Rośl. Oleiste, XIX(1): 159–165.

Pastuszewska B., Raj S. 2003. *Śruta rzepakowa jako pasza białkowa i energetyczna – ograniczenia i perspektywy*. Rośl. Oleiste, XXIV(2): 525–536.

Smulikowska S., Van Nguyen C. 2003. *Przydatność paszowa nasion i wytłoków rzepakowych w żywieniu drobiu i świń i ich wpływ na jakość* produktów zwierzęcych. Rośl. Oleiste, XXIV(1): 11–22.

Stein H.H., Lagos L.V., Casas G.A. 2016. *Nutritional value of feed ingredients of plant origin fed to pigs*. Anim. Feed. Sci. Tech., 218: 33–69.

Usydus Z., Korniewicz D., Dobrzański Z., Korniewicz A. 2006. *Wpływ koncentratu rybno-mineralnego (KR-M) na wykorzystanie podstawowych składników pokarmowych i mineralnych u tuczników*. Acta Sci. Pol., Medicina Veterinaria, 5(1): 33–41.

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RESPONSE OF SOME PHYSIOLOGICAL PARAMETERS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) TO VARIATION IN THE LIGHT ENVIRONMENT

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Key words: defoliation, dry matter allocation, plant density, R:FR ratio, stem elongation.

Abstract

Light is considered as an important source of energy for all types of plants, and any change in its quality or quantity such as R:FR ratio affects plant growth through physiological, morphological, and biochemical processes. To examine the impact of changes in light quality on sunflower canopy, a factorial experiment with three replications was conducted at the Research Farm of Faculty of Agriculture, University of Birjand, in spring and summer 2014. The treatments included planting density (5 and 10 plants per square meter), leaf arrangement (no defoliation, and defoliating 50% of leaves of each plant when heads became visible), and the colors of optical filters wrapped around the shoot (blue cellophane with R:FR = 1.84 passing blue spectrum with the wavelength 480.62 nm, white cellophane as a control treatment for the blue one with R:FR = 2.40 which transmit the whole spectrum contained in the sunlight, and a control where no filter was used). The use of blue filters resulted in greater stem elongation, reduced stem diameter, and reduced amounts of plant dry matter allocated to each sunflower head as well as the achene number per capitulum. Blue filters had no significant impact on 100-grain weight. During the growing period, reduction in the dry matter allocated to stem was smaller when blue filters were used, especially for the defoliation treatments, compared to where no filter or white filters were used. Defoliation in combination with using blue filters at a density of 5 plants m^{-2} increased specific leaf area (SLA) due to larger leaf areas. It seems any variation in R:FR, plant density, and/or leaf density can influence sunflower yield through affecting photomorphological processes.

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Introduction

As an important environmental factor, light not only acts as a source of energy but also regulates morphological processes in plants (ALABYEV et al. 2002). Quality of light affects growth, flowering, and morphology of plants (KUBOTA et al. 2000). Quality and quantity of light are altered through plant canopy. Quantity of light drops in shades while quality of light changes through increase in quantity of infrared light (700–800 nm) and reduction in blue (400–500 nm), and red light (600–700 nm) (SARALA et al. 2007). Green leaves absorb red light and reflect infrared. Therefore, closed and dense canopies have lower red to far red ratio (R:FR) and reflect larger quantities of infrared light (KASPERBAUER 1987, KASPERBAUER and KARLEN 1994, MALIAKAL et al. 1999). Allocation of dry matter to different parts of a plant under farming conditions is associated with the R:FR ratio received during the growing period (KASPERBAUER 1987).

Red light-intercepting films have been designed to decrease R:FR (WILSON and RAJAPAKSE 2001). There are instances where light quality variations were employed to develop desirable changes in plant products in the market. For example, FR increased chlorophyll content and leaf length as two important factors for marketable *Allium wakegi* (YAMAZAKI et al. 2000). Lowered R:FR (0.01, 043, and 0.65) has been reported to improve growth and flowering in *Eustoma grandiflorum*, while evidence suggested that increased R:FR delayed its growth and flowering (YAMADA et al. 2008). Plants that receive lower R:FR often have longer leaves with smaller width, longer stems, and smaller roots. Stem elongation is the first observed response to reduced R: FR even when light cannot directly touch the stem (KASPERBAUER and KARLEN 1994). Such responses may negatively affect crop productivity as reduction in R: FR may limit available resources for growth of harvestable organs of sunflower (LIBENSON et al. 2002).

A large body of research has focused on responses of photoreceptors and phytochromes to FR and R (ZHOU and SINGH 2002). Reduced R:FR ratios received through phytochromes at the base of grassy plants shoot (LIBENSON et al. 2002) are known to affect stem elongation in response to crowding and vegetation shading, and this is believed to be a form of adaptive phenotypic plasticity. Stem elongation allows a plant to develop its leaves above those of the adjacent plants to receive more light.

Using phytochrome family of photoreceptors, plants are able to sense changes in light quality (MALIAKAL et al. 1999). Irradiating the plant with blue light will cause canopy to grow toward the light. This phenomenon, referred to as phototropism, is one of the most well-known responses in

plants (VOLKOV et al. 2004). A phototropic response is composed of four processes: receiving light signal, signal transduction, transforming the signal into physiological response, and creating a directional growth response (VOLKOV et al. 2004). Irradiation of soybean with blue light plays an effective role in positive phototropism. In addition, researchers have pointed out that K^+ and Ca^{2+} channel blockers, like tetraethyl ammonium chloride and $ZnCl_2$, can block propagation of action potentials induced by blue light and may inhibit phototropism in soybean (VOLKOV et al. 2005).

Leaf epinasty is controlled by cell elongation on the abaxial epidermis triggered by blue light irradiation of the axial side of the leaf. It is observed that blue light, under red light conditions, created a larger leaf area in lettuce (FUKUDA et al., 2008). The rate of O_2 evolution by wheat seedlings with blue light, compared to red light, was over 50% (optimal temperature) and 60% (after exerting 45°C) (ALYABYEV et al. 2002). A study on incomplete mutants produced in response to specific spectral wavelength indicated that blue and far-red lights are effective in blocking ethylene impacts (VANDENBUSSCHE and VAN DER STRAETEN 2004). The phytohormone abscisic acid, cytoplasmic concentration of the secondary messenger Ca^{2+}, as well as blue and red lights modulate sensitivity of stomatal guard cells to internal CO_2 (LÜTTGE 2007).

Reduced leaf area is a common response to fungal and arthropod attack, hails, herbivory, etc. A number of studies tried to explain the effects of defoliation on plants (MORIONDO et al. 2003). Defoliation studies have provided a better understanding of physiological processes involved in vegetative and reproduction growth in several corps (CRUZ-CASTILLO et al. 2010). For sunflower (*Helianthus annuus* L.) there has been a large emphasize on the link between the reduction in leaf number and final amount of plant production (MORIONDO et al. 2003, ALIMOHAMMADI and AZIZOV 2011). In this regard, the aim of this work was to analyze sunflower responses to artificial manipulation of R:FR ratio, blue light, plant density, and defoliation for better understanding of some plants physiological processes, such as source-sink relationships, dry matter allocation, and compensation manner.

Materials and Methods

The experiment was carried out in Research Farm of Faculty of Agriculture, University of Birjand, Iran, during spring and summer 2014. The farm is located 8 km far from Birjand, adjacent to Birjand-Kerman Road (32°56′N, 59°13′E, 1480 m elevation). Based on Emberger's classification,

the region is a hot arid region. The soil contained 20% clay, 19.4% silt, 60.6% sand, with an electrical conductivity (EC) of 3.85 dS m^{-1} and a pH of 7.97. The preceding crop in the experimental field was sugar beet. The common farming operations were used to prepare the seed bed. The same amount of fertilizer was applied to all plots based on soil tests before planting, including 30 kg ha^{-1} phosphorus (P), 75 kg ha^{-1} potassium (K), and 138 kg ha^{-1} N. One third of the nitrogen fertilizer plus all P and K fertilizers were applied before the planting and the remainder of N fertilizer was added to the soil at 6-8 leaves stage along with irrigation. Weeds were removed by hand hoeing over the entire growing season. No indication of disease and insect damages were observed from germination to final harvest. Irrigation method was the same for all treatments (7-days irrigation intervals).

The study conducted using a factorial experiment based on randomized complete block design with three replications. Each plot consisted of 7 planting rows. The treatments included planting density (5 and 10 pl m^{-2}), leaf arrangement (no defoliation, and defoliating 50% of leaves of plants (every other leaf) at flowering stage), and the colors of optical filters wrapped around the stem (blue cellophane with R:FR = 1.84 passing blue spectrum with the wavelength 480.62 nm, white cellophane as a control treatment for the blue one with R:FR = 2.40 which passed the whole spectrum contained in the sunlight, and a control where no filter was used with R:FR = 2.40). The plant rows were spaced at 50 cm with a space of 40 cm (for 5 pl m^{-2}) and 20 cm (for 10 pl m^{-2}) between two adjacent plants. To plant the crop, 3 to 5-cm deep holes were dug at the pre-specified spacing. Three sunflower (Euroflor cultivar) seeds were sown at each hole on 29 April and emerged plants were thinned to one plant at 2-4-leaf stage. Euroflor is an oily single cross hybrid, French origin, with intermediate maturity and lodging resistance (YOUSEFPOOR and YADAVI 2014). At the beginning of the flowering stage (20 June) and after exerting leaf density treatment, cellophane filters were wrapped around the stems, leaving a 2-cm space to let the air flow. The wrappings were maintained until stem elongation was completed.

Samples were taken at three stages: head-visible stage (HV, 26 June), pollination (PO, 14 July), and physiological maturity (PM, 20 August). At each stage, three plants were randomly selected from the second, fourth, and sixth rows of each plot by leaving 50 cm at either side (to allow for marginal effect). Before each sampling, the radiation flux density was measured at the top and bottom of canopy using Sun scan (AccuPAR LP-80, DECAGON devices, Made in USA) at 11:00 to 13:00 (Table 1). Furthermore, number and area of leaves, stem length and diameter, as well as

Table 1

Radiation intensity [μmo m^{-2} s^{-1}] at above and bottom of the sunflower canopy, measured at different growth stages. Plants were sown at two different plant density (5 and 10 pl m^{-2}) and all measurements were carried out between 11:00 and 13:00

Specification	Measuring place		
	above the canopy	bottom of the canopy	
Growth stages		10 pl m^{-2}	5 pl m^{-2}
Head visible (26 June)	1420	300	540.33
Pollination (14 July)	1332	490.66	798.5
Physiological maturity (20 August)	1066.5	451	566.25

the dry weight of leaves, stem, and inflorescence were measured, separately. At the third sampling, achene number per capitulum and weight of 100-grain were also measured. Leaf area meter (WD_3-R_3 model, Delta-T Devices, UK) was used to measure leaf area. The stem length was measured using ruler, stem diameter was determined with caliper, and achenes were counted by seed counter (Contador Model, Pfeuffer GmbH, Germany). To measure dry weight, plant parts were first placed in an oven set at 72°C for 48 hours. SLA, leaf weight ratio (LWR) and capitulum weight ratio (CWR) were obtained using Equations (1), (2), and (3), respectively.

$$SLA\ [cm^2\ g^{-1}] = LA/LDW \quad (1)$$

$$LWR\ [g\ g^{-1}] = LDW/TDW \quad (2)$$

$$CWR\ [g\ g^{-1}] = TCW/TDW \quad (3)$$

Where, LA and LDW are leaf area and leaf dry weight, respectively; TDW and TCW indicate total and capitulum dry weights, respectively.

The data were normalized and analyzed by Genstat (V.9) and the means were compared using FLSD.

Results and Discussion

In the present study, the first growth response of sunflower to increase in density from 5 to 10 pl m^{-2}, was an increase in the length and reduction in the diameter of stem; however with the progress of growing stages, the difference between the two levels of density reduced (Table 2). Increased plant densities decreases light penetration inside the canopy and increases the level of competition between individual plants for water and nutrients and meantime, plants react to increase in crowding with different mechanisms including morphological changes such as changes in stem length and specific leaf area (SLA) (LAMBERS et al. 2008). There was

a negative correlation between the length and diameter of the stem (-0.80, -0.32 and -0.29 in the stages of emerging inflorescence, pollination and physiological maturity, df = 35). With increase in plant density and decrease in light penetration into the canopy, the competition for absorbing light will increase and the plants, especially those sensitive to shade, will increase their height in order to receive more light as a mechanism for shade avoidance (ROSHDI et al. 2009, XIAO et al. 2006, SMITH 1982, MILLER and FICK 1978). This will mainly be accomplished to the cost of reduction in the stem diameter (BABAEI-AGHDAM et al. 2009). It is known that phytochrome B has a significant role in forming this response, even though two phytochromes D and E are also involved in inducting the mentioned reaction (FRANKLIN and WHITELAM 2004).

Increasing plant density per area did not have a considerable effect on the number of leaves in the plant, even though a minor reduction was observed with the progress of growing season. Nevertheless, this density increase primarily caused an increase in the leaf area in the stages of florescence emergence and pollination, which could be a response to reduction to the radiation availability at these stages. On the other hand, as growth continued, as a result of density increase, the leaves number and area reduced at the physiological maturity, indicating the leaves abscission in high density after the pollination (Table 2). This may be a result of increase in competition and shading in the density of 10 pl m^{-2} which exerts a high pressure on available sources. Leaf area reduction in plants as a result of density increase has previously been reported in sunflower (BANGE et al. 1997) and corn (SANGOI et al. 2002). Basically, plants growth and development in response to competition for light is much more effective than any other factor in controlling leaf area expansion (BALDISSERA et al. 2014).

Despite increasing leaf area and decreasing leaf dry weight at high density, no significant difference was observed in LWR between the two plant densities (Table 2). This indicates that plants allocated the same rate of produced assimilates to the leaves at high and low densities. These factors together caused an increase in SLA at higher densities in all three growth stages in spite of allocating the same amount (and a lower amount) of dry matter to the leaves. This indicates that plants struggle to enhance their leaf area through decreasing the leaves thickness, which is a response to increased competition for incident radiation, leading to more light absorption in each unit of weight assigned for leaf production. However, in the next growing stages , the difference of SLA between the two densities became lower (Table 2), which might be owing to the indirect effect of defoliation treatment (Table 2) and increase in light penetrating through canopy (Table 1).

Table 2

Average growth parameters for sunflower at 5 and 10 pl m^{-2}, measured in three stages during crop cycle

Sampling stage§	HV			PO			PM		
Plant density [pl m^{-2}]	5	10	LSD	5	10	LSD	5	10	LSD
Shoot length [cm]	57.16±4.91*	76.25±3.5	7.064	84.13±0.28	92.83±0.28	5.46	94.02±2.08	102.52±2.66	5.57
Shoot diameter [cm]	0.97±0.18	0.69±0.14	0.152	1.12±0.03	0.99±0.05	0.08	1.40±0.05	1.16±0.06	0.15
Leaf number [no pl^{-1}]	17.33±1.58	18.33±0.44	2.483	10.91±0.29	10.55±0.24	0.913	10.61±0.41	9.25±0.37	0.98
Leaf area [cm^2 pl^{-1}]	955.06±116.12	1148.35±72.06	71.698	479.05±31.0	632.19±10.8	102.90	506.35±24.1	407.04±27.8	65.95
Leaf dry weight [g pl^{-1}]	11.71±2.52	10.18±2.80	0.559	11.48±0.8	8.77±0.33	1.29	10.41±0.86	7.62±0.14	1.55
Shoot dry weight [g pl^{-1}]	19.68±2.96	15.51±2.80	1.548	20.20±0.79	15.65±1.35	3.08	28.36±2.41	20.20±1.20	3.78
Capitulum dry weight [g]	4.39±2.97	3.71±2.83	0.249	17.89±0.76	13.77±1.57	2.88	47.47±1.11	39.48±3.17	5.87
SLA [cm^2 g^{-1}]	81.51±29.77	112.77 ±46.93	78.572	43.39±6.34	71.11±3.05	9.91	48.62±7.58	53.42±13.46	20.263
LWR [g g^{-1}]	0.327±0.02	0.346±0.03	0.072	0.231±0.007	0.229±0.01	0.0219	0.120±0.014	0.113±0.010	0.0169
CWR [g g^{-1}]	0.122±0.05	0.126±0.05	0.0202	0.36±0.010	0.36±0.016	0.0577	0.55±0.004	0.58±0.016	0.042
Grains per Capitulum	–	–	–	–	–	–	459.86	374.38	49.87
Grain weight [g 100 $achene^{-1}$]	–	–	–	–	–	–	5.03	3.65	0.84

* Means ±standard error

§ Three sampling stages refer to head-visible stage (HV), pollination (PO), and physiological maturity (PM)

SLA increase as a result of increase in plant density has also been observed in other plants such as potatoes (VOS 1995) and tomatoes (HEUVELINK et al. 1999), the reason of which has ascribed to the reduction in the average of the light received by leaf area in high densities (LEE and HEUVELINK 2003). Generally speaking, high densities cause a response similar with the situation being plants in the shade; the plants growing in shade, have more tendency to expand their leaf area, and their leaves are rather thin and thus they have high SLA and low leaf mass density (LAMBERS et al. 2008). Reduction in sunflower leaf dry weight in response to increased densities is caused by photosynthates deviation to acquire resources such as radiation, water and nutrients, as a result of intensification of interplant competition (POURSAKHY and KHAJEPOUR 2014).

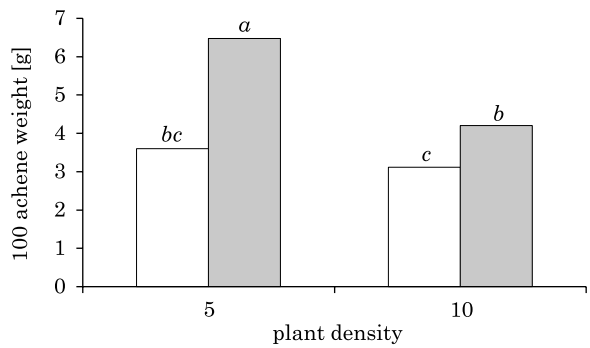

Fig. 1. A comparison of 100-grain weight [g] for 5 and 10 pl m^{-2} during physiological maturity stage under two situations: no defoliation (shaded bars) and defoliation (white bars). Bars with at least one common letter indicate no significant difference according to $LSD_{5\%}$

In addition to the leaf weight, the dry weight of the stem and capitulum of sunflower or, in other words, the dry weight of the whole plant, also decreased in response to density increase (Table 2). The stem dry weight in the density of 10 pl m^{-2} were 21.2, 22.5 and 28.7 percent lower than the values gained in the density of 5 pl m^{-2} in the stages of head visible, pollination and physiological maturity, respectively (Table 2). POURSAKHY and KHAJEPOUR (2014) observed that increase in the density of sunflower plants caused reductions in stem dry weight of individual plants. Similarly, at the physiological maturity, high plant density (10 pl m^{-2}) caused small capitulums with less weight which was most likely due to a lower achene number (18.5%) and lower 100-achene weight (27.4%) compared to the density of 5 pl m^{-2} (Table 2 and Figure 1). achene weight reductions at high densities indicates a drop in production capability of individual plants (source limitation) and reduction of the whole photosynthates partitioned for seed filling (BARROS et al. 2004). The interesting point is that the capitulum weight ratio (CWR) did not show significant differences between the

two densities at any growth stages (Table 2). Basically, thinner leaves with higher SLA produced at high densities have a shorter life span (LAMBERS et al. 2008), which in turn limits the source activity of plants. Negative effect of increased density on weight reduction of achene in sunflower has also previously been reported (GHOLINEZHAD et al. 2009, HOLT and ZENTNER 1985). IBRAHIM (2012) found that low density of sunflower is effective in height reduction and increase in achene weight, leaf area, diameter of capitulum, oil percentage, unsaturated fatty acids percentage (oleic and linoleic) and the length of maturation period. He stated the reason of such changes was the greater usage of assimilates for competition at high densities compared to lower densities. BARROS et al. (2004) reported that two densities of 3.5 and 4.6 pl m^{-2} of sunflower produced the same number of achenes per m^{-2}, with a lower achene weight at higher density. They attributed this lower achene weight at higher density to less produced dry matter in that density, which reduced remobilization of assimilates during achene filling period. This condition will finally lead to a reduction in radiation use efficiency (RUE) as a result of increase in plant density (MORRISON and STEWART 1995).

Using blue filter caused more reductions in R/FR (7.10%) in comparison with white filter (4.7%) and the control (0.075%). Physiological and morphological responses of sunflower to the quality of radiation were the same in both stages of pollination and physiological maturity. White filter did not induce a significant difference in stem length compared to the control in the two mentioned stages, whereas blue filter caused a significant increase in stem length compared to the control (Table 3). On the other hand, control plants had the highest stem diameter and using white and blue filters caused 5 and 16.5 % decrease in stem diameter compared to the control, respectively, while this reduction was only significant in blue filter treatment (Table 3). This has also been observed in soybean plants in which the decrease of red to far-red ratio reduced stem diameter (YANG et al. 2014). It seems that a reduction in R/FR ratio during the plant growth is one of the most substantial factors causing elongation in plant height and at the same time reduction in stem diameter. In the plant canopy, the quality of radiation transmitted through the leaves or reflected is changed. This is because the plants absorb red light and transmit or reflect far-red radiation. This causes a reduction in R/FR under shaded environments (TAIZ and ZEIGER 2006). As blue filter absorbs and reflects a part of incident visible light spectrum, it can induce the effects of increased far-red radiation (or reduction in R/FR ratio) in the plant. Increase in the internodes length and stem elongation as a result of reduced R/FR ratio have been reported in *Sinapis alba, Chenopodium album* and *Datura ferox* (BOOTH et al. 2003).

Table 3

Average growth parameters for sunflower: impacts of optical filters at pollination and physiological maturity stages

Treatments		Shoot length [cm]	Shoot diameter [cm]	Leaf no.	Leaf area [cm^2 pl^{-1}]	LDW [g pl^{-1}]	SDW [g pl^{-1}]	CDW [g pl^{-1}]	LWR [g g^{-1}]	CWR [g g^{-1}]	GPC
Pollination											
Light filters	blue	98.958	0.915	12.7	688.5167	12.287	24.298	11.033	0.258	0.231	–
	white	85.375	1.096	10.3	526.770	9.1	15.460	17.083	0.218	0.410	–
	control	81.125	1.163	9.2	451.587	9.008	14.024	19.396	0.212	0.457	–
	LSD	6.69	0.13	1.16	126.03	1.58	3.78	3.53	0.0279	0.073	–
Physiological maturity											
light filters	blue	105.62	1.02	11.791	569.7	10.93	30.15	33.25	0.144	0.449	327.5
	white	95.125	1.32	9.25	414.98	8.29	22.39	46.74	0.100	0.641	450.12
	control	94.083	1.5	8.75	385.4	7.82	20.29	50.44	0.106	0.610	473.75
	LSD	6.82	0.24	1.49	80.78	1.90	4.63	7.1	0.021	0.054	81.96

LDW – leaf dry weight; SDW – shoot dry weight; CDW – capitulum dry weight; LWR – leaf weight ratio; CWR – capitulum weight ratio; GPC – grains per capitulum

BALOCH et al. (2009) stated that phytochromes and photoreceptors families are responsible for perceiving the R/FR ratio throughout canopy and decrease in this ratio can lead to stimulating the elongation of internodes and consequently increasing the stem height .They believed that the effects of far-red radiation on stem growth can be considered as a part of shade avoidance response. Similarly, the high amounts of intercepted far--red radiation stimulates the plants, through decreasing P_{fr}/P_{total}, to allocate more assimilates to the stem in order to reach a greater height (TAIZ and ZEIGER 2006) (Figure 2). It is thought that phytochrome B is involved

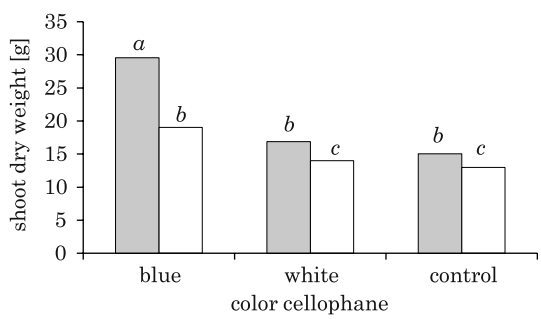

Fig. 2. A comparison of shoot dry weight [g] at pollination stage for three treatments (blue filter, white filter, and the control treatment) under two situations: no defoliation (shaded bars) and defoliation (white bars). Bars with at least one common letter indicate no significant difference according to $LSD_{5\%}$

in intensifying the response of stem elongation to a reduction in R/FR ratio (XIONG et al. 2002). It has also been observed that R/FR reduction can increase the content of gibberellic acid (GA_1) and auxin (IAA) phytohormones in internodes and leaves of sunflower, which in turn reduces the level of ethylene in internodes, (KUREPIN and WALTON 2007, KUREPIN et al. 2007) through influencing DNA (PRICE and JOHNSTON 1996). Increase in stem length, plant fresh and dry weight and leaf area as affected by far-red radiation has also been observed in watermelon (HEATHER et al. 1997).

Reducing R/FR using blue filter caused more increase in SLA at maturity compared with white filter and the control (Table 4). Similarly, the blue filter increased LWR, leaf number and area, and leaf and stem dry weights during plant growth, whereas it significantly reduced the capitulum weight ratio (CWR) (Table 3 and Table 4, Figure 3). Similar results have been reported in different plants, for example, OYAERT et al. (1999) reported that using blue filter increased leaf number per plant, plant dry and fresh weight, and LWR in chrysanthemums. Increased stem dry and fresh weights under high levels of far-red radiation has also been reported

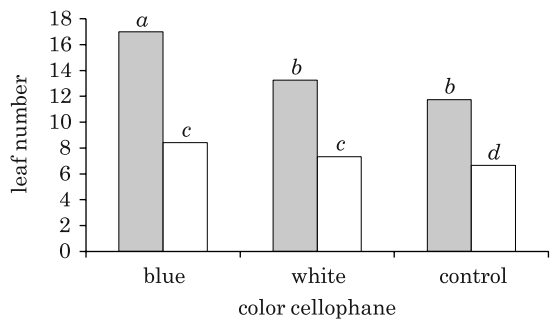

Fig. 3. A comparison of leaf number in pollination stage for three treatments (blue filter, white filter, and the control treatment) under two situations: no defoliation (shaded bars) and defoliation (white bars). Bars with at least one common letter indicate no significant difference according to $LSD_{5\%}$

in lettuce (KRIZEK and ORMROD 1980). The variation rate in different traits caused by blue filter and the control treatment at the pollination stage was different as compared with the physiological maturity stage. For example, using blue filter caused more increase in some traits in the pollination stage compared to the physiological maturity, including number of leaves (38%, 34%), leaf area (52.4%, 47.8%), stem dry weight (73.2%, 48.5%) and stem length (22%, 12.2%). On the other hand, rate of increase in some other traits resulting from using blue filter was lower in the pollination stage compared to physiological maturity including leaf dry weight (36.4%, 39.7%) and then LWR (21.6% and 35.8%). In contrast, there was

a sharp decline in capitulum diameter at pollination (49.4%) and stem diameter at physiological maturity (32%) as a result of using blue filter (Table 3). It seems that greater increase in the characteristics such as leaf number, leaf area, stem dry weight and stem length resulting from blue filter (compared to the control) at pollination stage is owing to the greater importance of shade avoidance responses at this stage, which leads to more assimilate allocation to stem elongation and leaf area expansion in this critical phase of plant growth. Given that blue filter creates a lower level of R/FR ratio, it induces the sensing of being under high density or shade conditions by plants, and the morpho-physiological responses that arise in these conditions are similar to the responses resulted by cultivating plants at high density to some extent. LAMBERS et al. (2008) stated that the plants growing in shade invest a large portion of their assimilate and other resources in leaf area; these plants have a high leaf area ratio, rather thin leaves with a low mass density. Elongating stems is another strategy employed by plants growing under shade conditions. It has been stated that low R/FR ratios caused stem elongation in *Vigna sinesis,* and it seems that preventing stem elongation by light is related to decrease in responsibility of tissues to Gibberlines (OLSZEWSKI et al. 2002).

Table 4

Average values for some physiological traits measured at pollination (PO) and physiological maturity (PM) stages, as influenced by optical filter, plant density, and leaf density treatments

Light filters		Blue				White				Control			
Plant density [pl m^{-2}]		5		10		5		10		5		10	
Leaf density		*D**	*C*	*D*	*C*	*D*	*C*	*D*	*C*	*D*	*C*	*D*	*C*
Total dry weight [g]	PO	44.34	63.28	34.28	48.57	41.75	53.23	32.00	39.60	39.34	55.58	35.30	39.50
	PM	62.20	104.05	56.50	74.62	71.46	103.14	57.89	77.23	67.62	109.05	57.86	79.74
Shoot dry weight [g]	PO	22.26	33.93	15.82	25.18	15.13	19.29	12.87	14.55	13.3	17.3	12.65	12.85
	PM	26.58	45.08	21.57	27.37	21.87	32.08	13.2	22.45	16.23	28.32	15.9	20.74
Leaf dry weight [g]	PO	11.43	16.67	8.83	12.22	8.18	11.63	6.8	9.78	7.82	13.18	7.17	7.87
	PM	8.4	16.68	6.62	12.05	8.02	11.83	5.3	8.02	7.25	10.3	5.48	8.25
Capitulum dry weight [g]	PO	10.65	12.683	9.633	11.166	18.433	22.3	12.33	15.27	18.22	25.1	15.48	18.78
	PM	27.22	42.28	28.31	35.2	41.58	59.23	39.39	46.76	44.13	70.43	36.48	50.75
Leaf area [cm^2 pl^{-1}]	PO	468.58	758.45	569.35	957.68	346.32	537.08	431.68	792.00	306.52	457.37	450.78	591.68
	PM	561.05	724.47	390.28	603.03	367.63	508.55	310.87	472.90	370.23	506.18	258.52	406.68
SLA [cm^2 g^{-1}]	PO	43.27	46.08	64.42	77.45	45.35	46.08	63.17	81.71	39.50	40.11	62.91	75.22
	PM	73.86	44.09	79.95	51.03	48.19	42.37	72.62	61.99	55.84	49.80	60.71	51.20
LWR [g g^{-1}]	PO	0.26	0.26	0.25	0.26	0.20	0.22	0.21	0.25	0.20	0.23	0.19	0.22
	PM	0.13	0.16	0.12	0.16	0.11	0.12	0.09	0.10	0.09	0.11	0.09	0.10
CWR [g g^{-1}]	PO	0.25	0.20	0.28	0.24	0.44	0.42	0.40	0.39	0.46	0.45	0.20	0.18
	PM	0.43	0.40	0.50	0.46	0.58	0.57	0.68	0.61	0.65	0.65	0.43	0.39

* *D* – defoliation; *C* – no defoliation (control)

The reduction in R/FR through using blue filter also caused a reduction in capitulum weight, achenes per capitulum and CWR (Table 3), while it had no significant effect on achene weight (data not shown). This effect is similar to the response where sunflower is cultivated at high densities which lead to a reduction in the achenes per capitulum and achene weight (Table 2). Competition between adjacent plants at high densities usually causes a drop in produced biomass and also assigning more resources for vegetative growth and, as a result, a reduction in achenes per capitulum (GARDNER et al. 1985). It seems that reducing R/FR ratio using blue filter in this study, through simulating crowding and competition between plants, has forced them to allocate more dry matter to vegetative organs such as stem and leaves (LIBENSON et al. 2002) and to remobilize assimilates from reproductive organs to promote vegetative growth (TAIZ and ZEIGER 2006). Obviously, this will lead to reduced reproductive growth and subsequently less achene set in sunflower plants. In this case, achene yield and achenes number per plant will decrease, with no effect on achene weight. Under these conditions, the competition that occurs between vegetative and reproductive structures is harmful for seed setting and seed filling (LIBENSON et al. 2002). It has been proved that in response to R/FR reduction, the level of soluble metabolites and structural carbohydrates (cell-wall carbohydrates) increase and sucrose concentration reduces in sunflower stem, which expectedly are in the favor of carbon unloading from stem phloem (MAZZELLA et al. 2008).

Defoliation, beside its direct effects on reducing leaf number, area and dry weight per plant, caused a significant reduction in stem and capitulum dry weights at pollination and physiological maturity, and also decreased SLA, achenes per capitulum and achene weight at pollination stage, while did not have a significant impact on the stem length and diameter (Table 5). It is acceptable that a damage such as removing leaves can affect a number of plant physiological processes, like sink and source balance, hormone production and changes in radiation absorption in different layers of canopy. In both stages of pollination and physiological maturity, defoliation treatment was accompanied with a compensative response to increase single leaf area per plant; in the way that in the stage of pollination, the average area of single leaf in defoliated plants and in the control treatment were 57.4 and 48.7 cm^2, respectively, which reduced to 52.3 and 42.4 cm^2 at physiological maturity. This indicates that the defoliated plants increased the single leaf area in order to compensate the manipulated reduction in their leaf area (Table 5). By increasing radiation entry into the canopy and increase in R/FR, defoliation caused SLA reduction at the pollination stage. Forming thick leaves with low SLA appears to be

a kind of avoiding from high radiation damage which have been previously observed in different plants such as tobacco (BALLARE et al. 1994) and *Plantago lanceolata* (VAN HINSBERG and VAN TIELEREN 1997), that is the result of forming thick ladder tissue consisting of two cell layers in the growing leaves (TERASHIMA et al. 2006, YANO and TERASHIMA 2001).

Table 5
Average growth parameters for sunflower: impacts of leaf density at pollination and physiological maturity stages

Sampling stage	Pollination			Physiological maturity		
Leaf density	defoliation	control	LSD 5%	defoliation	control	LSD 5%
Shoot length [cm]	88.36	88.611	5.249	96.611	100	5.352
Shoot diameter [cm]	1.044	1.072	0.103	1.186	1.377	0.193
Leaf number [no pl^{-1}]	7.472	14	0.95	7.194	12.66	1.21
Leaf area [cm^2 pl^{-1}]	428.872	682.377	102.90	376.43	536.9	65.95
Leaf dry weight [g pl^{-1}]	8.488	11.775	1.29	6.844	11.188	1.55
Shoot dry weight [g pl^{-1}]	15.336	20.518	3.08	19.22	29.34	3.78
Capitulum dry weight [g]	14.125	17.550	2.15	36.18	50.77	5.87
SLA [cm^2 g^{-1}]	52.201	62.307	7.39	65.194	50.080	20.263
Grains per capitulum	–	–	–	378.47	455.77	49.87
Grain weight [g 100 $achene^{-1}$]	–	–	–	3.35	5.33	0.84

In the maturity stage, more leaves shed in control plants and the SLA severely increased in defoliated plants. All of these factors together caused a reduced difference between control and defoliated plants at the maturity stage (Table 5). This indicates that sunflower is able to compensate the damage caused by defoliation in certain growing stages through increase in leaf area or delay in its senescence and that the effect of defoliation on crop yield mostly depends on the growing stage (in which defoliation has occurred), the rate of defoliation and genotype (POLAT et al. 2011, SCHNEITER and JOHNSON 1994, SCHNEITER et al. 1987, MORIONDO et al. 2003). When the reproductive phase begins, the vegetative phase does not stop completely and as the demand for the carbohydrates in reproductive organs increases, the plant will be able to meet the sinks demands by maintaining its remaining leaves (SEVERINO et al. 2010). Although it has been stated that leaf removal will enhance photosynthesis and growing of remained leaves (ALKIO et al. 2003), it seems that resources deviation from reproductive growth toward compensatory mechanisms has caused the plant not to have a chance to recover its yield (number and weight of achenes) – Table 5. Defoliation caused a 37% reduction in leaf area at

the pollination stage, while the capitulum dry weight and total biomass decreased 19.5 and 23.4%, respectively, indicating the high compensation ability of sunflower at this growth stage.

In the physiological maturity, cutting half of the leaves caused a 30% decrease in leaf area, while the achenes per capitulum dry weight, achene weight and total biomass (capitulum + leaf + stem) decreased 16.9, 28.7, 37 and 31.8 percent, respectively, indicating the greater sensitivity of achene weight to defoliation treatment than achene number (Table 5). Decrease in capitulum diameter (POLAT et al. 2011) and achene number per capitulum (NEZAMI et al. 2008) have been reported as two important components in determining sunflower achene yield under defoliation conditions; which is due to the influence of leaf removal in reducing source to sink ratio (ALKIO et al. 2003). Eventually, decrease in photosynthesizing area as well as loss of stored carbohydrates in shedding structures and increase in energy consumption for compensatory processes as the result of defoliation will cause severe reduction of biomass and achene (both number and weight) production. This may indicate that sunflower yield production is a source-limited process. It has been reported that reduction in source-sink ratio through shading over soybean plants (increased density) or defoliation can reduce the size of cotyledon cells, leading to lower seed weights (LINDSTRÖM et al. 2006).

While defoliation in 5 pl m^{-2} density caused 44.3% decrease in 100-achenes weight, this came down to 25.9% in 10 pl m^{-2} density, indicating more impact of increasing density on the control plants (Figure 1). Lower reduction of achene weight as the result of defoliation at high density might be correlated to the compensatory role of greater density for the enhanced radiation penetration into the canopy due to defoliation. A similar reaction was seen in leaf dry weight under the influence of density and defoliation at the pollination stage (Figure 4). Due to the loss of some sources of

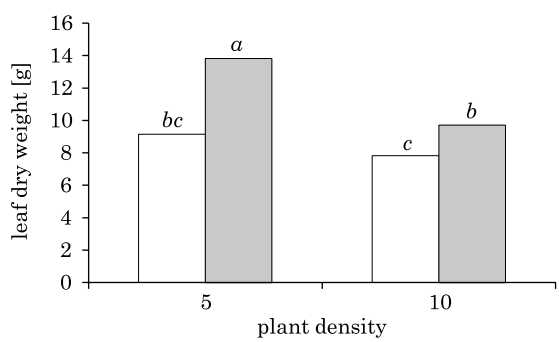

Fig. 4. A comparison of leaf dry weight (gr) for 5 and 10 pl m^{-2} during pollination stage under two situations: no defoliation (shaded bars) and defoliation (white bars). Bars with at least one common letter indicate no significant difference according to $LSD_{5\%}$

assimilates (IBRAHIM 2012), defoliation caused a decrease in achene weight and leaf dry weight in both densities. However, by increasing the radiation penetration into canopy, the difference of the achene and leaf weight at high density (10 pl m^{-2}) to low density (5 pl m^{-2}) was reduced. One of the possible reasons for this decreased difference between two densities can be an increase in the proportion of sunlit leaves and better radiation distribution through canopy under defoliation treatment. Using blue filter in both groups of control and defoliated plants, which simulates the shade and high density conditions, resulted in a greater dry stem weight, whereas defoliation accompanied with blue filter, white filter and control (without filter) caused 35.5, 17.2 and 13.9 percent reduction in stem dry weight compared to non-defoliated plants, respectively (Figure 2). The highest number of leaves were also observed in plants covered with blue filter (Figure 3). In both the total above-ground dry weight and number of leaves, the greatest difference between control and defoliated plants was observed where the blue filter was used. As the blue filter decreases R/FR in canopy and, on the other hand, defoliation increases radiation penetration into the canopy and therefore the R/FR ratio, it seems that defoliation has mitigated the effects of used blue filter on the leaves number and stem dry weight of sunflower.

Even though defoliation and a reduction in plant density caused more light penetration through canopy, blue filter covering on the stems caused the plants not to be able to completely perceive this increase in radiation level in canopy and then as a result, plants showed a response similar to the condition of low R/FR ratio in canopy. Using blue filter combined with defoliation and density of 5 pl m^{-2} led to considerable increase in stem dry weight at the pollination and physiological maturity compared with white filter and control (Table 4). Previous findings indicate that increase in R/FR ratio causes reduction in stem dry weight in chrysanthemum (*Dendranthema* × *grandiflorum* (Ramat.) Kitamura) and bell pepper (*Capsicum annuum* L.) (LI et al. 2000). On the other hand, the cumulative effect of decrease in R/FR ratio on promoting dry matter allocation to the stems has been previously reported (HURD 1974, KASPERBAUER 1987).

Decrease in R/FR ratio is effective in accelerating leaves senescence, and perceiving an increase in R radiation by phytochromes in sunflower delays this phenomenon (BALLARE and CASAL 2000). At the end of the growth (stage 3), the leaf area in the defoliated plants at 5 pl m^{-2} in all optical filter treatments, unlike the non-defoliated plants, still was increasing, while at high density, leaf area showed a decreasing trend (Table 4). It seems that leaf removal, especially in combination with blue filter and low density, has slowed the aging process of leaves by activating the mecha-

nisms of compensating for source shortage. By removing the leaves, it appears that radiation is distributed more uniformly inside the canopy and the lower leaves that received little radiation, especially at high density, compensate for detached leaves. In high density, as well, a much less decline in leaf area was occurred in the defoliated filtered plants than intact ones (Table 4).

In comparison with control (intact) plants, defoliation in both densities and in all applied filters caused a reduction in LWR or, in other words, dry matter allocation to the leaves in both pollination and physiological maturity stages. As a compensatory mechanism for this leaf area removal and reduction in partitioning, SLA increased at the maturity stage in all filters in the density of 5 pl m^{-2} that can be a response to increase in the intensity of the radiation intercepted by remained leaves (Table 4). While the number of leaves in defoliation treatment decreased to half, the amount of decrease in leaf area in different treatments ranged between 22 to 40%, and SLA in all treatments in the stage of physiological maturity increased from 12 to 67% as compared to its previous stage, which indicates a severe reduction in leaf thickness to compensate the leaf area reduction at the physiological maturity stage (Table 4). Two mechanisms have been proposed for increasing leaf area: changing the allocation of assimilates during leaf growth through decreasing dry matter allocated to the stem, or increasing the SLA and more leaf area accompanied with the same biomass invested in leaves (MORIONDO et al. 2003).

In all levels of density and defoliation, using blue filter caused a severe increase in leaf area as compared to white filter and control (without filter) in similar levels of density and defoliation. In the physiological maturity, using blue filter accompanied with defoliation in both densities led to a higher SLA as compared to its corresponding treatments in white filter and control (Table 4). As previous studies showed the effect of a reduction in R/FR ratio on increasing the leaf area (HEATHER et al. 1997), it is expected that using blue filter with reducing R/FR ratio exerts more influence on increasing leaf area than its dry weight.

Increased density effectively decreased SLA in all three filters (blue, white and control) and the amount of SLA reduction in non-defoliated plants at the physiological maturity stage compared to the previous stage was higher in 10 pl m^{-2} than 5 pl m^{-2}. Using blue filter in each density, with or without defoliation, increased leaf dry weight compared with using white filter and control at similar levels of density and defoliation (Table 4). According to BRITZ and SAGER (1990) when plants are growing under reduced levels of blue light, transport of photosynthates outside the leaves is reduced, thus their leaves dry weight would be increased. As blue filter

Table 6

Correlation coefficients between measured traits in three sampling stages (n = 36)

	Shoot length	Shoot diameter	Leaf number	Leaf area	Leaf dry weight	Shoot dry weight	Capitulum dry weight	SLA	LWR	CWR	Achene per Capitulum	100 Achenes weight
Heading visible stage												
1	1											
2	-0.817 ns	1										
3	0.005 ns	0.343 ns	1									
4	0.799 ns	-0.719 ns	-0.509 ns	1								
5	-0.431 ns	0.839*	0.213 ns	-0.244 ns	1							
6	-0.595 ns	0.858*	0.094 ns	-0.317 ns	-0.947**	1						
7	-0.047 ns	0.334 ns	0.307 ns	0.383 ns	0.743 ns	0.753 ns	1					
8	0.629 ns	-0.940**	0.374 ns	0.614 ns	-0.880*	-0.824*	-0.374 ns	1				
9	-0.213 ns	0.451 ns	0.616 ns	-0.576 ns	0.241 ns	0.028 ns	-0.390 ns	-0.550 ns	1			
10	0.143 ns	0.061 ns	-0.442 ns	0.582 ns	0.515 ns	0.558 ns	0.952**	-0.103 ns	0.624 ns	1		
Pollination stage												
1	1											
2	-0.324*	1										
3	0.295*	-0.219 ns	1									
4	0.557**	-0.294*	0.766**	1								
5	0.139 ns	-0.213 ns	0.675**	0.380*	1							
6	0.387**	-0.199 ns	0.681**	0.502**	0.746**	1						
7	-0.609**	0.386*	-0.015 ns	-0.313*	0.069 ns	-0.262 ns	1					
8	0.385*	-0.183 ns	0.249 ns	0.669**	-0.383*	-0.121 ns	-0.314*	1				
9	0.370*	-0.416**	0.310*	0.345*	0.595**	0.262 ns	-0.477**	-0.103 ns	1			
10	-0.657**	0.365*	-0.434**	-0.504**	-0.490**	-0.746**	0.787**	-0.078 ns	-0.612**	1		
Physiological maturity stage												
1	1											
2	-0.294*	1										
3	0.169 ns	-0.018 ns	1									
4	0.313*	-0.067 ns	0.604**	1								
5	-0.058 ns	0.025 ns	0.831**	0.621**	1							
6	0.204 ns	-0.093 ns	0.755**	0.713**	0.692**	1						
7	-0.351*	0.682**	0.332*	0.007 ns	0.261 ns	.145 ns	1					
8	0.431**	-0.085 ns	-0.402**	0.174 ns	-0.590**	-.139 ns	-.265 ns	1				
9	-0.011 ns	-0.310*	0.493**	0.382*	0.745**	0.305*	-0.321*	-0.602**	1			
10	-0.391**	0.493**	-0.452**	-0.626**	-0.493**	-0.676**	0.557**	0.050 ns	-0.645**	1		
11	-0.091 ns	0.581**	0.019 ns	0.081 ns	-0.079 ns	0.064 ns	0.545**	0.145 ns	-0.462**	0.400**	1	
12	0.156 ns	0.311*	0.543**	0.346*	0.424**	0.644**	0.429**	-0.091 ns	-0.039 ns	-0.168 ns	0.418**	1

** and * means correlation is significant at the 0.01 and 0.05 probability level, respectively;
ns means correlation is not significant

reflects blue light, using this filter may face plants with the blue light deficiency. Simultaneously, as growth proceed, leaf dry weight and LWR decreased and leaf area and stem dry weight increased under blue filter+ leaf removal +low density, as compared to the previous stage (Table 4). Therefore, as low density and leaf removal causes more radiation penetration and increase in R/FR ratio through canopy, which in turn is effective in increasing SLA (MORIONDO et al. 2003), the reduction in leaf thickness and increase in leaf area (SLA increase) after limiting the source (leaf defoliation) in the above-mentioned treatment combination is an effort for compensating the decrease in radiation interception during the growth season and preventing from a sharp drop in growth of reproductive organs.

In all filters and plant densities, defoliation reduced capitulum dry weight as compared to the control. In each stage, using blue filter in all levels of density and defoliation caused severe reduction in capitulum dry weight in comparison with the white filter and control (without filter) treatments. In the contrary, the highest LWR was obtained using blue filter in each level of defoliation and density (Table 4). A negative correlation between LWR and CWR at the pollination (-0.61^{**}) and physiological maturity (-0.64^{**}) stages indicated that using blue filter will cause more assimilates allocation to the leaves (higher LWR) and a lower allocation to capitulum (reduction in CWR). Moreover, considering negative correlation of LWR with capitulum dry weight (-0.32^{*}) and achenes per capitulum (-0.46^{**}) one could say that blue filter can decrease the amount of photosynthates allocated to the reproductive organs by increasing allocation to the leaves and stem (Table 6). The greatest amount of assimilates allocated to leaves (the highest LWR) and stem (the highest stem dry weight) was observed in blue filter treatment with the density of 5 pl m^{-2} without defoliation (Table 4). LIBENSON et al. (2002) reported that low R/FR ratios cause a decline in sunflower yield as the result of decrease in the number of achenes produced in plant. They stated that at high densities, stimulating stem growth in low R/FR ratios may decrease the available resources for the seed yield.

Conclusion

As with other crops, the morphology, physiology, yield and yield components of sunflower are all affected by its density. By increase in plant's population at high densities (crowding), the competition between the plants for growth will lead to taller plants competing for light (IBRAHIM 2012). In the present study, the reduction in radiation penetration into the canopy

through increasing plant density or using blue filter caused a reduction in R/FR ratio through canopy which, in turn, triggered the shad-avoidance mechanisms in sunflower. Under these conditions, the plant managed to increase leaf area and stem length to better benefit from radiation by increasing photosynthate allocated to leaf and stem and also decreasing leaf thickness. On the other hand, these increased allocation to vegetative organs effectively reduced the share of reproductive structures from photo--assimilates, resulting in reduced achene weight, number and capitulum weight. Among these, grain weight was affected more than its number. Decrease in plant density and leaf defoliation causes increase in R/FR ratio through canopy by increasing radiation penetration, thereby moderates shade-avoidance responses to some extent. However, the findings of this study showed that defoliation was not able to completely compensate the decrease in R/FR ratio entering the canopy caused by using blue filter.

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References

ALIMOHAMMADI R., AZIZOV I. 2011. *Defoliation effects on yield and yield components of sunflower cultivars (Helianthus annuus L.).* Int. J. AgriScience., 1(7): 361–365.

ALKIO M., SCHUBERT A., DIEPENBROCK W., GRIMM E. 2003. *Effect of source-sink ratio on seed set and filling in sunflower (Helianthus annuus L.).* Plant Cell Environ., 26: 1609–1619. doi: 10.1046/j.0016-8025.2003.01077.x.

ALYABYEV A.JU., LOSEVA N.L., JAKUSHENKOVA T.P., RACHIMOVA G.G. 2002. *Comparative effects of blue light and red light on the rates of oxygen metabolism and heat production in wheat seedlings stressed by heat shock*. Thermochimica Acta, 394: 227–231.

BABAEI AGHDAM J., ABDI M., SEIFZADEH S., KHIAVI, M. 2009. *Effects of different levels of nitrogen and plant density on yield and yield components of sunflower, Azargol cultivar in Takestan region*. Iranian. J. New Agric. Sci., 14: 1–12.

BALDISSERA T.C., FRAK E., CARVALHO P.C.D.F., LOUARN G. 2014. *Plant development controls leaf area expansion in alfalfa plants competing for light*. Ann. Bot., 113: 145–157.

BALLARE, C.L., CASAL J.J. 2000. *Light signals perceived by crop and weed plants*. Field Crop Res., 67: 149–160.

BALLARE C.L., SCOPEL A.L., JORDAN E.T., VIESTRA R.D. 1994. *Signaling among neighboring plants and development of size inequalities in plant population*. Proc. Natl. Acad. Sci. USA, 91: 10 094–10 098.

BALOCH J., QASIM KHAN M., ZUBAIR M., MUNIR M. 2009. *Effects of different shade levels (light integrals) on time to flowering of important ornamental annuals*. Int. J. Agric. Biol., 11(2): 138–144.

BANGE M., HAMMER P.G.L., RICKERT K.G. 1997. *Effect of radiation environment on radiation use efficiency and growth of sunflower*. Crop Sci., 37: 1208–1214.

BARROS J.F.C. CARVALHO M., BASCH G. 2004. *Response of sunflower (Helianthus annuus L.) to sowing date and plant density under Mediterranean conditions*. Europ. J. Agron., 21: 347–356.

BOOTH B.D., MURPHY S.D., SWANTON C.J. 2003. *Weed ecology in natural and agricultural systems*. Bristol: CAB International, Wyvern 21 Ltd.

BRITZ S.J., SAGER J.C. 1990. *Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources*. Plant Physiol., 94: 448–454.

CRUZ-CASTILLO J.G., WOOLLEY D.J., FAMIANI F. 2010. *Effects of defoliation on fruit growth, carbohydrate reserves and subsequent flowering of 'hayward' kiwifruit vines*. Sci. Hort., 125: 579–583.

FRANKLIN K.A., WHITELAM G.C. 2004. *Light signals, phytochromes and cross-talk with other environmental cues*. J. Exp. Bot., 55: 271–276.

FUKUDA N., FUJITA M., OHTA Y. 2008. *Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition*. Sci. Hort., 115: 176–182.

GARDNER F.P., PEARCE R.B., MITCHELL R.L. 1985. *Physiology of crop plant*. Ames. USA, Iowa State University Press.

GHOLINEZHAD E., AYNABAND A., HASSANZADE GHORTAPEH A., NOORMOHAMAD, G., BERNOUSI I. 2009. *Study of the effect of drought stress on yield, yield components and harvest index of sunflower hybrid Euroflor at different levels of nitrogen and plant population*. Not. Bot. Horti. Agrobot. Cluj. Napoca., 37(2): 85–94.

HEATHER A., GRAHAM H., DECOTEAU D.R. 1997. *Young watermelon plant growth responses to end-of-day red and far-red light are affected by direction of exposure and plant part exposed*. Sci. Hort., 69: 41–49.

HEUVELINK E. 1999. *Evaluation of a dynamic simulation model for tomato crop growth and development*. Ann. Bot., 83: 413–422.

HOLT N.M., ZENTNER R.P. 1985. *Effect of plant density and row spacing on agronomic performance and economic returns of non-oil seed sunflower in southeastern Saskatchewan*. Can. J. Plant Sci., 65: 501–509.

HURD R.G. 1974. *The effect of an incandescent supplement on the growth of tomato plants in low light*. Ann. Bot., 38: 613–623.

IBRAHIM H.M. 2012. *Response of some sunflower hybrids to different levels of plant density*. APCBEE Procedia., 4: 175–182.

KASPERBAUER M.J. 1987. *Far-red light reflection from green leaves and effects on phytochrome mediated assimilate partitioning under field conditions*. Plant Physiol., 85: 350–354.

KASPERBAUER M.J., KARLEN D.L. 1994. *Plant spacing and reflected far-red light effects on phytochrome-regulated photosynthate allocation in corn seedlings*. Crop Sci., 34: 1564–1569.

KRIZEK D.T., ORMROD D.P. 1980. *Growth response of grand rapids lettuce and first lady marigold to increased far-red and infrared radiation under controlled environments*. J. Am. Soc. Hortic. Sci., 105: 936–939.

KUBOTA S., YAMATO T., HISAMATSU T., ESAK S., OI R., ROH M.S., KOSHIOKA M. 2000. *Effect of red-and far-red-rich spectral treatment and diurnal temperature alternation on the growth and development of petunia*. J. Japan. Soc. Hort. Sci., 69(4): 403–409.

KUREPIN L.V., WALTON L.J. 2007. *Interaction of red to far red light ratio and ethylene in regulating stem elongation of Helianthus annuus*. Plant Growth Regul., 51: 53–61.

KUREPIN L.V., NEIL EMERY R.J., PHARIS R.P., REID D.V. 2007. *Uncoupling light quality from light irradiance effects in Helianthus annuus shoots: putative roles for plant hormones in leaf and internode growth*. J. Exp. Bot., 58(8): 2145–2157.

LAMBERS H., CHAPIN F.S., PONS T.L. 2008. *Plant physiological ecology (2nd ed).* New York, USA, Springer-Verlag.

LEE J.H., HEUVELINK E. 2003. *Simulation of leaf area development based on dry matter partitioning and specific leaf area for cut Chrysanthemum*. Ann. Bot., 91: 319–327.

LI S., RAJAPAKSE N.C., YOUNG R.E., OI, R. 2000. *Growth responses of chrysanthemum and bell pepper transplants to photoselective plastic films*. Sci. Hort., 84: 215–225.

LIBENSON S., RODRIGUEZ V., LÓPEZ PEREIRA M., SÁNCHEZ R.A., CASAL J.J. 2002. *Low red to far-red ratios reaching the stem reduce grain yield in sunflower*. Crop Sci., 42: 1180–1185.

LINDSTRÖM L.I., PELLEGRINI C.N., AGUIRREZÁBAL L.A.N., HERNÁNDEZ L.F. 2006. *Growth and development of sunflower fruits under shade during pre and early post-anthesis period*. Field Crops Res., 96: 151–159.

LÜTTGE U. 2007. *Carbon dioxide signaling in plant leaves*. C. R. Biologies, 330: 375–381.

MALIAKAL S.K., MCDONNELL K., DUDLEY S.A., SCHMITT J. 1999. *Effect of red to far-red ratio and plant density on biomass allocation and gas exchange in Impatiens capensis*. Int. J. Plant Sci., 160(4): 723–733.

MAZZELLA M.A., ZANOR M.I., FERNIE A.R., CASAL J.J. 2008. *Metabolic responses to red/far-red ratio and ontogeny show poor correlation with the growth rate of sunflower stems*. J. Exp. Bot., 59(9): 2469–2477.

MILLER J.F., FICK G.N. 1978. *Influence of plant population on performance of sunflower hybrids*. Can. J. Plant Sci., 58: 597–600.

MORIONDO M., ORLANDINI S., VILLALOBOS F.J. 2003. *Modelling compensatory effects of defoliation on leaf area growth and biomass of sunflower (Helianthus annuus l.)*. Europ. J. Agron., 19: 161–171.

MORRISON M.J., STEWART D.W. 1995. *Radiation-use efficiency in summer grape*. Agron. J., 87: 1139–1142.

NEZAMI A., KHAZAEI H.R., BOROUMAND REZAZADEH Z., HOSSEINI A. 2008. *Effects of drought stress and defoliation on sunflower (Helianthus annuus) in controlled conditions*. Desert., 12: 99–104.

OLSZEWSKI N., SUN T., GUBLER, F. 2002. *Gibberellin signaling: biosynthesis, catabolism, and response pathways*. Plant Cell. Suppl.: S61–S80.

OYAERT E., VOLCHAERT E., DEBERGH, P.C. 1999. *Growth of chrysanthemum under coloured plastic films with different light qualities and quantities*. Sci. Hort., 79: 195–205.

POLAT T., OZER H., OZTURK E. 2011. *Responses of hybrid and open pollination sunflowers (Helianthus annus l.) to defoliation*. Aust. J. Crop Sci., 5(2): 132–137.

POURSAKHY N., KHAJEPOUR M. 2014. *Effect of planting pattern and plant density on growth and yield of sunflower (hisun-36) hybrid*. Agron. J., 104: 54–61.

PRICE H.J., JOHNSTON J.S. 1996. *Influence of light on dna content of Helianthus annuus linnaeus*. Proc. Natl. Acad. Sci. USA., 93: 11264–11267.

ROSHDI M., REZADOOST S., KHALILI MAHALLEH J., HAJI HASANI ASL N. 2009. *Effects of plant density and defoliation at different developmental stages on yield and yield components of nuts sunflower*. Iranian J. New Agric. Sci., 15: 41–54.

SANGOI L., GRACIETTI M.A., RAMPAZZO C., BIANCHETTI P. 2002. *Response of Brazilian maize hybrids from different areas to changes in plant density*. Field Crop Res., 79: 39–51.

SARALA M., TAULAVUORI K., TAULAVUORI E., KARHU J., LAINE K. 2007. *Elongation of scots pine seedlings under blue light depletion is independent of etiolation*. Environ. Exp. Bot., 60: 340–343.

SCHNEITER A.A., JOHNSON B.L. 1994. *Response of sunflower plants to physical injury*. Can. J. Plant Sci., 74(4): 763–766.

SCHNEITER A.A., JONES J.M., HAMMOND J.J. 1987. *Simulated hail research in sunflower defoliation*. Agron. J., 79: 431–434.

SEVERINO L.S., FREIRE M.A.O., LUCENA A.M.A., VALE L.S. 2010. *Sequential defoliations influencing the development and yield components of castor plants (Ricinus communis l.)*. Ind. Crops Prod., 32: 400–404. doi: 10.1016/j.indcrop.2010.06.007.

SMITH H. 1982. *Light quality, photoperception, and plant strategy*. Ann. Rev. Plant Physiol., 33: 481–518.

TAIZ L., ZEIGER E. 2006. *Plant Physiology. 4th Ed.* Sunderland, Massachusetts, USA, Sinauer Assoiciates Inc.

TERASHIMA I., HANBA Y.T., TAZOE Y., VYAS P., YANO S. 2006. *Irradiance and phenotype: comparative ecodevelopment of sun and shade leaves in relation to photosynthetic* co_2 *diffusion.* J. Exp. Bot., 57: 343–354.

VAN HINSBERG A.A., VAN TIELDEREN P. 1997. *Variation in growth from in relation to spectral light quality (red/far red ratio) in Plantago lanceolata l. In sun and shade population.* Oecologia, 111: 452–459.

VANDENBUSSCHE F., VAN DER STRAETEN D. 2004. *Shaping the shoot: a circuitry that integrates multiple signals.* Trends Plant Sci., 9: 499–506.

VOLKOV A.G., DUNKLEY T.C., LABADY A.J., BROWN C.L. 2005. *Phototropism and electrified interfaces in green plants.* Electrochim. Acta., 50: 4241–4247.

VOLKOV A.G., DUNKLEY T.C., MORGAN S.A., RUFF II D., BOYCE Y.L., LABADY A.J. 2004. *Bioelectrochemical signaling in green plants induced by photosensory systems.* Bioelectrochemistry, 63: 91– 94.

VOS J. 1995. *The effects of nitrogen supply and stem density on leaf attributes and stem branching in potato (Solanum tuberosum l.).* Potato Res., 38(3): 271–279.

WILSON S.B., RAJAPAKSE N.C. 2001. *Growth regulation of sub-tropical perennials by photoselective plastic films.* J. Environ. Hort., 19(2): 65–68.

XIA S., CHEN S., ZHAO L., WANG, G. 2006. *Density effects on plant height growth and inequality in sunflower population.* Agron. J., 48: 513–519.

XIONG, J. PATIL G.G., MOE R. 2002. *Effect of dif and end- of- day light quality on stem elongation in Cucumis sativus.* Sci. Hort., 94: 219–229.

YAMADA A., TANIGAWA T., SUYAMA T., MATSUNO T., KUNITAKE T. 2008. *Improvement of Eustoma grandiflorum (raf.) Shinn. Cut flower quality for early-autumn shipping with long-day treatment using light sources that delay flower bud formation.* J. Jpn. Soc., 77: 296–303.

YAMAZAKI H., OI R., HAMANO M., YAMATO Y., MIURA H. 2000. *Inhibition of bulb development of Allium wakegi araki by covering with far- red- intercepting film in summer.* J. Japan. Soc. Hort. Sci., 69(3): 250–254.

YANG F., HUANG S., GAO R., LIU W., YONG T., WANG X., WUA X., YANG W. 2014. *Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio.* Field Crop Res., 155: 245–253.

YANO S., TERASHIMA I. 2001. *Separate localization of light signal perception for sun or shade type chloroplast and palisade tissue differentiation in Chenopodium album.* Plant Cell Physiol., 42: 1303–1310.

YOUSEFPOOR Z., YADAVI A. 2014. *Effect of biological and chemical fertilizers of nitrogen and phosphorus on quantitative and qualitative yield of sunflower.* Iranian J. Agric. Sci. Sustain. Prod., 24(1): 95–112.

ZHOU Y., SINGH B.R. 2002. *Red light stimulates flowering and anthocyanin biosynthesis in American cranberry.* Plant Growth Reg., 38: 161–171.

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EFFECT OF FOLIAR SPRAY OF ZnO-NPs ON THE PHYSIOLOGICAL PARAMETERS AND ANTIOXIDANT SYSTEMS OF *LYCOPERSICON ESCULENTUM*

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Key words: catalase, *Lycopersicon esculentum*, zinc oxide nanoparticles, physiological parameters, superoxide dismutase.

Abstract

The nature of nanoparticles and their effective application has been given considerable attention by researchers in various fields, mainly agriculture. The present investigation examined the foliar effect of zinc oxide nanoparticles (ZnO-NPs) on plant growth profiling, photosynthetic machinery and associated biochemical changes in tomato (*Lycopersicon esculentum*) following growth in various concentrations (10, 50, 100 and 200 ppm ZnO-NPs). After 15 days of transplantation, ZnO-NPs sprayed to the foliage of tomato plant for five days (35-39 DAS). Treated plants at days 45 and 60 (pre-flowering stage), registered an increase in growth and biomass over their respective control. Among different concentrations of ZnO-NPs [0 (control), 10, 50, 100 and 200 ppm], 50 ppm proved to be the optimum foliar spray treatment and increase the SPAD chlorophyll (27% and 32%), net photosynthetic rate (31% and 35%), leaf protein content (17% and 22%), catalase (CAT, 55% and 61%), peroxidase (POX, 68% and 75%) and superoxide dismutase (SOD, 50% and 55%) activity. Interestingly, significant increases in lycopene (23%), β-carotene (25%) content followed by a decrease in the content of ascorbic acid (38%) in response to above treatments. Number of fruits and fruit yield in the treated plants were also higher (21% and 28%) as compare to respective controls. These results suggest that ZnO-NPs interact with meristematic cells triggering biochemical pathways conductive to an enhancement of growth attribute. Further studies are needed to investigate the mechanisms and the side effects of ZnO-NPs on tomato plants.

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Introduction

In the recent years, NPs has been attractive area of research due to their exclusive properties, such as better electrical conductivity, plasticity, roughness, and formability of ceramics, escalating the hardness and potency of metals and alloys, and by increasing the radiant effectiveness of semiconductors (RITTNER and ABRAHAM 1998). Alongthis, some other properties, such as small size, high surface-to-volume ratio and unique physio-chemical properties, leads to the use of NPs in industries and wide range of consumer products (STAMPOULIS et al. 2009).

The demand of agriculture production is increasing day by day due to uncontrolled growth of world population. Therefore, it is necessary to increase the productivity of the crop by using modern technologies. Nanotechnology is one of the most promising areas which can be exploited to achieve the goal. It has been studied by various scientists, that NPs has the potential to increase the productivity of the crop (Scott and CHEN 2002, BATSMANOVA et al. 2013). In plants, NPs either harmful or beneficial, it mainly depends upon the given manner and used nanomaterials (MONICA and CREMONINI 2009). Limited reports are available on nanomaterials effect on flora (BERNHARDT et al. 2010). NPs Study in flora have recognized that NPs may be taken-up (KUREPA et al. 2010, SCHWAB et al. 2015), transported, (WANG et al. 2012, ZHAO et al. 2012) and concentrated in vacuoles, nuclei and plasmodesmata (KUREPA et al. 2010, SCHWAB et al. 2015), which modify physiological processes of plant as well as growth and development (BURKLEW et al. 2012, GARCIA-SANCHEZ et al. 2015). In plants, reactive oxygen species (ROS) is formed as a natural by-product of the normal metabolism of O_2 and have very promising roles in cell signalling and homeostasis (RAY et al. 2012). Imbalance in ROS causes oxidative stress, higher formation of ROS damage to DNA, proteins and lipid and finally cell death (TRIPATHY and OELMULLER 2012). To overcome the toxic effect of oxidative stress plant activates enzymatic (CAT, POX and SOD) and non-enzymatic (proline) antioxidants (TRIPATHY and OELMULLER 2012, SEWELAM et al. 2016). These enzymes are the key elements in the defence mechanism (ANDRE et al. 2010). ZnO-NPs included as a third most widely used NPs with an estimation of total global production about 550 and 33,400 tons per annum (BONDARENKO et al. 2013, CONNOLLY et al. 2016, PENG et al. 2017). ZnO-NPs included as a bio-safe materials that give their impacts on the biological and chemical species from the photo-oxidizing and photo-catalysis (SIRELKHATIM et al. 2015).

The data present a new approach to evaluate the impact of ZnO-NPs on the performance of *Lycopersicon esculentum* in respect of growth param-

eters, photosynthetic attributes, biochemical parameters and yield characteristics. The hypothesis of this study is that the effect of ZnO-NPs on plant production is directly related to the photosynthesis process and indirectly in context with the defence system of the plant.

Material and Methods

Plant material and treatment

Seeds of *Lycopersicon esculentum* var. PKM-1 procured from Department of Horticulture, Indian Agricultural Research Institute, (New Delhi), were surface sterilized with 1% sodium hypochlorite solution for 10 minutes, followed by repeated washings with double distilled water (DDW). The experiment was arranged in a completely randomized design in the net house of the Department of Botany of Aligarh Muslim University, Aligarh, India, under natural environmental condition. In earthen pots, sterilized seeds were sown to make nursery. At the stage of 20 days, tomato seedlings were transplanted to the maintained pots, filled with soil and farmyard manure (6:1). The plants were treated with DDW (control) and 10, 50, 100 and 200 ppm of ZnO-NPs as foliar spray at 35-39 DAS at evening. Each plant was sprayed thrice at a time. The nozzle of the sprayer was adjusted in such a way that it pumped out about 1 cm^3 of the solution in a single spray. Therefore, each plant received about 3 cm^3 of DDW or ZnO-NPs solution. Each treatment was replicated five times with three plants per replicate and plants were sampled at 45 and 60 DAS to assess various growth, photosynthetic parameters, biochemical characteristics as well as the yield.

Source of NPs

Characterization and manufacturing of ZnO-NPs as KHAN et al. (2016) described. Material study and properties are included under the process of characterization. Widely separation, microscopy and spectroscopy used in this procedure (FABREGA et al. 2011). ZnO-NPs were procured from Sigma-Aldrich Chemicals Pvt. Ltd. India. A stock solution of 200 ppm was prepared by dissolving required amount of ZnO-NPs in 10 ml DDW in 100 ml volumetric flask, and make total volume 100 ml adding DDW. Other required concentrations were prepared by diluting the stock solution.

Plant growth analysis

The plants were detached from pots along attached soil and were dipped in a water filled bucket. Soil removed gently and the lengths of root and shoot were measured by using a meter scale. The plants were stored in oven and run 24 hours at 80°C, and weighed the dry plant weight. Through the meter of leaf-area (ADC Bio scientific, Hoddesdon, UK), deliberate leaf area.

Determination of chlorophyll content and photosynthetic attributes

The SPAD value of chlorophyll in newly leaf was calibrate through SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan). Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) at each selected stage, was measured in fully expanded leaves of the plants by using portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA). The atmospheric conditions during measurement were photosynthetic active radiation, 1,016 µmol $m^{-2}s^{-1}$, air temperature, 25°C, relative humidity, 85%, CO_2 concentration, 600 ppm and photosynthetic photon flux density (PPFD), 800 µmol mol^{-2} s^{-1}, respectively. All the measurements were made between 11:00 to 12:00 h under the clear sun light.

Analysis of NR and CA activities

Activity of NR was compute by JAWORSKI (1971) procedure. A mixture of newly form leaf (0.1 g), phosphate buffer (pH 7.5), KNO_3, and isopropanol was store in incubator at 30°C for 2 h. Sulfanilamide and N-1-napthylethylenediamine hydrochloride mixture were added to the incubated mixture. At 540 nm read absorbance with a spectrophotometer (Spectronic 20D; Milton Roy, USA). CA action in leaves was measured through DWIVEDI and RANDHAWA (1974) procedure. Leaf was slash into minute pieces in a cysteine hydrochloride solution. They were blotted and conveyed in a test tube, pursue phosphate buffer addition (pH = 6.8), 0.2 M $NaHCO_3$, bromothymol blue, and red indicator of methyl. 0.5 N HCl used for titrating.

Protein content estimation

1 g newly formed leaf, homogenized in freeze buffer extraction, which is consisted of 40 mM tris-HCl (pH = 7.5), 0.07% β-mercaptoethanol, 2% polyvinylpyrrolidone, 0.5% Triton X-100 and 1 mM phenylmethane sulfo-

nyl floride (PMFS), 1 mM ethylenediaminetetraacetic acid (EDTA) by pestle and mortar. Centrifuged at 20,000 xg for 10 minutes, and supernatant was collected to valuation the protein by BRADFORD'S (1976) method.

Antioxidative enzymes assay

In antioxidant enzymes estimation, the leaf tissue (0.5 g) was homogenized in a 50 mM phosphate buffer (pH = 7.0) containing 1% polyvinylpyrrolidone. Centrifuged at 15,000 x *g* for 10 minutes at 4ºC, resulting supernatant used as a source of enzymes like CAT, POX and SOD.

For the estimation of POX activity, the enzyme extract (0.1 mL) was added in the reaction mixture of pyrogallol, phosphate buffer (pH = 6.8), and 1% H_2O_2. The change in the absorbance was read at every 20 seconds for 2 minutes at 420 nm (CHANCE and MAEHLY 1956). A control mixture was prepared by adding DDW instead of enzyme extract. The reaction mixture for CAT consisted of phosphate buffer (pH = 6.8), 0.1 M H_2O_2, and enzyme extract (0.10 mL). H_2SO_4 was added to the reaction mixture, after its incubation for 1 minute at 25ºC, and it was titrated against potassium permanganate solution (CHANCE and MAEHLY 1956). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of BEAUCHAMP and FRIDOVICH (1971). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 20 µM riboflavin, 75 mM nitroblue tetrazolium (NBT), 13 mM methionine, and 0.1 mM ethylenediaminetetraacetic acid (EDTA) were irradiated under two fluorescent light tubes (40 µmol m^{-1} s^{-1}) for 10 min. The absorbance was measured at 560 nm with a UV-visible spectrophotometer (KONO 1978) with slight modifications. Blanks and controls were also run in the same manner but without illumination and enzyme, respectively. The amount of SOD activity that gave half-maximal inhibition of NBT reduction was defined as one unit of SOD activity.

Determination of proline content

BATES et al. (1973), method was used for identification of proline amount in newly form leaves. Leaves extracted in sulfosalicylic acid, an equal volume of glacial acetic acid and ninhydrin solutions was added. The sample was heated at 100°C, to which 5 ml of toluene was added. The absorbance of the aspired layer was read at 528 nm on a spectrophotometer.

Determination of Lycopene, β-carotene and ascorbic acid content

In ripe fruits amount of lycopene measured by the method given by RANGANNA (1976). In this procedure, pigment was extracted in acetone and transferred to petroleum ether layer in a separating funnel. Absorbance of the petroleum ether layer was recorded on spectrophotometer at 503 nm. Petroleum ether is also used as a blank. Lycopene content in the sample was calculated by the formula-

$$\text{Lycopene [mg g}^{-1}\text{]} = \frac{31.20 \cdot \text{absorbance}}{\text{weight of sample [g]}}$$

β-carotene content in a fruit h described by method given by SADASIVAM and MANICKAM (1997). Pigment was extracted in acetone and hexane and absorbance was read at 436 nm with the help of spectrophotometer. The amount of carotene in the sample was calculated by using standard curve prepared from pure carotene and expressed in µg g^{-1} FM.

In the mature fruit content of ascorbic acid was determined through the procedure given by RAGHURAMULA et al. (1983). The samples were homogenized with 4% oxalic acid and then centrifuged. Supernatant was titrated against 2, 6-dichlorophenol indophenol dye. The amount of dye used was placed in the following formula for calculating ascorbic acid content

$$\text{Amount of ascorbic acid [µg g}^{-1}\text{ FM]} = \frac{0.5\text{ mg}}{V_1\,\text{cm}^3} \cdot \frac{V_2}{5\text{ cm}^3} \cdot \frac{100\text{ cm}^3}{\text{Wt of sample}} \cdot 100$$

where:
V_1 – liter value of working standard
V_2 is the liter value of the sample
Wt – the weight of the sample.

Results were expressed as milligrams per gram on fresh mass basis.

Yield characteristics

At the stage of harvesting (180 DAS or post flowering stage), 9 plants (3 plant from each pot) representing each treatments were randomly sampled and counted for the number of fruits and per plant and weight to assess fruit yield per plant.

Statistical analysis

The experiment was conducted according to simple randomized block design. Each treatment was replicated five times. Data were statistically

analysed for analysis of variance (*ANOVA*) using *SPSS*, *17.0 for Windows* (*SPSS*, Chicago, IL, USA). Least significant difference (LSD) was calculated to separate the means.

Results

Growth biomarkers

ZnO-NPs treated plants showed an obvious increased in the growth of the plant, and the increased in the growth were positively related with the concentrations of ZnO-NPs applied upto certain concentrations (Figures 1a–*f*, Figure 2*a*). The maximum increase in shoot length (30.1%), shoot fresh mass (27.7%), shoot dry mass (29.0%), root length (28.7%), root fresh mass (26.1%), root dry mass (24.6%) and leaf area (24.1%) at 60 DAS was recorded in the plants treated with 50 ppm of ZnO-NPs over their control. The maximum decrease was reported in the plant sprayed with 200 ppm of ZnO-NPs.

SPAD value

With the progress of time from 45 to 60 days stage, SPAD chlorophyll content increased and also increased in the presence of ZnO-NPs in a concentration dependent manner (Figure 2*b*). Moreover, the maximum increase (32.1%) in SPAD chlorophyll values was found in the plants sprayed with 50 ppm of ZnO-NPs over their control.

Leaf gas-exchange traits

The foliar application of 50 ppm of ZnO-NPs proved best and increased the values of P_N (35.1%), gs (29.1%), Ci (31.2%) and E (32.4%) over their non-treated control plants at 60 DAS (Figures 2 *c–f*). The pattern of response of plants for photosynthesis and related attributes are 50 ppm > 100 ppm > 10 ppm > 0 ppm > 200 ppm respectively.

Activities of CA and NR

Plants raised from foliar application of ZnO-NPs had significantly higher activity of CA and NR over their respective controls. Out of 2 stages of analysis (45 and 60 DAS), the maximum CA (25.9%) and NR (28.5%) activities were noted at the stage of 60 days (Figures 3*a–b*). 50 ppm ZnO-NPs demonstrated to be best.

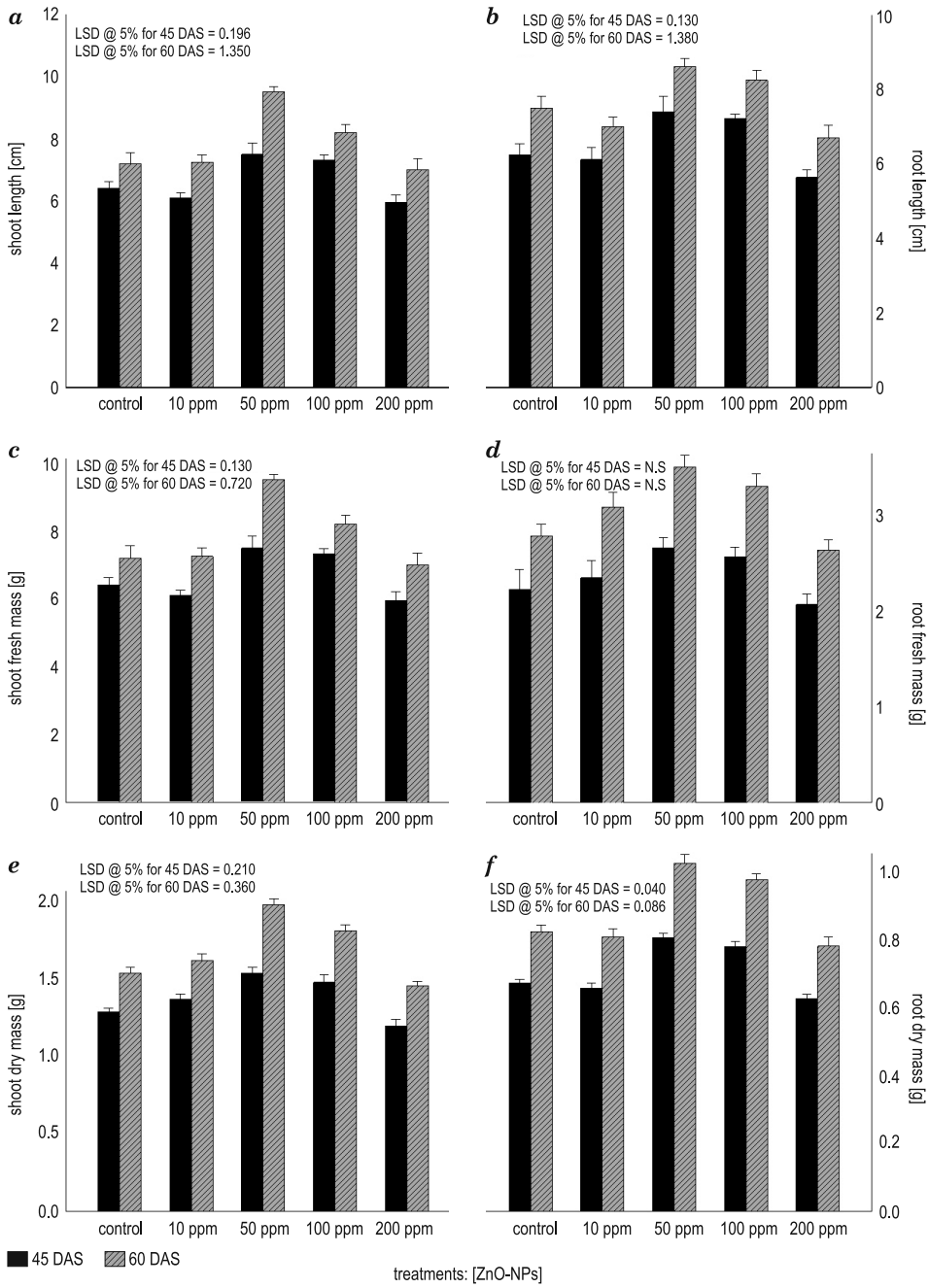

Fig. 1. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) shoot length, (*b*) root length, (*c*) shoot fresh mass, (*d*) root fresh mass, (*e*) shoot dry mass, and (*f*) root dry mass in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

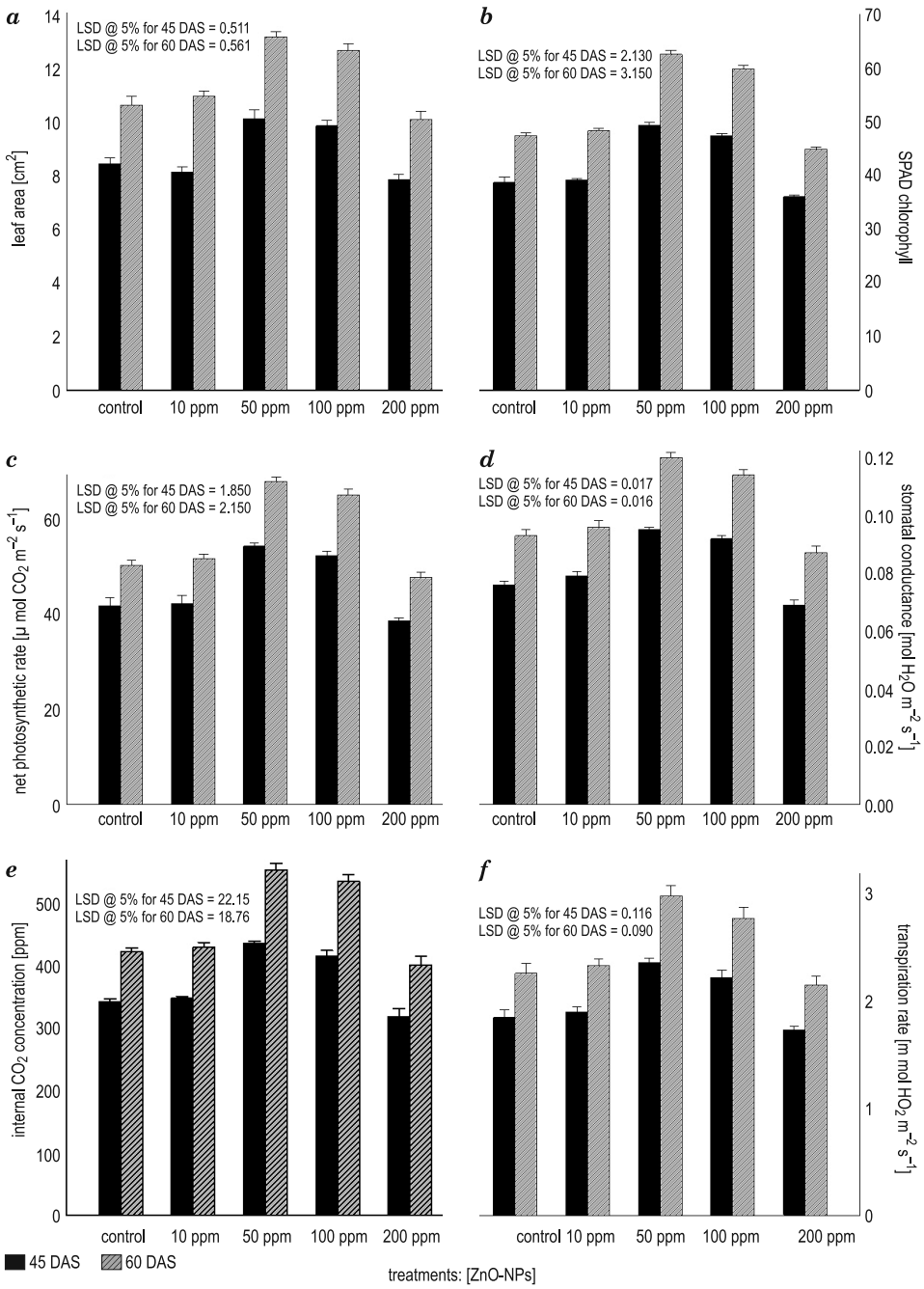

Fig. 2. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) leaf area, (*b*) SPAD chlorophyll, (*c*) photosynthetic rate, (d) stomatal conductance, (*e*) internal CO_2 concentration, and (*f*) transpiration rate in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

Protein content

It is induced from the Figure 4*a* that protein content in the leaves increased with the advancement of age irrespective of treatment. The maximum value of protein content was recorded at 60 DAS in the plant treated with 50 ppm of ZnO-NPs as foliar application. The pattern of response of plants for ZnO-NPs is 50 ppm > 100 ppm > 10 ppm > 0 ppm > 200 ppm respectively.

Activity of antioxidant enzymes

Activity of antioxidant enzymes (CAT, POX and SOD) increased with age of the plant. Effect of ZnO-NPs varies from concentration to concentration in all the antioxidant enzymes. The maximum activity of CAT, POX and SOD (60%, 74% and 55%) was recorded in the plant sprayed with 50 ppm of ZnO-NPs at 60 DAS (Figures 3*c–e*).

Proline content

In tomato leaves content of proline was significantly elevated with application of ZnO-NPs. The leaves of plants exposed to the 50 ppm ZnO-NPs possessed the higher concentration of proline as compare to the non-treated (control) plants at 60 DAS which was 54 % higher (Figure 3*f*).

Lycopene, *β*-carotene and ascorbic acid content

Fruits grown from ZnO-NPs treated plants had higher content of lycopene and *β*-carotene as compared to control plants. Maximum value of lycopene and *β*-carotene was noted in plants whose leaf exposed with 50 ppm of ZnO-NPs. The respective increase was 22.6% and 24.9% over their controls at 180 DAS (Figures 4*d–e*). Ascorbic acid content decreased in the fruits in proportion to the treatment of ZnO-NPs (Figure 4*f*). Foliar application of ZnO-NPs had a negative impact on ascorbic acid content, and decreased the ascorbic acid content as compared to that of control plants.

Number of fruits and fruit yield

Graph 4*b* and *c* clearly revealed that the plants treated with ZnO-NPs had significantly higher fruits number and yield at harvest. ZnO-NPs (50 ppm) greatly increased fruit numbers per plant (21.1%) and fruit yield (19.4%) over their respective controls.

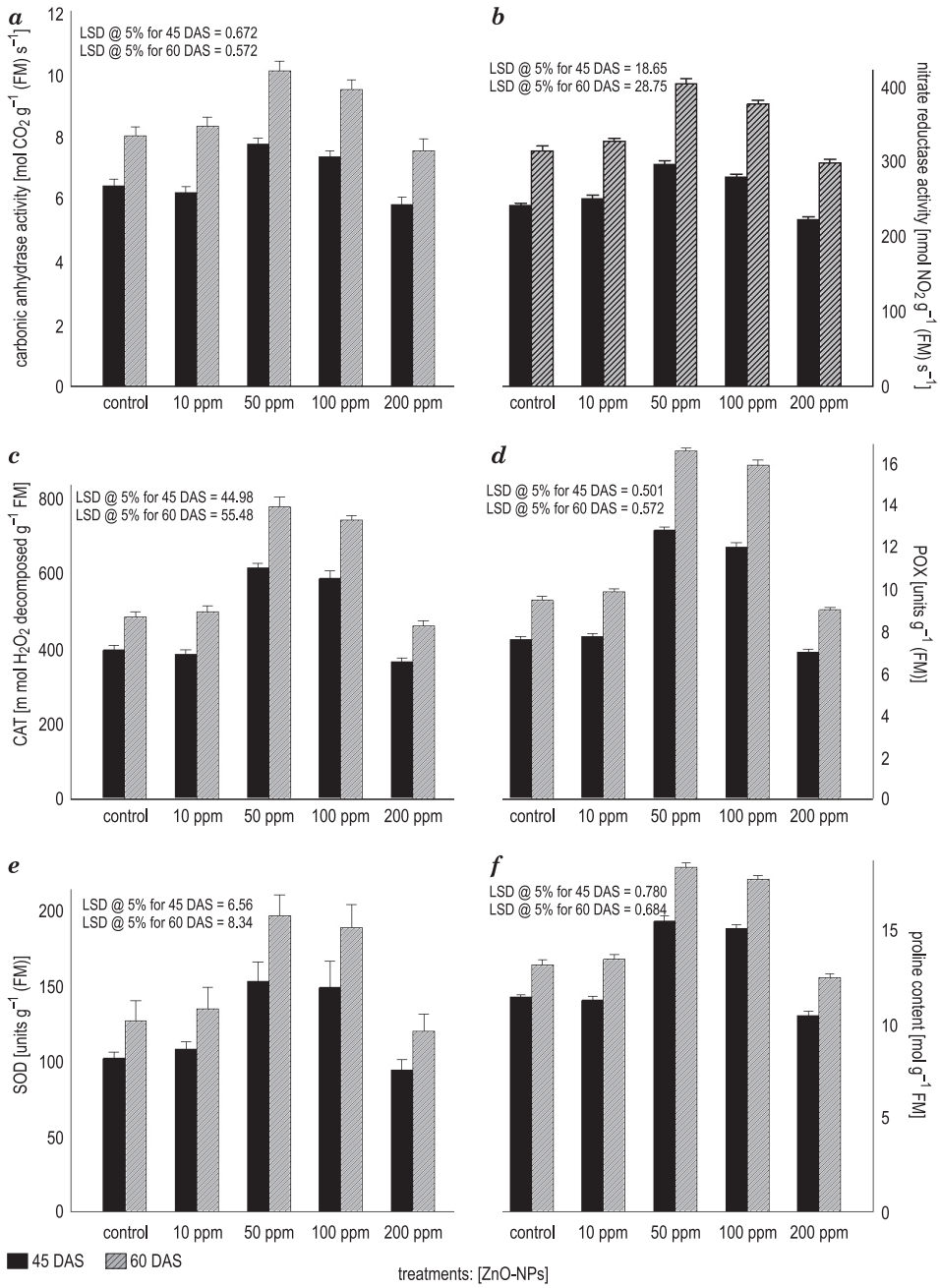

Fig. 3. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) CA activity, (*b*) NR activity, (*c*) catalase, (*d*) peroxidase, (*e*) superoxide dismutase, and (*f*) proline content in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

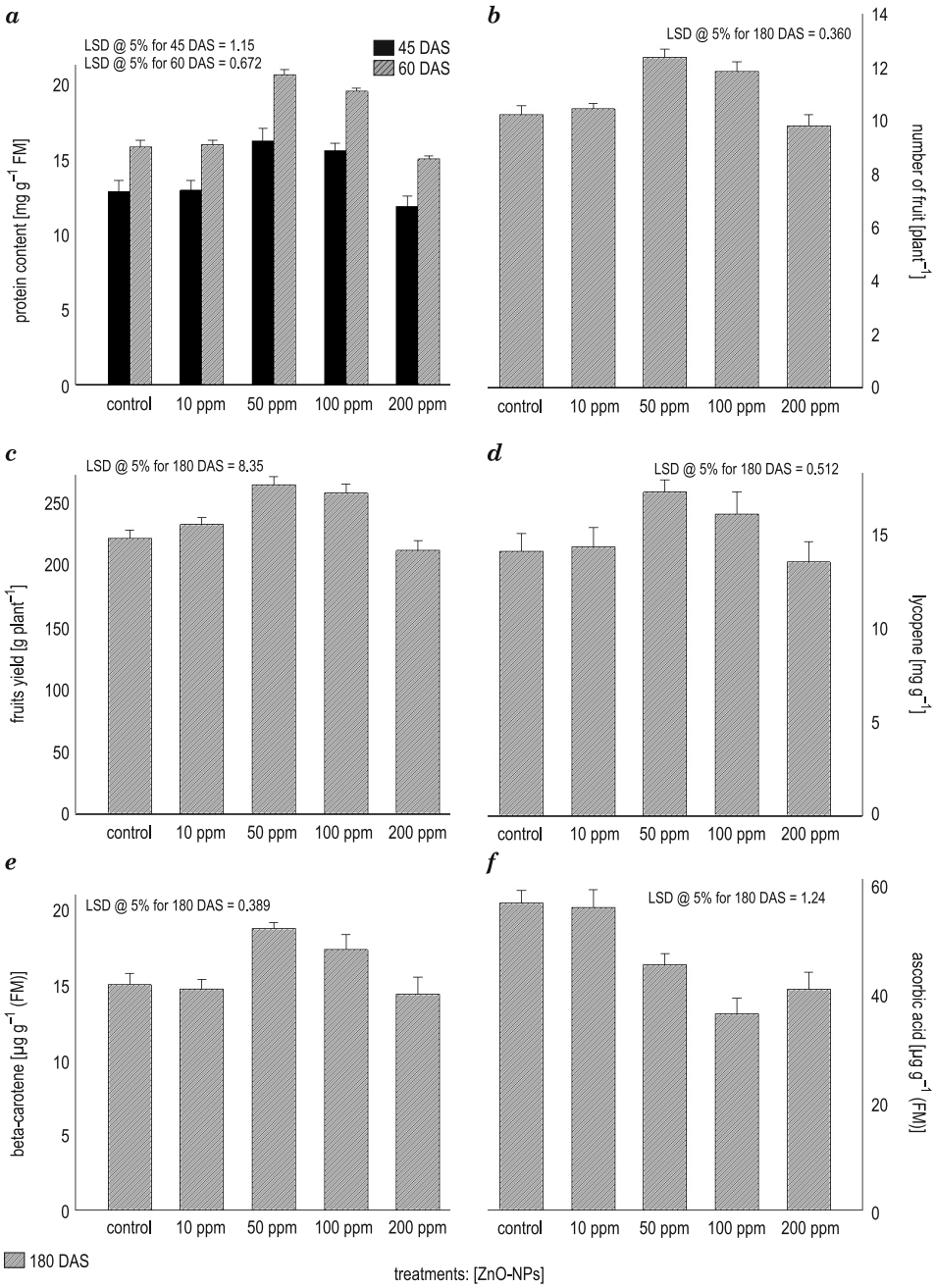

Fig. 4. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) protein content in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS, (*b*) number of fruits, (*c*) fruit yield, (*d*) lycopene content, (*e*) *β*-carotene, (*f*) ascorbic acid in the leaves of *Lycopersicon esculentum* seedlings at 180 DAS. Vertical bars indicate standard errors between the replicates

Discussions

Nanotechnology is a one of the new discipline and NPs have become a centre of attraction for researchers due to its exclusive physico-chemical properties compared to their large particles (MONICA and CREMONINI 2009). Zn used in various functions like protein synthesis, membrane activity, elongation of cell etc. (CAKMAK 2000). In recent years scientist work in the field of nanotechnology and try to increase the crop productivity through changes in physiological and biochemical parameters. In plants, NPs increased crop productivity by alteration in photosynthetic machinery, biochemical and growth parameters. This increment mainly based on the concentration of NPs and its size (KHODAKOVSKAYA et al. 2012). Therefore, the present study was undertaken.

In the present study the value of growth biomarkers (root & shoot length, leaf area, fresh and dry masses of plants and leaf area) increased significantly when exposed to the ZnO-NPs. Application of ZnO-NPs could enhance the rate of photosynthesis of the plants, which leads the increased cell division and ultimately enhanced the biomass of the plant (ALLOWAY 2004). However, this study is further corroborated by the study of MARSCHNER (1993), where ZnO-NPs increased the values of shoot length, root length, shoot and root biomass in Cyamopsis tetragonoloba plant. Alongthis various other researchers also confirmed the present observations (PANDEY et al. 2010, PRASAD et al. 2012, SEDGHI et al. 2013, MUKHERJEE et al. 2014, RAMESH and TARAFDAR 2014, RASKAR and LAWARE 2014, FAIZAN et al. 2018) on various other crops. The chlorophyll content in leaves was measured as an indicator of the plants photosynthetic performance. ZnO-NPs (50 ppm), treated plant leaves showed maximum increase in chlorophyll content (SPAD value; Figure 2b). It is hypothesized that ZnO-NPs involved in the enhancement of transcription and/or translation, this leads the increase chlorophyll content in plants, and more efficiently for the synthesis of photosynthetic pigments which increased the rate of photosynthesis (Figure 5). This statement is corroborated by the study of AN et al. (2008), where NPs treatment increased the chlorophyll content in Asparagus plant. In sustainable agriculture routine, NPs arbitrate changing mechanism, need to additional investigation.

The photosynthetic machinery is the back bone of plant system; therefore if any enhancement in photosynthesis occurs due to ZnO-NPs, the related attributes such as gs, E and Ci will also increases. In this observation, photosynthetic machinery shows irregularity in contrast with different treatments of ZnO-NPs. Application of ZnO-NPs increased the formation of chlorophyll pigment and encourages Ribulose 1,5-bisphosphate

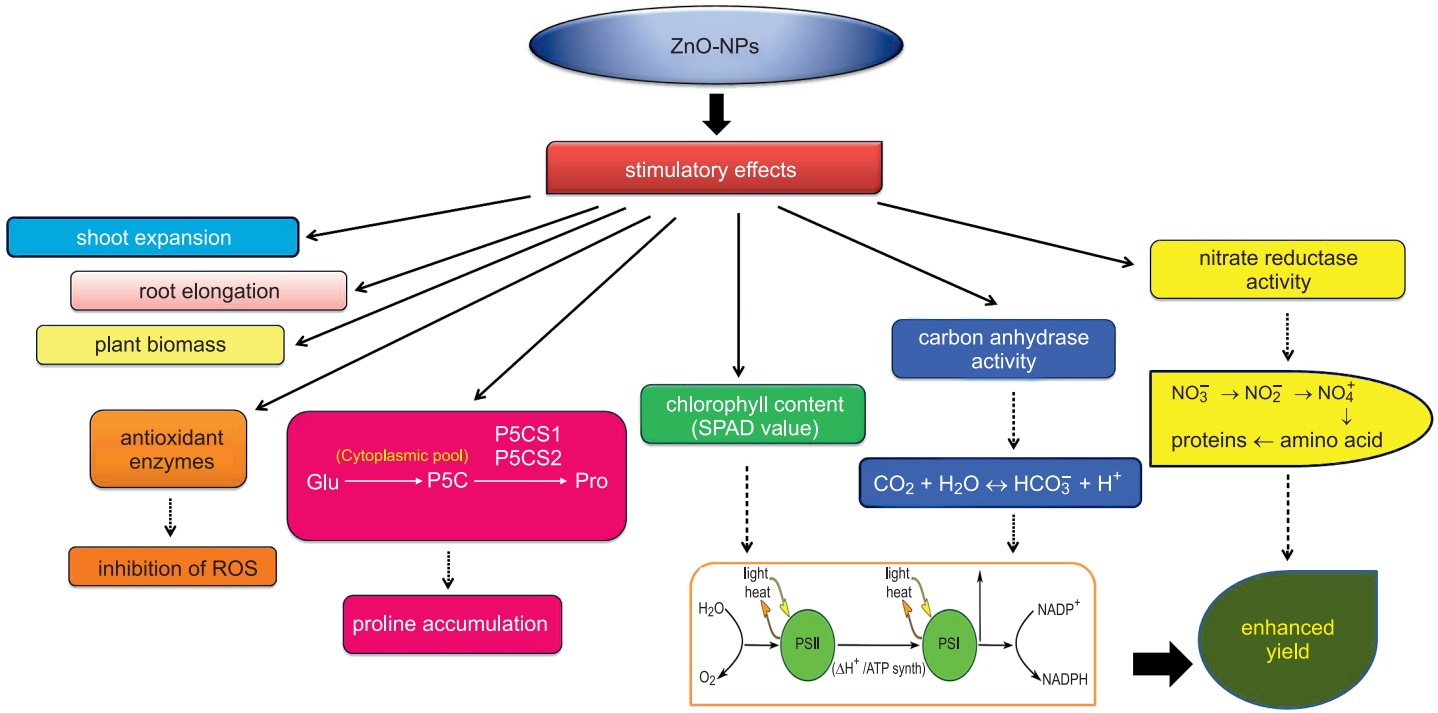

Fig. 5. Effect of ZnO-NPs on the shoot expansion, root elongation, plant biomass, carbonic anhydrase activity, nitrate reductase activity, antioxidant enzymes activity, content of proline, chlorophyll content, photosynthetic rate and yield: CO_2 – carbon dioxide; H_2O – water; HCO^{3-} – carbonic acid; O_2 – oxygen; PSI – photosystem 1st; PSII – photosystem 2nd; $NADP^+$ – Nicotinamide adenine dinucleotide phosphate; Glu – glutamate; Pro – proline; P5C – 1-Pyrroline-5-carboxylic acid; P5CS1 and 2 – delta-1-pyrroline-5-carboxylate synthase 1 and 2; NO_2^{-} – nitrite; NO_3^{-} – nirate; NO_4^{+} – ammonia

carboxylase (Rubisco) activity which stimulates the photosynthetic rate in plants. Moreover NOJI et al. (2011) suggested that silica nano particles obligated with PS II and induce constant performance of oxygen evolving reactions of photosynthetic machinery, signify the light directed transport of electron to quinine molecules from water. NOJI et al. (2011) also suggested that complexity of PS II have potential to form photosensors and imitated photosynthetic system. In this study, photosynthetic efficiency increased by the combined effect of all these altered processes (Figures 2*c–f*). Higher activity of photosynthetic system in plants would lead the higher yield of the plants (Figure 5).

In this study it was found that foliar application of different concentrations of ZnO-NPs treatment enhanced the photosynthesis and related attributes along with the CA activity (Figure 3*a*). There are several factors which determine the activity of CA, like hormonal signalling, light intensity, availability of Zn and regulation of genetic expression of the transcripts (TIWARI et al. 2005). Moreover, ZnO-NPs enhanced the assimilatory rate of CO_2, which are helpful to increase the CA activity. This can be supported by the result of Faizan et al. (2018) where ZnO-NPs treatment enhances CA activity in tomato. SiO_2-NPs also improve the photosynthetic rate by improving activity of CA (SIDDIQUI et al. 2014, XIE et al. 2012). Nitrate reductase is a primary enzyme in nitrate assimilation pathway and plays a role as a limiting factor of plant growth and development. NR activity provides an excellent estimation of N_2 amount in plant and is relevant to plant development and yield (SRIVASTAVA 1980). In above observation ZnO-NPs increased the activity of NR at certain concentration (Figure 3*b*). The total content of organic N_2 and head nitrogenous metabolites enhanced quantitatively during higher supply of NO_3^- in plants, this is reflected on increased plant growth and development (BOSE and SRIVASTAVA 2001). It is hypothesized that ZnO-NPs enhanced the activity of NR enzyme and NO_3^- uptake, this leads to higher growth and yield of the plant (Figure 5). Alongthis, the increase in chlorophyll content and CA activity by the application of ZnO-NPs enhanced the rate of photosynthesis, which finally increased the overall growth to the plants (Figure 5).

Plant yield depends on the ability of plant to adapt various types of environmental adversities, which mostly cause oxidative stress. Environmental stresses stimulate the formation of reactive oxygen species (ROS) in plant cell and cause dangerous oxidative damage in plants, which ultimately inhibit the growth and development of the plant (CAVERZAN et al. 2016). To overcome the production of ROS, plant produces several enzymes i.e. CAT, POX and SOD (CAVERZAN et al. 2016). However, application of ZnO-NPs considerably increased the formation of antioxidant

enzymes (Figure 3*c–e*) as compare to the non-treated plants. It is believed that increased activity of antioxidant enzymes is due to Zn, which maintain the protein and biomembranes stability which balance the production of scavenging ROS (KHAN et al. 1998). In stressed condition plant accumulate compatible solute i.e. proline. This solute is highly soluble and non-toxic. Proline provides protection against stress by maintaining cellular osmotic adjustment. ZnO-NPs increase the accumulation of proline in plant cell (Figure 3*f*) by conversion of glutamate into proline. It is hypothesized that ZnO-NPs increased the phosphorylation of glutamate, and this glutamate reduced into glutamic-5-semialdehyde (GSA) through the enzyme D1-pyrroline-5-carboxylate synthetase (P5CS), and randomly catalyzed into pyrroline-5-carboxylate (P5C). This P5C finally reduced into proline (Figure 5). Protein protects the plant cell from potential oxidative damage. Application of ZnO-NPs in tomato plants enhanced the content of protein through the activation of transcriptional and/or translational processes (Figure 4*a*). These results are lined with the earlier findings of RALIYA and TARAFDAR (2013) and MUKHERJEE et al. (2016), where treatment of ZnO-NPs increases the protein content in cluster bean and green pea respectively.

ZnO-NPs treated plants had higher level of lycopene and *β*-carotene in the mature fruits, over their respective controls (Figure 4*d–e*) and 50 ppm of ZnO-NPs proved best. Unlike lycopene and *β*-carotene the treatment (50 ppm) significantly decreased the ascorbic acid content in the fruits. It assumes that the increasement in lycopene and *β*-carotene is due to the ethylene-mediated alteration. A similar observation was found (KOLE et al. 2013) in bitter melon, in which they found an increase of 82% lycopene by carbon based fullerol NPs. Number of fruits and fruit yield elevated by the treatments of ZnO-NPs (Figure 4 *b–c*). The best concentration was noted to be the feeding of 50 ppm. The fruit bearing capacity of the plants is mainly determined by the density of the flowers retained in the plant (ZHAO et al. 1987). Therefore, higher yield of plants could be due to the better flowering and fruit development and improved photosynthetic rate (Figure 5).

Conclusion

The present study provides some important clues about the physiological role of ZnO-NPs in plants. NPs promote the photosynthetic rate and antioxident system of tomato plants at different concentrations. Out of the concentrations tested (0, 10, 50, 100 and 200 ppm); 50 ppm ZnO-NPs

increased the efficiency of photosynthesis and enhanced the antioxidant system of the tomato plants more significantly. Beyond this concentration, response was not very promising. The highest concentration tested (200 ppm ZnO-NPs) had an adverse effect on growth of tomato plants, and therefore should be considered as a toxic concentration. Consequently, it is concluded that 50 ppm ZnO-NPs is the preferred concentration to be used for enhancement of plant growth and development.

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References

ALLOWAY B.J. 2004. *In zinc in soil and crop nutrition. International Zinc Association*. Brussels, Belgium.

AN J., ZHANG M., WANG S., TANG A. 2008. *Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles*. PVP, LWT, Food Sci. Tech., 41: 1100–1107.

ANDRE C.M., LARONDELLE Y., EVERS D. 2010. *Dietary antioxidants and oxidative stress from a human and plant perspective: a review*. Curr. Nutri. Food Sci., 6: 2–12.

BATES L.S., WALDEEN R.P., TEARE I.D. 1973. *Rapid determination of free proline water stress studies*. Plant Soil, 39: 205–207.

BATSMANOVA L.M., GONCHAR L.M., TARAN N.Y., OKANENKO A.A. 2013. *Using a colloidal solution of metal nanoparticles as micronutrient fertiliser for cereals. Proceedings of the International Conference on Nanomaterials. Applications and properties, September Crimea*, Ukraine.

BEAUCHAMP C.O., FRIDOVICH I. 1971. *Superoxide dismutase: improved assays and assays applicable to acrylamide gels*. Ann. of clin. Biochem., 44: 276–287.

BERNHARDT E.S., COLMAN B.P., HOCHELLA M.F., CARDINALE B.j., NISBET R.M., RICHARDSON C.J., YIN L. 2010. *An ecological perspective on nanomaterial impacts in the environment*. J. Environ. Qual., 39: 1–12.

BONDARENKO O., JUGANSON K., IVASK A., KASEMETS K., MORTIMER M., KAHRU A. 2013. *Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review*. Arch. Toxicol., 87(7): 1181–1200.

BOSE B., SRIVASTAVA H.S. 2001. *Absorption and accumulation of nitrate in plants. Influence of environmental factors*. Ind. J. Exp. Biol., 39: 101–110.

BRADFORD M. 1976. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding*. Ann. clin. Biochem., 72: 248–254.

BURKLEW C.E., ASHLOCK J., WINFREY W.B., ZHANG B. 2012. *Effects of aluminum oxide nanoparticles on the growth, development, and microRNA expression of tobacco* (*Nicotiana tabacum*). *PLoS ONE7*.

CAKMAK I. 2000. *Role of zinc in protecting plant cells from reactive oxygen species*. New Phytol., 146: 185–205.

CAVERZAN A., CASASSOLA A., BRAMMER S.P. 2016. *Antioxidant responses of wheat plants under stress*. Genet. Mol. Biol., 39(1): 1–6.

CHANCE B., MAEHLY A.C. 1956. *Assay of catalase and peroxidases*. Methods Enzymol., 2: 764–775.
CONNOLLY M., FERNANDEZ M., CONDE E., TORRENT F., NAVAS J.M., FERNANDEZ-CRUZ M.L. 2016. *Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout*. Sci. Total Environ., 551–552, 334–343.
DWIVEDI R.S., RANDHAWA N.S. 1974. *Evaluation of rapid test for hidden hunger of zinc in plants*. Plant Soil, 40: 445–451.
FABREGA J., LUOMA S.N., TYLER C.R., GALLOWAY T.S., LEAD J.R. 2011. *Silver nanoparticles: behaviour and effects in the aquatic environment*. Environ. Int., 37: 517–531.
FAIZAN M., FARAZ A., YUSUF M., KHAN S.T., HAYAT S. 2018. *Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants*. Photosynthetica, 56(2): 678–686.
GARCIA-SANCHEZ S., BERNALES I., CRISTOBAL S. 2015. *Early response to nanoparticles in the Arabidopsis transcriptome compromises plant defence and root-hair development through salicylic acid signalling. BMC Genom,* 16: 341.
JAWORSKI E.G. 1971. *Nitrate reductase assay in intact plant tissues*. Biochem. Biophy. Res. Comm., 43: 1274–9.
KHAN J., BRENNAND D.M., BRADLEY N., GAO B., BRUCKDORFER R., JACOBS M. 1998. *3 Nitrotyrosine in the proteins of human plasma determined by an ELISA method*. J. Biochem., 330: 795–801.
KHAN S.T., AHMAD J., AHAMED M., MUSARRAT J., AL-KHEDHAIRY A.A. 2016. *Zinc oxide and titanium dioxide nanoparticles induce oxidative stress, inhibit growth and attenuate biofilm formation activity of Streptococcus mitis*. J. Biol. Inorg. Chem., 21: 295–303.
KHODAKOVSKAYA M.V., DE SILVA K., BIRIS A.S., DERVISHI E., VILLAGARCIA H. 2012. *Carbon nanotubes induce growth enhancement to tobacco cells*. ACS Nano, 6: 2128–2135.
KOLE P., RANDUNU K.M., CHOUDHARY P., PODILA R., CHUN K.P., RAO A.M., MARCUS R.K. 2013. *Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon* (*Momordica charantia*). BMC Biotechn., 13–37.
KUREPA J., PAUNESKU T., VOGT S., ARORA H., RABATIC B. M., LU J. 2010. *Uptake and distribution of ultrasmall anatase TiO_2 Alizarin red S nanoconjugates in Arabidopsis thaliana. Nano Lett.,* 10: 2296–2302.
MARSCHNER H. 1993. *Zinc uptake from soil*. In: *Zinc in soils and plants*. Ed. A.D. Robson. Kluwer Academic Publishers, Dordrecht, pp. 59–79.
MONICA R.C., CREMONINI R. 2009. *Nanoparticles and higher plants*. Caryol., 62: 161–165.
MUKHERJEE S., CHOWDHURY D., KOTCHERLAKOTA R., PATRA S.B.V., BHADRA M.P., SREEDHAR B., PATRA C.R. 2014. *Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system)*. Theranos., 4: 316e335.
MUKHERJEE A., SUN Y., MORELIUS E., TAMEZ C., BANDYOPADHYAY S., NIU G., WHITE J.C., PERALTA-VIDEA J.R., GARDEA-TORRESDEY J.L. 2016. *Differential toxicity of bare and hybrid ZnO nanoparticles in green pea (Pisum sativum L.). A Life Cycle Study*. Front. Plant Sci., 6: 1242.
NOJI T., KAMIDAKI C., KAWAKAMI K., SHEN J.R., KAJINO T., FUKUSHIMA Y., SEKITOH T., ITOH S. 2011. *Photosynthetic oxygen evolution in mesoporous silica material adsorption of photosystem II reaction center complex into 23 nm nanopores in SBA*. Lang., 27: 705–713.
Pandey R.R., Saini K.K., Dhayal M. 2010. *Using nano-arrayed structures in sol-gel derived Mn^{2+} doped TiO_2 for high sensitivity urea biosensor*. J. Biosen & Bioelect., 2155: 6210.
PENG Y.H., TSAI Y.C., HSIUNG C.E., LIN Y.H., SHIH Y. 2017. *Influence of water chemistry on the environmental behaviors of commercial ZnO nanoparticles in various water and wastewater samples*. J. Hazard. Mater., 322: 348–356.
PRASAD T.N.V.K.V., SUDHAKAR P., SREENIVASULU Y., LATHA P., MUNASWAMY V., REDDY, K.R., SREEPRASAD T.S.P., SAJANLAL R., PRADEEP T. 2012. *Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut*. J. Plant Nutri., 35: 905–927.
RAGHURAMULU N., NAIR M.K., KALYANASUNDARUM S. 1983. *A Manual of laboratory techniques*. National Institute of Nutrition, Silver Prints Hyderabad.

RALIYA R., TARAFDAR J.C. 2013. *ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in clusterbean (Cyamopsis tetragonoloba L.)*. Agric. Res., 2(1): 48–57.

RAMESH R., TARAFDAR J.C. 2014. *ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in clusterbean (Cyamopsis tetragonoloba L.)*. Agric. Res., 2: 48–57.

RANGANNA S., 1976. *Manual of analysis of fruit and vegetable products*. McGraw Hill, New Delhi, pp. 77.

RASKAR S.V., LAWARE S.L. 2014. *Effect of zinc oxide nanoparticles on cytology and seed germination in onion*. Int. J. Curr. Micr. Appl. Sci., 3: 467–473.

RAY P.D., HUANG BO-WEN, TSUJI Y. 2012. *Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling*. Cell Signal., 24(5): 981–990.

RITTNER M.N., ABRAHAM T. 1998. *Nanostructed materials: an overview and commericial analysis*. Int. J. f Min., Met, Mat., 50: 37–38.

SADASIVAM S., MANICKAM A. 1997. *Carotenes*. In: *Biochemical methods*. New Age International Publishers, New Delhi, pp. 187–188.

SCHWAB F., ZHAI G., KERN M., TURNER A., SCHNOOR J.L., WIESNER M.R. 2015. *Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants*. Critical review Nanotox., 10: 257–278.

SCOTT N., CHEN H. 2002. *Nanoscale science and engineering for agriculture and food systems*. National Planning Workshop November 18–19 Washington DC.

SEDGHI M., HADI M., TOLUIE S.G. 2013. *Effect of nano zinc oxide on the germination of soybean seeds under drought stress*. Ann. West Uni Timisoara ser. Biol., 2: 73–78.

SEWELAM N., KAZAN K., SCHENK P.M. 2016. *Global plant stress signaling: reactive oxygen species at the cross-road*. Front. Plant Sci., 7: 187.

SIDDIQUI M.H., AL-WHAIBI M.H., FAISAL M., AL SAHLI A.A. 2014. *Nanosilicon dioxide mitigates the adverse effects of salt stress on Cucurbita pepo L.* Environ. Toxicol. Chem., 33: 2429–2437.

SIRELKHATIM A., SHAHROM M., AZMAN S., NOOR H.M.K., CHUO A.L., SITI K.M. B., HABSAH H., DASMAWATI M. 2015. *Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism*. Micro Nano Lett., 7(3): 219–242.

SRIVASTAVA H.S. 1980. *Regulation of nitrate reductase activity in higher plants*. Phytochem., 19: 725–733.

STAMPOULIS D., SINHA S.K., WHITE J.C. 2009. *Assay-dependent phytotoxicity of nanoparticles to plants*. Environ. Sci. Tech., 43: 9473–9479.

TIWARI S.B., WANG S., HAGEN G., GUILFOYLE T.J. 2005. *Transfection assays with Arabidopsis protoplasts containing integrated reporter genes*. In: Arabidopsis protocols. Eds. J. Salinas, J.J. Sanchez-Serrano. Totowa, NJ: Humana Press (in press).

TRIPATHY B.C., OELMÜLLER R. 2012. *Reactive oxygen species generation and signaling in plants*. Plant Signal. Behav., 7: 1621–1633.

WANG Z., XIE X., ZHAO J., LIU X., FENG W., WHITE J.C. 2012. *Xylem- and phloem-based transport of CuO nanoparticles in maize (Zea mays L.)*. Environ. Sci. Techn., 46: 4434–4441.

XIE Y., LI B., ZHANG Q., ZHANG C. 2012. *Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of Indocalamus barbatus McClure*. J. Nanjing Forest Univ. (Natural Science Edition), 2: 59–63.

ZHAO L.J., PERALTA-VIDEA J.R., REN M.H., VARELA-RAMIREZ A., LI, C.Q., HERNANDEZ-VIEZCAS J.A. 2012. *Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: electron microprobe and confocal microscopy studies*. J. Chem. Engin., 184: 1–8.

ZHAO Y.J., LUO W.H., WANG Y.Q., XU R.J. 1987. *Retarding effects of brassinolide on maturation and senescence of hypocotyls segments of mung bean seedlings*. Acta Physiol. Sinica, 13: 129–135.

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EVALUATION OF THE SENSITIVITY OF SELECTED PATHOGENIC FUNGI TO HERBAL ADDITIVES AND THE PRESENCE OF AN ANTAGONISTIC FUNGUS IN *IN VITRO* CONDITION

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Key words: biological methods of plant protection, *Trichoderma viride,* cinnamon.

Abstract

In the counteract pathogenic fungi, alternative methods of plant protection are becoming more and more important, using both antagonistic microorganisms and vegetable active biological substances. The purpose of the work was to determine the effect of dried three plants on the growth and development of 8 species of phytopathogenic fungi, economically important (*Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. oxysporum, F. solani, Monilinia fructigena, Sclerotinia sclerotiorum, Trichothecium roseum*). For fungi cultures, the method of poisoned substrates was used, in which macerates of plants in a concentration of 2% and 5% were added to the glucose-potato substrate (PDA). The experiment was carried out for two weeks in duplicate. The growth of mycelium and the ability to form spores were assessed. The research shows that plant additives have a different degree of impact on the studied phytopathogens. The most effective turned out to be cinnamon, which completely inhibited the growth of all the fungi examined. The presence of the antagonistic fungus *Trichoderma viride* has definitely influenced the development of the studied fungi. In all cases, the rapid growth of *T. viride* mycelium was noted and inhibition of phytopathogenic fungal growth.

Introduction

In modern agriculture, according to the integrated plant protection in force since 2014, in the fight against phytopathogens, biological methods of plant protection are becoming increasingly important. These methods use natural antagonistic mechanisms between microorganisms: antibio-

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sis, competition and parasitism, as well as phytoncides present in plants. Therefore, research on alternative and effective plant protection agents focuses on the search for antagonistic microorganisms secreting antibiotic substances, volatile compounds and lytic enzymes, as well as plant resistance inducing agents (SANTOS and MARQUINA 2004, EL-TARABILYA and SIVASITHAMPARAMB 2006, MATYJASZCZYK and SOBCZAK 2011, WALKOWIAK and KRZYŚKO-ŁUPICKA 2014), thus inhibiting the growth of pathogenic microorganisms. Important biological agents with antibiotic, competitiveness and parasitic abilities include Trichoderma (KASHYAP et al. 2017). Numerous studies prove different efficacy of these fungi (*T. harzianum* Rifai*, T. koningii* Oudem.*, T. viride* Pers.) in relation to numerous phytopathogens (WOJTKOWIAK-GĘBAROWSKA 2006). Therefore, biopreparations are available on the market based on *Trichoderma* spp. (BRIAN and HEMMING 2008), which can be successfully used, among others in greenhouses, in crops under covers, conducted using conventional methods as well as in organic crops. Recent studies show on this subject is also focused on substances of plant origin (KRĘCIDŁO and KRZYŚKO-ŁUPICKA 2017). The compounds contained in various species of plants – phytoncides, have been known for a long time as a natural passive chemical defense against phytopathogens (KOZŁOWSKA 2007). Numerous of them with fungistatic properties have been used to develop biological agents, such as Biosept 33 based on grapefruit seed extract or Bioczos based on garlic (SZOPIŃSKA et. al. 2007, MARJANSKA-CICHON and SAPIEHA-WASZKIEWICZ 2010, ZYDLIK 2008). However, new, effective plant compounds are still being sought for acting against numerous dangerous pathogenic fungi.

The aim of the work was to determine the effect of three plant additives with different concentrations, as well as the influence of the *Trichoderma viride* saprotrophic fungus on the growth and development of several species of phytopathogenic fungi, economically important.

Material and Methods

Eight fungal species were selected for the study: *Botrytis cinerea* (Pers.)*, Fusarium avenaceum* (Fr.) Sacc.*, F. culmorum* (W.G. Sm.) Sacc.*, F. oxysporum* (Schltdl)*, F. solani* (Mart.) Sacc.*, Monilinia fructigena* (Honey)*, Sclerotinia sclerotiorum* (Lib.) de Bary and *Trichothecium roseum* (Pers.) Link. The strains were isolated from diseased crop plants (pumpkin, apple, carrot, wheat, vines). For the isolation of fungi the method of moist chambers was used, to which sick parts of plants were laid out. After 24h, in order to obtain pure cultures, fungal isolates were passaged onto

standard PDA medium used in phytopathological diagnostics (MARCINKOWSKA 2003). In order to carry out the experiment, cultures were carried out in petri dishes using the poisoned substrate method. The dry mass of three plant species: mint (*Mentha*), cinnamon (*Cinnamomum*) and English herb (*Pimenta dioica*), were added to 15 ml of liquid PDA medium in two variants:

– 2% concentration: the substrate PDA prepared in 3 flasks was successively added mint, cinnamon, allspice (2 g of dry matter/100 ml of medium);

– 5% concentration: the substrate PDA prepared in 3 flasks was successively added mint, cinnamon, allspice (5 g of dry matter/100 ml of medium).

The experiment was carried out in a few repetitions and in 2 variants.

Dry mass were obtained by grinding the dried parts of plants in a sterile mortar until fine dust was obtained. The control sample consisted of isolates of the tested fungal species sown on a PDA medium without herbal additives. In order to determine the effect of saprotrophic *Trichoderma viride*, the tested species of phytopathogenic fungus and *T. viride* were placed next to each other on Petri dishes with PDA substrate. The Petri dish with isolates was sealed with parafilm. The experiment was carried out for two weeks, in duplicate, at room temperature of 22°C. During daily observations, the diameter of the mycelium was measured and the time of spore formation was recorded. For this purpose, preparations from Gerlach's culture were made. Under sterile conditions, pieces of adhesive tape with a length of about 3 cm were prepared and imprints of grown mushroom colonies were made. This material was transferred to the primary slide, with the adhesive side up, a drop of blue with lactofenol applied and covered with a coverslip. The preparations were viewed under an OLYMPUS Bx4 optical microscope.

On the basis of the obtained results, the percentage of mycelial growth inhibition was calculated according to the formula:

$$\text{pzw} = \frac{K_0 - F}{K_0} \cdot 100$$

where :
pzw – percent inhibition of growth;
K_0 – diameter of the culture in the control combination (culture covering the whole pan);
F – diameter in combination with plant additive.

The given values were averaged.

Results and Discussions

Different effects of plant additives on the development of the fungi studied were found. In all cases, total inhibition of mycelial growth was noted by the addition of cinnamon at a concentration of 2% and 5% (Table 1).

Table 1

Percentage of growth inhibition (pzw) of the fungi examined on the substrate with plant additives

Herbal additives / Species of fungi	Cinnamon		English herb		Mint	
	2%	5%	2%	5%	2%	5%
B. cinerea	100%	100%	55%	100%	77%	100%
F. avenaceum	100%	100%	68%	100%	2%	55%
F. culmorum	100%	100%	66%	100%	44%	10%
F. oxysporum	100%	100%	44%	81%	1%	50%
F. solani	100%	100%	31%	81%	22%	44%
M. fructigena	100%	100%	66%	100%	83%	100%
S. sclerotiorum	100%	100%	77%	66%	44%	55%
T. roseum	100%	100%	44%	77%	33%	66%

Available literature indicates that the ground cinnamon bark is fungistatic and bactericidal. It results from the oil substances contained in it – mainly cinnamon oil (ZOHREH et. al. 2011, PIEKUTOWSKA 2017), which includes cinnamaldehyde. Research conducted in 2005, by JHAM et. al., showed that cinnamaldehyde is the main fungicide of cinnamon bark, while other components addively or synergistically affect the total fungisticity of cinnamon. Similar results were obtained by other scientists testing the effectiveness of cinnamon bark extracts in inhibiting the growth of fungi of the genus *Fusarium*. The largest inhibitory effect, *in vitro* and *in vivo*, using cinnamon was recorded by EL-MOUGY and ABDEL-KADER (2007).

The addition of English herb (5%) to four fungal species was also successful: *Botrytis cinerea, Fusarium avenaceum, Fusarium culmorum* and *Monilinia fructigena*. The most important phytoncide contained in fruits of English herb is essential oil, which consists of: eugenol (60–80%), methyl-eugenol (20–30%), feldenne, cineole, cariophylene and palmitic acid known for its antimicrobial and antifungal properties (CZERWIŃSKA and PIOTROWSKI 2005, BERTHOLD-PLUTA and KURZYŃSKA 2010).

The weakest fungistatic properties were observed in the case of mint, the addition of which (5%) inhibited the growth of only *B. cinerea* and *M. fructigena* (Figure 1, Figure 2). Mint leaves contain about 3% essential oil, tannins, phenolic acids, triterpenes, carotenoids and flavonoids.

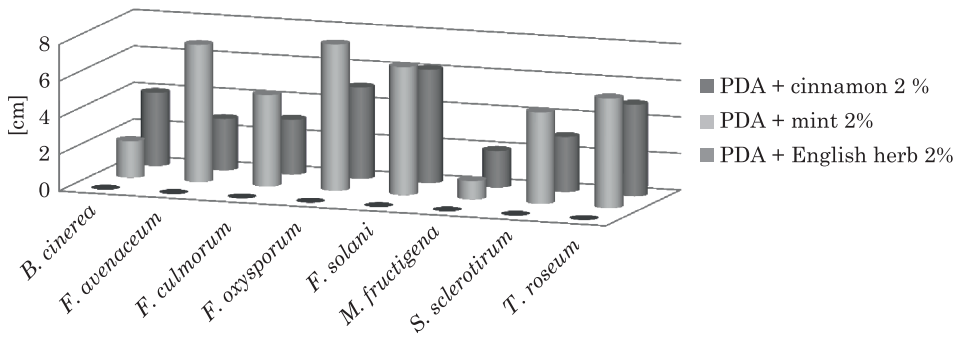

Fig. 1 Effect of vegetable additives (2%) on PDA substrate for mycelium development

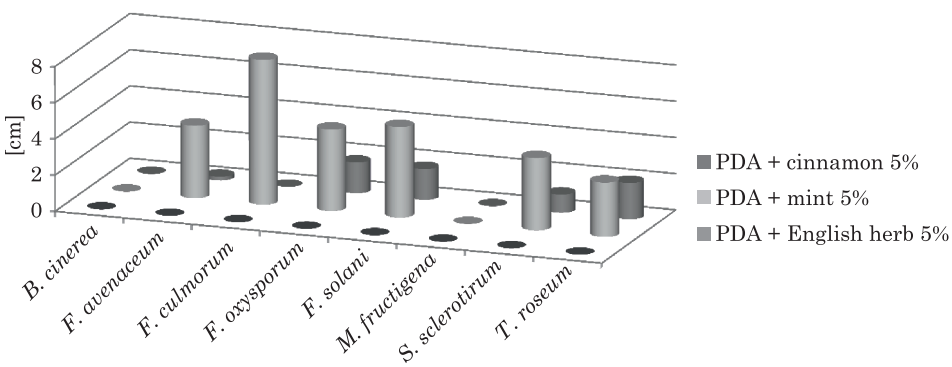

Fig. 2. Effect of vegetable additives (5%) on PDA substrate for mycelium development

Contained in the essential oil – menthol, has strong antimicrobial properties. Some authors claim that this oil affects the growth of mycelia of yeast-like and mold fungi (KUSIAK et. al. 2010), and also inhibits the production of mycotoxins, e.g. ochratoxin A by *Aspergillus parasiticus* (FERDES and UNGUREANU 2012). Studies carried out by FERDES and UNGUREANU (2012) showed a strong effect of peppermint oil on inhibiting the growth of *F. oxysporum* mycelium, which was not observed in our own studies. This is probably due to the fact that FERDES and UNGUREANU (2012) used a pure, extracted peppermint oil in which the concentration of active compounds was very high, which had an effect on inhibiting the growth of the *Fusarium* fungi tested. However, the higher concentration

of dried mint leaves added to the medium increased the degree of mycelial growth inhibition of the phytopathogens tested. EJECHI et. al. (1997) also drew a similar conclusion, stating the effectiveness of biopreparations with increasing their concentration.

The antagonistic effect of *Trichoderma viride* on the development of the fungi studied was found. The largest was demonstrated in the case of *F. solani*, whose diameter of the mycelium was only 1 cm (pzw = 88%). In the remaining species, the percentage of mycelial growth inhibition ranged from 66% to 82%. The exception was *F. culmorum* mushroom, which in the presence of *T. viride* developed abundant mycelium (pzw = 44%) – Figure 3.

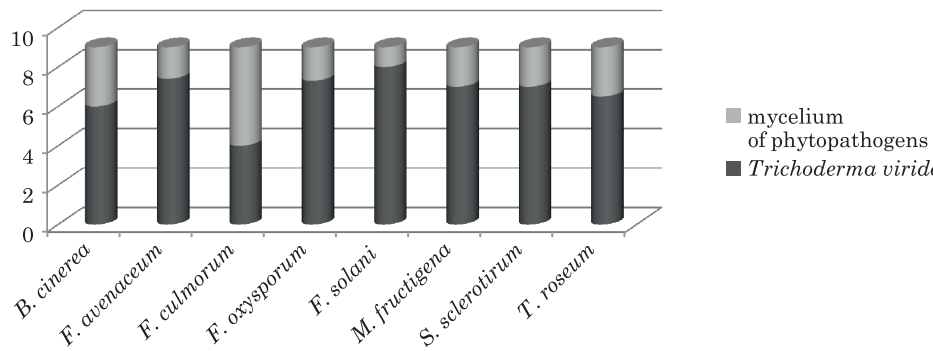

Fig. 3. Effect of *Trichoderma viride* on PDA substrate for fungal growth

Trichoderma viride is a rhizosphere species. Its antagonistic effect on pathogens results from aggressive competition for nutrients and space, as well as from the production of various active substances that inhibit the growth of other pathogens. These substances include lytic enzymes that interact with antibiotics (WOJTKOWIAK-GĘBAROWSKA 2006).

An inhibition of spore growth was observed in 3 examined fungal species. The addition of 5% English herb delayed the formation of *Fusarium solani* spores by 6 days and at *F. oxysporum* by 3 days compared to the control. In the case of the addition of 5% mint, the delay in spore formation was noted in *M. fructigena*, whereas in *F. avenaceum* no fungal spores were observed in the presence of this plant supplement. In the remaining species studied, forming conidial spores, their formation was not inhibited compared to the control.

Conclusions

Summing up, the results obtained in the conducted study indicate the different properties of fungistatic compounds contained in plants. The most effective was the addition of cinnamon, which already at a concentration of 2% completely inhibited the growth of mycelium of the tested isolates. Further research should be directed to the use of this herbal supplement as an available biological agent in the protection of plants against phytopathogens. *T. viride* significantly reduced the growth of mycelium of all tested fungi, with the exception of *F. culmorum*.

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References

BERTHOLD-PLUTA A., KURZYŃSKA D. 2010. *Jakość mikrobiologiczna rynkowych przypraw i ziół.* Postęp Tech. Przetw. Spoż., 2: 42–46.

BRIAN P.W., HEMMING H.G. 2008. *The effect of maleic hydrazide on the growth response of plants to gibberellic acid.* Annals of Applied Biology, 45(3): 489–497.

CZERWIŃSKA E., PIOTROWSKI W. 2005. *Czystość mikrobiologiczna przypraw i ich aktywność bakteriostatyczna*. Zeszyty Naukowe Wydziału Budownictwa i Inżynierii Środowiska, nr 22, Seria Inżynieria Środowiska, Wydawnictwo Politechniki Koszalińskiej, pp. 253–263.

EJECHI B.O., OJEATA A., OYELEKE S.B. 1997. *The effect of extracts of some Nigerian spices biodeterioration of Okro (Abelmoschus (L) Moench) by fungi*. J. Phytopathol., 145(11/12): 469–472.

EL-MOUGY S.N., ABDEL-KADER M.M. 2007. *Antifungal effect of powdered spices and their extracts on growth and activity of some fungi in relation to damping-off disease control*. Journal of Plant Protection Research, 47(3): 267–278.

EL-TARABILYA K.A., SIVASITHAMPARAMB K. 2006. *Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters*. Soil Biol. Biochem., 38: 1505–1520.

FERDES M., UNGUREANU C. 2012. *Antimicrobial activity of Essentials oils against four food-borne fungal strains*. U.P.B. Sci. Bull., ser. B, 74(2): 87–98.

JHAM N.G., DHINGRA D.O., JARDIM M.C., VALENTE M.M.V. 2005. *Identification of the major fungitoxic komponent of cinnamon bark oil*. Fitopatol. Bras. [online], 30(4): 404–408.

KASHYAP P.L., RAI P., SRIVASTARA A.K., KUMAR S. 2017. *Trichoderma for climate resilient agriculture*. World Journal of Microbiology and Biotechnology, 33: 155.

KOZŁOWSKA M. 2007. *Fizjologia roślin. Od teorii do nauk stosowanych*. Wyd. PWRiL, Poznań.

KRĘCIDŁO M., KRZYŚKO-ŁUPICKA T. 2017. *Wpływ środka dezynfekcyjnego Divosan Forte na przyrost biomasy środowiskowego szczepu Trichoderma viride*. Proceedings of ECOpole, 11(1): 193–199.

KUSIAK A., KĘDZIA A., MOLĘDA-CISZEWSKA B., KĘDZIA W.A., MACIEJEWSKA K., WŁODARKIEWICZ A., KWAPISZ E. 2010. *Działanie olejku z mięty pieprzowej na bakterie beztlenowe*. Dent. Med. Probl., 47(3): 334–338.

MARCINKOWSKA J. 2003. *Oznaczanie rodzajów grzybów ważnych w patologii roślin*. Fundacja „Rozwój SGGW".

MARJANSKA-CICHON B., SAPIEHA-WASZKIEWICZ A. 2010. *Skuteczność preparatów biotechnicznych opartych na ekstrakcie z czosnku w zwalczaniu szarej pleśni na truskawce*. Progress in Plant Protection, 50(1): 378–382.

MATYJASZCZYK E., SOBCZAK J. 2011. *Środki ochrony roślin zawierające substancje aktywne pochodzenia naturalnego*. Bydgoskie Towarzystwo Naukowe, pp. 307–310.

PIEKUTOWSKA M. 2017. *Potencjał naturalnych preparatów pochodzenia roślinnego dla poprawy zdrowotności i żywotności materiału siewnego roślin rolniczych [Potential of natural plant extracts for health and vigour improvement of seed material of agricultural crops]*. Problemy Drobnych Gospodarstw Rolnych – Problems of Small Agricultural Holdings, 3: 43–59. doi: http:// dx.doi.org/10.15576/PDGR/2017.3.43

SANTOS A., MARQUINA D. 2004. *Killer toxin of Pichia membranifaciens and its possible use as a biocontrol agent against grey mould disease of grapevine*. Microbiology, 150: 2527–2534.

SZOPIŃSKA D., DORNA H., TYLKOWSKA K. 2007. *The effects of grapefruit extract on germination, vigour and health of cabbage, onion and zinnia seeds*. Roczniki Akademii Rolniczej w Poznaniu – CCCLXXXIII, Ogrodn., 41: 631–636.

WALKOWIAK W., KRZYŚKO-ŁUPICKA T. 2014. *Nowe rozwiązania w ochronie zbóż przed fuzariozami*. Progress in Plant Protection, 54(2): 127–134.

WOJTKOWIAK-GĘBAROWSKA E. 2006. *Mechanizmy zwalczania fitopatogenów glebowych przez grzyby z rodzaju Trichoderma*. Post. Mikrobiol., 45(4): 261–273.

ZOHREH D., MOHAMMAD A., MOHAMMAD A., AMIRCHAGHMAGHI M., FALAKI F., MOZAFARI M.P., HAMZEI M.F. 2011. *In vitro comparison of inhibitory activity of 10 plant extracts against Candida albicans*. Australian Journal of Basic and Applied Sciences, 5(5): 930–935.

ZYDLIK P. 2008. *Wykorzystanie preparatów pochodzenia naturalnego w zwalczaniu niektórych chorób roślin sadowniczych*. Nauka Przyr. Technol. Ogrodn., 2(1): 1–6.

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THE FATTY ACIDS COMPOSITION OF SELECTED FISH OILS USED AS DIETARY SUPPLEMENTS

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Key words: EFA, supplementation, DHA, EPA, fish oil.

Abstract

The paper presents the qualitative composition of the fatty acids in the selected fish oil available on the Polish pharmaceutical market. Determination of the fatty acids composition was performed by gas chromatography (GC-FID). The results showed that all the tested fish oil composition contained essential fatty acids (EFA). All tested fish oils were characterized by high content of fatty acids from the group of n-3 and n-6. Differences in the composition of fatty acids in the tested fish oil were observed. The Norwegian fish oil was the richest in n-3 acids, i.e. eicosapentaenoic acid (34.43%) compared to the other samples tested: Icelandic fish oil (7.95%); Scandinavian fish oil (7.01%); Norwegian lemon fish oil (8.34%); kaps fish oil (7.95%); Norwegian fish oil forte (10.90%) and Scandinavian multi-tabs fish oil (6.11%). The highest share of acids from the group EFAs ie. linoleic acid n-6 (6.07%) and α-linolenic acid, n-3 (1.02%) was found in Scandinavian multi-tabs fish oil.

Introduction

Until the end of the 19th century, it was thought that the only and the most important role of fats in the body is the supplying of energy. Fats also have many other important functions, i.e. facilitate the perception of taste and food swallowing; inhibit stomach cramps and secretion of acidic gastric juice; they build cell membranes in the white mass of the brain; protect against excessive heat loss (as subcutaneous fat); stabilize the kidneys and other organs inside the body (as organ fat); provide essential fatty acids, from which tissue hormones regulating processes in the cells of

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various parts of the body are produced; determine the efficiency of the cardiovascular system by increasing the blood flow through the coronary vessels of the heart; affect the condition of the skin and hair and are a carrier of some vitamins (A, D, E, K), facilitating their absorption from food (MARCINIAK-ŁUKASIK and KRYGIER 2004).

An important role in the proper development and functioning of the human body act essential fatty acids (EFAs) from the series n-3 and n-6. They should be provided in the diet because they are not produced by the human body. Among them, the following are considered to be basic: α-linolenic acid (C18:3) from the n-3 family, which is a precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and linoleic acid (C18:2) from the n-6 family as a precursor of arachidonic acid (AA) (MATERAC at al., 2013). The rich source of these acids are fish products, seeds and nuts and fats obtained from them.

n-3 fatty acids

Polyunsaturated fatty acids (PUFA) from the group of n-3 and n-6 are part of the phospholipids of cell membranes and their quantitative relation depends on the proportion in the diet. These acids are released from the phospholipid material for the synthesis of eicosanoids: prostaglandin (PG), prostacyclin (PGI), tromboksants (TXA), leukotrienes (LT). The health effects of EFAs are due in large part, to the effects of the activity of eicosanoids – tissue hormones. EPA and DHA influence on various metabolic effects in the human body. EPA affects the cardiovascular system by the synthesis of eicosanoids. DHA is an important structural component of the nervous tissue, especially the brain and retina cortices. Also it has an important role during development of the nervous system that occurs in early childhood and foetal life. Too low level of DHA in the diet of women may shorten the period of pregnancy, and may lead to low birth weight of the child. Mother's milk is the source of EPA and DHA for young children, and their content depends mainly on diet and ranges from 0.05% to 0.7% and even 1.9% at women which consume large amounts of fish. Scientific reports show that low intake of DHA affects the lower intelligence rate. Declining with age D4 desaturase enzyme activity leads to the inhibition of the synthesis of DHA and of disorders of the central nervous system of the elderly. Therefore, an adequate level of intake of n-3 DHA is especially important for the elderly people. In addition, the beneficial effects of DHA is the prevention of stress, depression and aggression (THIES et al. 2001, COREY et al. 2015, SZPONAR et al. 2007).

Significant action in the human body show the long-chain fatty acid forms (LC PUFA). The beneficial effect of n-3 LC PUFA on the functioning of the human body were observed for the first time in the 1970s. DYERBERG et al. (1975) conducted experiments among Greenland Eskimos have observed a very low incidence of cardiac diseases, psoriasis, cancer, allergy and lack of atherosclerosis in this population compared to the population of Denmark. An important fact is that the Eskimo diet is rich in cholesterol due to the high consumption of fish and marine mammals, and includes few fruits and vegetables, which were considered the main dietary risk factors for cardiovascular disease. Analysis of the results confirmed that the health effects are combined with a high level of n-3 LC PUFA in the diet. The fat fish and marine mammals are particularly rich in n-3 LC PUFA. Despite the health benefits of n-3 acids, their excessive consumption may lead to the development of certain health disorders such as: lengthening the bleeding time and the formation of bruising, blood clotting disorders, and the development of type II diabetes and most of all severity of lipid peroxidation within the body, especially the LDL-cholesterol fraction.

Fortification of food with n-3 acids

N-3 LC PUFA are obtained from fishes in the form of oil. The term "fish oil" is quite extensive, and its composition may vary depending on: the species of fish, their age, time and place of fishing and area of life. It is assumed that all marine fish, in particular fatty fish, have a significant content of n-3 LC PUFA. This includes some predatory species of freshwater fish (eg. from the salmonid family). The most important raw materials for the production of fish oil are: cod (langoustine), herring (menhaden), anchovies, mackerel, tuna and salmon., Two types of fish oil are obtained on an industrial scale. The first one are tissue oils obtained by extrusion of overcooked fish raw material, and the second are obtained from fish livers such as fish cod liver oil. They are the main source of vitamin A and D. Fish oil is used for the production of dietary supplements and is a rich source of squalene and alkylglycerols. Formerly, fish oil was considered as a by-product in the production of fishmeal. In industrial conditions, fish oil is subjected to hydrogenation. This process extends durability and changes its state from liquid to solid. Hydrogenation saturates the double bonds and causes the loss of health-promoting properties of LC PUFA. Fish oil used for the production of supplements and food additives shouldn't be subjected to a hydrogenation process.

International Society for the Study of Fatty Acids and Lipids recommends the consumption of n-3 fatty acids up to 0.65g DHA and EPA acid per day (at least 0.22 g per day). The ratio of n-6 to n-3 in the diet should be 4: 1 (MARCINIAK-ŁUKASIK 2011). Low fish consumption is observed in highly developed countries. For this reason, fish oil rich in long-chain n-3 PUFA is added to food products. Alternative sources are currently being sought of n-3 acids, e.g. from *Crypthecodiniumcohnii* microalgae cultures, which oil contains 40% DHA (with a small amount of other unsaturated fatty acids). This DHA source is called DHASCO. Food and Drug Administration qualified these products as GRAS (Generally Recognized as Safe) (MATERAC et al. 2013).

Food enriched in n-3-acids are produced in South Korea, Japan, European Union USA, Australia and Canada on industrial scale. In 1995 the first products with the addition of EPA and DHA appeared in Europe. The Spain offered a wide range of such products. Mainly used are preparations of n-3 acids to enrich fats for spreading bread, pastas, breakfast cereals, milk desserts, meats, confectionery, cottage cheese, eggs, milk, yogurt, bread, salad dressings, food concentrates, mayonnaise and instant products. In highly developed countries food with n-3 and n-6 acids appeared for pregnant women in the form of bars and for school-aged children (milk and bars). Research is still ongoing on the use of fish oil preparations for production: milk products, fruit juices, oils, sausages, smoked and seasoned products (MASZEWSKA and GAŃKO 2010, COLDER 2001).

Inadequate diet and pace of life causes deficiencies of appropriate nutrients in the human body. An alternative to supplement such deficiencies is supplementation, which of course can't to replace a well-balanced diet. Fish oils contains large amounts of n-3 and n-6 fatty acids as well as fat-soluble vitamins. It has a positive effect on the body, especially on the teeth, eyes, cardiovascular system, immune system and improves concentration. That is why fish oils are recommended by dieticians or doctors in order to supplement everyday menu with valuable active ingredients. Introducing new products rich in n-3 fatty acids may contribute to increasing the share of these acids in the diet (COREY et al. 2015, SZPONAR et al. 2007).

The aim of this study was to evaluate the qualitative composition of fatty acids in selected fish oils available on the Polish pharmaceutical market.

Materials and Methods

Determination of fatty acids

The material for research were 7 selected fish oils available on the Polish market. The characteristics of the trans containing the manufacturer's code (indicated by symbols *A* to *G*), the trade name and declared composition of the most important fatty acids shown in Table 1.

Table 1

Characteristics of selected fish oils

Producent code	The name of fish oils	Declared composition in one capsule (ca. 0.69 g)			
		DHA – docosahexaenoic acid [mg]	EPA – eicosapentaenoic acid [mg]	vit. A [µg]	vit. D [µg]
A	Icelandic fish oil	28	21	110	1.5
B	Scandinavian fish oil	57.5	46.5	120	1.45
C	Norwegian lemon fish oil	30	20	125	5
D	kaps fish oil	110	90	300	2.5
E	Norwegian fish oil	38.5	31.5	105	0.9
F	Norwegian fish oil forte	108	146.5	250	5
G	multi-tabs Scandinavian fish oil	70	50	120	1.25

About 100 mg of fat samples were weighed into glass ampules (20 ml capacity). A volume of 0.1 ml of hexane solution of internal standard (heptadecanoic acid – 10 mg ml^{-1}) was added to the extract (KOWALSKI 2007). Fat saponification and fatty acid esterification (with a 14% solution of BF3 in methanol) were performed in accordance to previously described procedures (*Animal and vegetable…* PN-EN ISO 12966-1:2015-01, *Animal and vegetable…* PN-EN ISO 12966-2:2011, KOWALSKI 2009).

GC was performed with Varian GC 450 gas chromatograph equipped with a flame-ionization detector FID. The fatty acids methyl esters were separated on 30 m × 0.32 mm × 0.25 µm film of Select^{TM} Biodiesel for FAME. A temperature gradient was applied (200ºC for 10 min, then incremented by 3ºC/min to 240ºC, 240ºC for 5 min) The injection port and detector temperatures were 250ºC and 300ºC; split ratio 1:50; Flow: carrier gas (helium) – 28 ml $min.^{-1}$, detector supply (hydrogen) – 30 ml $min.^{-1}$, detector supply (synthetic air) – 300 ml $min.^{-1}$; flow rates have been adjusted so that the ratio of gas flows (column + carrier gas): (detector supply): (air) was 1:1:10.

Quantitative analysis was performed with the method of internal normalisation, assuming that the sum of surface areas of peaks was 100%.

All tests were performed in triplicate.

Data were analyzed by analysis of variance (Duncan's test) at 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.).

Results and Discussion

Table 2 presents the results of the content of selected fatty acids in the tested fish oils.

The Figure 1 shows the percentage of n-3 and n-6 acids in the tested fish oils.

It has been shown that all of the tested fish oils in the composition contained a significant proportion of EFA with linoleic acid LA and α-linolenic ALA. The highest content of these fatty acids as compared to other characterized Scandinavian fish oil "G", respectively: LA acid – 6.07% and ALA – 1.02% acid. In contrast, the lowest content of these fatty acids was observed in Norwegian fish oil "F" i.e. LA – 1.28% and ALA – 1.11%. The remaining fish oils were demonstrated the following concentrations of LA and ALA respectively:

- Icelandic fish oil (*A*) – 2.57% and 1.06%;
- Scandinavian fish oil (*B*) – 1.6% and 1.03%;
- Norwegian lemon fish oil (*C*) – 2% and 1,01%;
- kaps fish oil (*D*) – 3.45% and 1.41%;
- Norwegian fish oil (*E*) – 3.18% and 1.37%.

GRELA and DUDEK (2007), confirmed that LA was dominant in the group of n-6 fatty acids in the meat of selected marine fish species. BAKES and NICHOLS (1995) showed in fish oil from shark liver oil the presence of LA in the share of 1.4%, while there no ALA acid was found. In fish oil, the percentage of LA was 1.43% and ALA 0.17% (GUIL-GUERRERO and BELARBI 2001). In other studies, no ALA was found and LA concentration was 1.6% (THORSTEINN et al. 2016). For comparison, in vegetable oils, ie., soybean, corn, sunflower, grape seed is from 55.07 to 65.90% linoleic acid (CICHOSZ and CZECZOT 2011). The above results show that oils obtained from fish are not a good source of these acids in comparison to vegetable oils.

The test fish oils are characterized by a high proportion of oleic acid, i.e. from 13.84% for *F* to "26.67" for *A*. The fishes are a source of oleic acid, for example 9.11% was found in carp fat and 8.89% in salmon fat, 6.23% in salmon fat, 11.6% in herring oil (ŁUCZYŃSKA et al. 2011). GRELA et al. (2010)

Table 2

Profile of selected fatty acids in the tested fish oils

Participation [%] (±SD)							
Fatty acid	*A**	*B*	*C*	*D*	*E*	*F*	*G*
C14:0 myristic acid	4.98 ± 0.44*b*	4.61 ± 0.07*bc*	4.49 ± 0.31*bc*	5.68 ± 0.64*a*	4.67 ± 0.18*bc*	3.52 ± 0.27*d*	4.20 ± 0.42*c*
C16:0 palmitic acid	12.99 ± 1.15*b*	11.72 ± 0.13*cd*	10.95 ± 0.59*d*	15.39 ± 1.18*a*	13.92 ± 0.38*b*	8.32 ± 0.31*e*	12.91 ± 0.18*bc*
C16:1 palmitooleic acid	9.72 ± 0.76*b*	8.63 ± 0.08*c*	10.70 ± 0.65*a*	8.59 ± 0.72*c*	7.56 ± 0.19*d*	4.63 ± 0.43*e*	8.52 ± 0.47*c*
C18:0 stearic acid	2.74 ± 1.39*cd*	1.75 ± 0.11*de*	1.65 ± 0.09*e*	2.28 ± 0.06*cde*	3.00 ± 0.19*bc*	3.79±0.14*ab*	4.57 ± 0.50*a*
C18:1 n9c oleic acid	26.67 ± 1.71*a*	25.35 ± 0.24*ab*	25.72 ± 1.22*ab*	21.94 ± 1.55*c*	24.59 ± 0.56*b*	13.84 ± 0.07*d*	25.54 ± 0.40*ab*
C18:2 n6c linoleic acid	2.57 ± 1.63*bcd*	1.60 ± 0.06*d*	2.00 ± 0.10*cd*	3.45 ± 0.24*b*	3.18 ± 0.12*bc*	1.28 ± 0.09*d*	6.07 ± 0.91*a*
C18:3 n3 (alpha) alpha-linolenic acid	1.06±0.07*b*	1.03±0.01*b*	1.01±0.05*b*	1.41±0.09*a*	1.37±0.06*a*	1.11±0.08*b*	1.02±0.07*b*
C18:3 n6 (gamma) gamma-linolenic acid	2.38 ± 0.15*bc*	1.99 ± 0.05*d*	2.51 ± 0.16*b*	2.41 ± 0.14*bc*	2.61 ± 0.09*b*	2.09 ± 0.28*cd*	3.55 ± 0.35*a*
C20:1 eicosenoic acid	**14.79 ± 0.57*a***	**13.92 ± 0.25*b***	**14.38 ± 0.51*ab***	**11.36 ± 0.70*c***	**8.98 ± 0.20*d***	**2.83 ± 0.02*e***	**14.42 ± 0.45*ab***
C20:5 eicosapentaenoic acid (EPA)	7.95 ± 0.47*c*	7.01 ± 0.27*d*	8.34 ± 0.56*c*	7.95 ± 0.35*c*	10.90 ± 0.34*b*	34.43 ± 0.29*a*	6.11 ± 0.11*e*
C22:1 n9 erucic acid	0.97 ± 0.02*ab*	1.06 ± 0.02*a*	0.84 ± 0.04*c*	1.04 ± 0.13*a*	0.07 ± 0.02*e*	0.33 ±0.02*d*	0.92 ± 0.05*bc*
C22:2 cis-13,16-docosadienoic acid	0.26 ± 0.18*a*	0.30 ± 0.08*a*	0.35 ± 0.03*a*	0.27 ± 0.10*a*	0.07 ± 0.05*b*	0.23 ± 0.12*ab*	–
C22:6 docosahexaenoic acid (DHA)	**8.32 ± 0.71*c***	**8.83 ± 0.61*c***	**6.88 ± 0.73*d***	**7.99 ± 0.42*c***	**11.88 ± 0.41*b***	**17.90 ± 0.20*a***	**8.05 ± 0.87*c***

* The designations are given in accordance with Table 1

a, *b*, *c*, *d* ... – values designated with the same letters do not significantly differ at 5% error (Duncan's test)

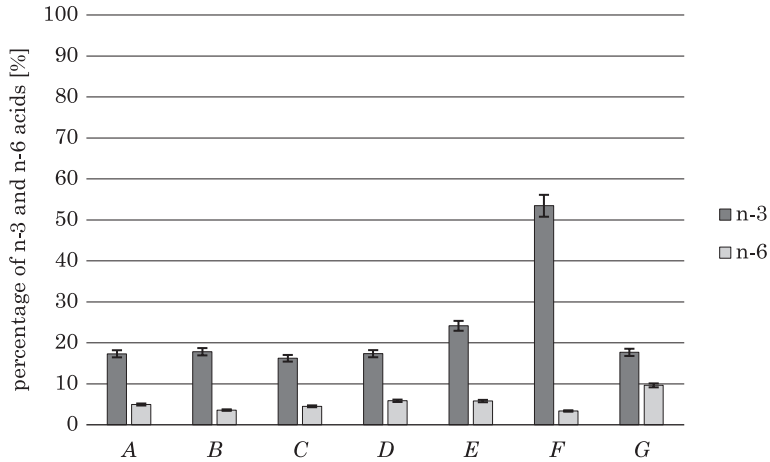

Fig. 1 Percentage of n-3 and n-6 acids in the tested fish oils labeled with letters *A* to *G* * the designations are given in accordance with Table 1

confirmed that the lipids of carp and bream were dominated by oleic acid 32.63% and 20.9% respectively, while in pike fat 17.4%. For shark liver oil, BAKES and NICHOLS (1995) have demonstrated its presence in the amount of 48.8%, whereas in fish oil, GUIL-GUERRERO and BELARBI (2001) showed in the composition of 14.5% oleic acid content. This result are similar to the obtained value for *F*. Higher content of oleic acid in fish oil was given in the work of THORSTEINN et al. (2016) at the level of 16.3%. Oleic acid is a fatty acid common in our diet and no deficiency is observed. A good source of oleic acid are vegetable oils such as olive oil (68.76%), rapeseed oil (57.14%) and animal fat – lard (43.20%).

Figure 2 shows percentage of n-3 and n-6 acids in the tested fish oils in relation to the composition declared by the manufacturers. The compo-

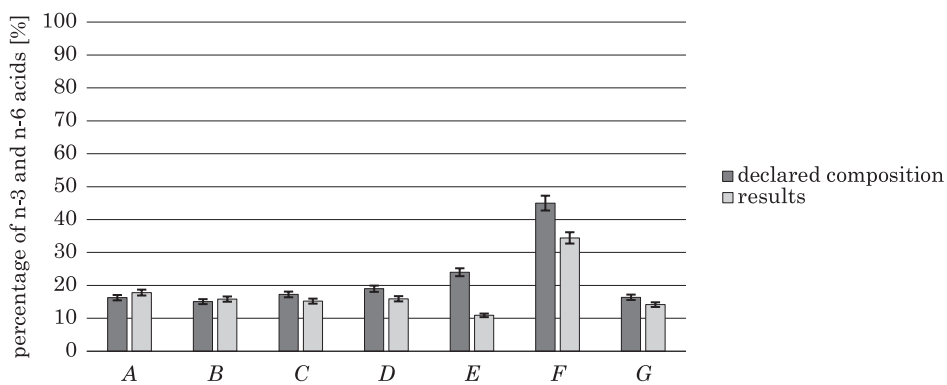

Fig. 2. Percentage of n-3 and n-6 acids in the tested fish oils in relation to the composition declared by the manufacturers * the designations are given in accordance with Table 1

sition of the tested fish oils and the content of n-3 acids (DHA and EPA) declared by the producers was mostly consistent with the results of the analysis (Figure 2). Fish oil *A a*ccording to the quantitative declaration for DHA and EPA acid should contain respectively 8.32% and 7.95%. In the present work were obtained for slightly higher levels of DHA – 10.18% and a similar concentration of EPA – 7.64%. In this study was obtained similar levels to the declared by the manufacturers contents of DHA and EPA acid for fish oils *B*, *C*, *D* and *G*. However, in fish oils *E* and *F*, lower levels than those reported by the manufacturers were found. According to the manufacturer fish oils *E* and *F* should contain respectively 24% and 45%. The obtained results of the analysis are at the level of: 10.9% *E* and 34.43% *F*. The variable acid content may be due to the raw material from which the fish oils were obtained. According to KRIS-ETHERTON et. al. (2003) fat content in tissues of various fish species depends not only on the sex, but also on the season and age of the individual.

Fish oil is the main source of long-chain n-3 PUFAs in the diet. It was found that the PUFAs: EPA and DHA acid have the better beneficial effect on the health due to the prevention of cardiovascular disease than a short-ALA acid (RUXTON et al. 2003, STEPHENSEN 2004). KOLANOWSKI (2007) and KOŁODZIEJCZYK (2007) report that fish, including fish oils, are the best source of polyunsaturated fatty acids n-3. The studies showed a high content of EPA acid. The highest content of EPA acid in respect of the others was found in the Norwegian fish oil forte *F* – 34.43%. In other fish oils observed comparable amount of the acid in the range of 7.01% to 10.90%. CICHOSZ and CZECZOT (2011), indicated the presence of EPA and DHA acids only in fish oil. ŁUCZYŃSKA et al. (2011) showed the highest content of DHA acid in trout muscles (14.5%), in salmon muscle tissues and carp, this acid was present in the amounts respectively of 9.93 and 3.39%. According to HALILOĞLU et al. (2002) trout are the best sources of DHA (19.17%) and EPA acid (3.07%) compared to other fish species. According to GRELA and DUDEK (2007), the group of n-3 in the meat of marine and freshwater fish are dominated by DHA and EPA acids. GRELA et al. (2010) showed the presence of DHA and EPA acids in poultry meat respectively (16.01%) and (13.64%) and in pike meat (15.73%) and (13.64%). BAKES and NICHOLS (1995) for fish oil, showed the presence of DHA and EPA acids respectively in 7% and 0.7%. GUIL-GUERRERO and BELARBI (2001) in fish oil showed that the amount of DHA acid in the study was 10.7% and EPA acid 8.89%. These values are similar to those obtained during the above analysis. THORSTEINN et al. (2016) observed a slightly higher content of these acids, in the amount of 9.6% – for EPA acid and 12.5% for DHA acid.

The analysis of the average diet of Europeans and Americans, too low intake of fatty acids from the n-3 family is observed, mainly DHA and EPA acids (MASZEWSKA and GAŃKO 2010). In many developed countries, the consumption of these acids is on average 0.15 g per day and is below the recommended level (THAUTWEIN 2001, KOLANOWSKI et al. 2004). The use of a daily diet of foods enriched with fish fats or using supplementation prevents the deficiency of nutrient DHA and EPA in the human body. The source of ALA from the n-3 group are leafy vegetables and oils, i.e. linseed, rapeseed and soybean, which are commonly found in our menu. However, the source of EPA and DHA acids are greasy sea fish, which can be replaced with encapsulated fish oils.

The obtained results confirm that acids in the n-3 family dominate in the fish oils. This is particularly evident for example, Norwegian fish oil forte "F", which contained fatty acids from the group n-3 in the amount of 53.44% (Figure 1). According to the FAO/WHO, the recommended dose of polyene acids (PUFA) in a healthy diet in daily nutrition is (5–10): 1 (n-6: n-3) (MARCINIAK-ŁUKASIK 2011). Although there is no established reference relationship, it is recommended to aim for the maximum consumption of omega-3s in relation to omega-6 (KOŚCIEJ et al. 2017). Appropriate amount of DHA acid in the diet can be provided by eating fatty fish such as herring, sprat and salmon 1-2 times a week. Recent research results show that early fish consumption promotes the development of immune tolerance andreduces the risk of allergies (SZAJEWSKA et al. 2014).

The fatty acid composition of all the analyzed fish oils were similar. However, individual deviations from the declared composition were reported, where the percentage of EPA and DHA acids differed from the others.

Summary and Conclusions

It was observed that manufacturers declare higher levels of fatty acid content compared to the results obtained during the analysis. The composition of the selected fish oil declared by the producers didn't differ slightly in the results obtained. The studies showed that all the fish oil are characterized a high content of acids from the group of n-3 and n-6. Fish oil had a higher content of n-3 acids than n-6. The average value was higher 4 times compared to the other tested fish oils. The content of other acid were at a similar level.

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References

Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 1. *Guidelines on modern gas chromatography of fatty acid methyl esters*. PN-EN ISO 12966-1:2015-01.

Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 2. *Preparation of methyl esters of fatty acids*. PN-EN ISO 12966-2:2011.

Bakes M.J., Nichols P.D. 1995. *Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters.* Comp. Biochem. Physiol., 1: 267–275.

Cichosz G., Czeczot H. 2011. *Stabilność oksydacyjna tłuszczów jadalnych – konsekwencje zdrowotne.* Bromat. Chem. Toksykol. – XLIV, 1: 50–60.

Luthringer C.L., Rowe L.A., Vossenaar M., Garrett G.S. 2015. *Regulatory monitoring of fortified foods. Identifying barriers and good practices.* Global Health: Science and Practice, 3(3):446–461.

Dyerberg J., Bang H.O., Hjorne N. 1975. *Fatty acid composition of the plasma lipids in Greenland Eskimos.* Am. J. Clin. Nutr., 28(9): 958–966.

Guil-Guerrero J.L., Belarbi El-H. 2001. *Purification process for cod liver oil polyunsaturated fatty acids.* JAOCS, 78: 477–484.

Grela E.R., Dudek R. 2007. *Składniki odżywcze i profil kwasów tłuszczowych mięsa wybranych gatunków ryb morskich i słodkowodnych.* Żyw. Człow. Metab., 34 (1/2): 561–565.

Grela E.R., Pisarski R.K., Kowalczuk-Vasilev E., Rudnicka A. 2010. *Zawartość składników odżywczych, mineralnych i profil kwasów tłuszczowych w mięsie wybranych gatunków ryb w zależności od terminu odłowu.* ŻYWNOŚĆ. Nauka. Technologia. Jakość, 4(71): 63–72.

Haliloğlu H.I., Aras N.M., Yetím H. 2002. *Comparison of muscle fatty acids of three trout species(Salvelinusalpinus, Salmo truttafario, Oncorhynchus mykiss) raised under the same conditions.*Turk. J. Vet. Anim. Sci., 26: 1097–1102.

Kolanowski W. 2007. *Długołańcuchowe wielonienasycone kwasy tłuszczowe omega-3. Znaczenie zdrowotne w obniżaniu ryzyka chorób cywilizacyjnych.* Bromatologia. Chemia. Toksykologia, (40): 229–237.

Kolanowski W., Uchman Z., Świderski F. 2004. *Intake of omega-3 polyunsaturated fatty acids among adult inhabitants of Warsaw.* Bromatologia. Chemia. Toksykologia, 37: 57–65.

Kołodziejczyk M. 2007. *Spożycie ryb i przetworów rybnych w Polsce – analiza korzyści i zagrożeń.* Roczniki Państwowego Zakładu Higieny, 58(1): 287–293.

Kris-Etherton P.M., Harris W.S., Lawrence J. 2003. *Fish consumption. Fish oil, omega-3 fatty acids, and cardiovascular disease.* Arterioscler Thromb. Vasc. Biol., 23: 20–31.

Kościej A., Skotnicka-Graca U., Ozga I. 2017. *Rola wybranych czynników żywieniowych w kształtowaniu odporności dzieci.* Probl. Hig. Epidemiol., 98(2): 110–117.

Kowalski R. 2007. *GC analysis of changes in the fatty acid composition in sun*flower *and olive oils heated with quercetin, caffeic acid and protocatechuic acid, and butylated hydroxyanisole*. Acta Chromatographica, 18, 15–23.

Kowalski R. 2009. *Silphium L. extracts – composition and protective effect on fatty acids content in sun*flower *oil subjected to heating and storage.* Food Chem., 112: 820–830.

Łuczyńska J., Tońska E., Borejszo Z. 2011. *Zawartość makro- i mikroelementów oraz kwasów tłuszczowych w mięśniach łososia (Salmo salar L.), pstrąga tęczowego (Oncorhynchus mykiss Walb.) i karpia (Cyprinus carpio L.).* Żywność. Nauka. Technologia. Jakość, 3(76): 162–172.

Marciniak-Łukasiak K., Krygier K. 2004. *Charakterystyka kwasów omega-3 i ich zastosowanie w żywności funkcjonalnej.* Przem. Spoż., 12(57): 32–36.

Marciniak-Łukasiak K. 2011. *Rola i znaczenie kwasów tłuszczowych omega-3.* Żywność. Nauka. Technologia. Jakość, 6(79): 24–35.

Maszewska M., Gańko I. 2010. *Kwasy tłuszczowe omega-3 rola w żywieniu, występowanie, zastosowanie.* Przemysł Spożywczy, 64: 28–31.

MATERAC E., MARCZYŃSKI Z., BODEK K.H. 2013. *Rola kwasów tłuszczowych omega-3 i omega-6 w organizmie człowieka.* Bromat. Chem. Toksykol., XLVI(2): 225–233.

RUXTON C.H., REED S.C., SIMPSON M.J., MILLINGTON K.J. 2004. *The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence*. Journal of *Human Nutrition* and Dietetics, 17: 449–459.

STEPHENSEN C. 2004. *Fish oil and inflammatory disease. Nutrition* Reviews, 62: 486–489.

SZAJEWSKA H., SOCHA P., HORVATH A. ET AL. 2014. *Zasady żywienia zdrowych niemowląt. Zalecenia Polskiego Towarzystwa Gastroenterologii, Hepatologii i Żywienia Dzieci.* Stand. Med. Pediatr., 3(11): 321–338.

SZPONAR L., STOŚ K., OŁTARZEWSKI M. 2007. *Suplementy diety w żywieniu dzieci i młodzieży*. Ped. Współcz., Gastroenterol., Hepatol. Żyw. Dziecka, 9(1): 41–44.

THAUTWEIN E.A. 2001. *N-3 fatty acids- physiological and technical aspects for their use in food. European Journal of Lipid Science and Technology*, 103: 45–55.

THIES F., NEBE-VON-CARON G., POWELL J.R., YAGOOB P., NEWSHOME E.H., COLDER P.C. 2001. *Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural healing cell activity in healthy subject aged >55.* Am. J. Clin. Nutr., 73: 539–548.

THORSTEINN L., BILJANA I., GUDRUN M.A., ORRI T.O., EINAR S. 2016. *Fatty acids from marine lipids. Biological activity, formulation and stability.* Journal of Drug Delivery Science and Technology, 34: 71–75.

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THE IMPACT OF DIFFERENT MATCHA GREEN TEA POWDER ADDITIONS ON SELECTED QUALITY FEATURES OF CORN PUFFS

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Key words: corn puffs, matcha green tea powder, quality features, physical properties, color analysis.

Abstract

The aim of this research was to assess the possibility of using matcha green tea powder in the production of corn puffs, as well as to determine its impact on the quality features of the extrudates.

The research material consisted of corn puffs (without and with 1%, 3% and 5% addition of matcha green tea powder) obtained by the extrusion process under laboratory conditions. The aim of the research was realized by examining the mass, geometrical characteristics, hardness parameters using the Universal Testing Machine (Instron) and color using Digital Image Analysis of the extrudates. In addition, moisture and water absorption capacity of finished products and raw materials were determined.

It was shown that matcha green tea powder causes a significant extension of the products (with simultaneous effect on reducing the width), the lower share of the additive also affects the increase of their weight and volume. Matcha green tea powder changes the strength characteristics of corn puffs, primarily increases their hardness, which consequently leads to deterioration or even disappearance (5% addition) of elasticity and flexibility. On the basis of the obtained results, it can be concluded that 1–3% addition of matcha green tea powder to corn puffs not influence significantly on the quality features, and may be use in extrusion process to obtain a product with an attractive green color and probably a higher nutritional value.

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Introduction

A snack is a small portion of food that is often eaten between meals. Nowadays, consumers want snacks to be tailored to their needs and requirements (PĘKSA et al. 2007). The use of extrusion in the food industry enables the creation of a new range of products, definitely other than potato chips and pretzels that dominate the food snacks market (CISNEROS and KOKINI 2002). Corn puffs are very popular among consumers both adults and kids, the intake is about 21% of the total snacks consumption (MRUK and TELEŻYŃSKA 2009). They are most often made from corn or rice grits and are liked by consumers due to their characteristic sensory and physical characteristics (PASTOR-CAVADA et al. 2011). Corn puffs are characterized by small size, various shapes and also have a crisp texture. In addition, extrudates are completely microbiologically safe and can also be stored for a relatively long period of time due to their low moisture content (FILLI and NKAMA 2007).

The production process of extruded snacks consists of several stages, including mixing of ingredients (according to the recipe), material dispensing, extrusion with appropriate parameters adapted to the type of snacks (also shaping the final product using molding dies) and possibly drying the products to the appropriate moisture content for safe storage. The production of corn puffs is carried out at moisture content of the raw materials of 14–17% and at temperatures above 100°C, which allows to obtain a crunchy consistency of product as a result of rapid evaporation of water (MITRUS and WÓJTOWICZ 2011). Unfortunately, the nutritional value of extruded products, including corn puffs, is relatively low, this causes attempts to be made to enrich the products with nutrients using various additives (PĘKSA et al. 2007). Currently, cereals and vegetable additives such as amaranth, pumpkin, flax, and dry legume seeds are very popular (CHÁVEZ-JÁUREGUI at al. 2003). In addition, the production of corn puffs also uses protein preparations obtained from whey, soy, yeast, or potato (MIKOŁAJCZAK 2016, PĘKSA 2006), vitamin preparations and fiber (BISHARAT et al. 2013).

Matcha or matcha green tea is becoming increasingly popular product among consumers (TOWNSEND et al. 2011). It is obtained from the plant *Camellia sinensis*, which is grown in areas with mild climatic conditions and in shady places. Three to four weeks before harvest, the plants are almost completely shaded, which improves the quality of tea as a result of prolonged puberty (COOPER et al. 2005). Matcha tea is a valuable source of phenolic compounds (WEISS and ANDERTON 2003), among which the catechins dominate (they constitute 80% of all polyphenols). In addition,

59% of all catechins consist of epigallocatechin gallate (EGCG), which is the most widespread and bioactive polyphenol in green tea (COOPER et al. 2005, KOO and NOH 2007). WIGHTMAN et al. (2012) found that EGCG can reduce heart rate and the level of oxygenated hemoglobin, SCHOLEY et al. (2012) showed that it increases calmness and reduces stress, and the last studies of other authors clearly indicate its antiproliferative effect in cancer cells (DU et al. 2012).

Besides polyphenols, matcha tea contains significant amounts of free amino acids (mainly *L*-theanine, accounts about 50% of the total amino acids), and caffeine (WEISS and ANDERTON 2003). Studies with a dose of *L*-theanine in an amount of 200–250 mg showed that it affects mood, alertness, mental fatigue, relaxation and anxiety level (GOMEZ-RAMIREZ et al. 2009, ROGERS et al. 2008). While caffeine, which is a stimulant all over the world, increases blood pressure (BARRY et al. 2005), affects cognitive functions (EINÖTHER et al. 2010), and also provides better performance, motivation and concentration in a short time (PAULUS et al. 2015).

Research on the use of matcha green tea powder in food products are taken to a small extent (DIETZ et al. 2017). The technology of extruded products production allows the use of various raw materials that have a positive effect not only on nutritional and sensory elements of products, but also can cause significant and not always beneficial changes in their physicochemical properties (PASTOR-CAVADA et al. 2011). The aim of this research was to assessment of the possibility of using matcha green tea powder as an additive in the extrusion process, as well as its impact on selected quality features of corn puffs such as water absorption capacity, mass, color, geometrical and strength characteristics.

Materials and Methods

Corn grits (Krupiec, Krzymów, Poland) and matcha green tea powder (Taheebo, Michałowice, Poland) were purchased in one of the supermarkets in Olsztyn. Both products were characterized by the current date of shelf life, appropriate organoleptic characteristics, and until the extrusion process they were stored in a dry, cool and darkened place.

Corn puffs (without any addition, and with 1%, 3%, 5% share of matcha green tea powder) were obtained by extrusion process using an extruder type S45A-12-10U (Metalchem, Gliwice, Poland) with a power of 10 kW and efficiency of 10 kg h^{-1}, whose basic working element was a cylinder (length 12.0 mm, nominal diameter 15.0 mm) connected to the discharge nozzle (diameter 4.5 mm). The extrusion process was carried out at

constant operating parameters: the cylinder temperature distribution profile was 105°C/130°C/110°C, the screw rotation was 80 rpm (revolutions per minute). Fresh extrudates were cooled (4 h at 19±2°C and relative humidity of 54±2%), packed in plastic bags and stored in cool and dry place until analyzes.

In raw materials (corn grits, matcha green tea powder) and corn puffs, the moisture content using the thermal research chamber KBC-100 W type (Wamed, Warsaw, Poland) according to the Polish Standard (*Chrupki – metody…* PN-A-88034:1998P) and water absorption capacity in accordance with the method given by EKIELSKI et al. (2013) were determined.

The mass of individual corn puffs was determined by weight using the laboratory weighing machine type PS600.3Y (Radwag, Radom, Poland), the volume by placing corn puffs in the cylinder, which was filled with a constant volume of amaranth seeds and read from the scale of the difference in volume, and geometrical features such as length and diameter were measured using a caliper. The specific density of corn puffs was determined by the weight-to-volume ratio of individual extrudates (MORSY et al. 2014). Based on the results of geometrical characteristics, the shape factor – elongation as the quotient of the length to the diameter of the product, as well as the expansion index as the quotient of the corn puff diameter to the diameter of the extruder nozzle were calculated (MORSY et al. 2014). The mechanical properties (displacement, hardness, energy compression) were determined by a Universal Testing Machine 4301 type (Instron, Massachusetts, USA), and the uniaxial compression test of individual product was used. The breaking stress was evaluated as the force divided by the cross-section area of corn puffs (LEE at al. 1999), while elasticity index as the product of strength (hardness) and displacement. The color of corn puffs (Figure 1) was measured with a Digital Image Analysis set and expressed in CIE L*a*b* color system (TAŃSKA et al. 2011). Images were acquired with a Nikon DXM-1200 (Nikon Instruments, Melville, USA) charge coupled device (CCD) color camera. Based on average values of CIE L*a*b* parameters for samples with green tea powder additive and control sample, estimated such color attributes as a total color difference

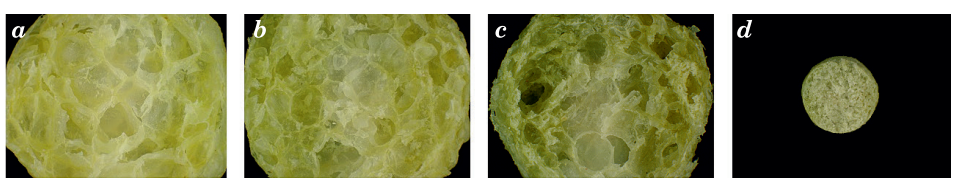

Fig. 1. Cross-section of obtained corn puffs: *a* – control; *b* – with 1% matcha green tea powder addition; *c* – with 3% matcha green tea powder addition; *d* – with 5% matcha green tea powder addition

(according to the equation given by TAŃSKA et al. (2017), a color saturation parameter (ΔC) and a saturation index (SI) (according to the equations given by GALUS and LENART 2012).

The results of all analyses (were made in five repetitions) were analyzed using Statistica 13.1 PL software (StatSoft, Kraków, Poland). The differences between the means were determined using analysis of variance (ANOVA) at a significance level α = 0.05 with Tukey's test.

Results and Discussion

Table 1 shows the values of basic parameters such as moisture and water absorption capacity for raw materials (corn grits, matcha green tea powder) and obtained corn puffs.

The highest moisture was characterized by corn grits (12.76%). The use of the extrusion process and matcha green tea powder additives (moisture was 7.04%) significantly affected ($p < 0.05$) the moisture of the obtained products, reducing the moisture by an average of 5% in relation to the basic raw material (corn grits). The moisture content of corn puffs with matcha green tea powder additions were lower (7.02–7.80%) than the moisture of control corn puffs (7.98%) and were characterized by a positive correlation according to the share of the additive used.

Table 1

Basic characteristics of raw materials and obtained corn puffs

Parameter	Corn grits	Matcha green tea powder	Corn puffs			
			control	1% MP	3% MP	5% MP
Moisture [%]	12.76^{a}±0.33	7.04^{d}±0.00	7.98^{b}±0.01	7.02^{d}±0.28	7.17^{c}±0.13	7.80^{b}±0.33
Water absorption capacity [%]	239.52^{f}±0.59	282.93^{d}±0.88	365.19^{c}±0.73	391.47^{b}±0.78	405.29^{a}±0.84	276.36^{e}±0.20

MP – matcha green tea powder addition; *a*, *b*, *c*… – average values in lines with the same letter are not significantly different at $p > 0.05$

Corn grits was characterized by the lowest water absorption capacity of the analyzed products and amounted to 239.52%. The water absorption capacity of control corn puffs was 365.19%, while the use of 1% and 3% matcha green tea powder additives resulted in almost 30 and 40% increase in water absorption capacity of the obtained products, respectively (significant differences, $p < 0.05$). The addition of 5% matcha green tea powder had similar water absorption capacity as raw materials, in particular matcha green tea powder (282.93%).

Matcha green tea powder is characterized by low moisture content, which can affect the low values of this parameters in obtained corn puffs. Moisture content in obtained corn puffs is typical for brittle and crunchy extruded products obtained from cereals. Similar values were found for crispbread (GONDEK et al. 2013, JAKUBCZYK et al. 2015), breakfast extrudates with a varied composition (CHANVIER et al. 2014) and many other products. According to literature data, this moisture content is optimal from the point of view of storage stability (GONDEK et al. 2017).

The water absorption capacity index is a measure of the ability to absorb and maintain water by samples. It depends to a large extent on the applied temperature of thermal processing during the production of snacks and the water content in raw materials (GAMBUŚ et al. 2000). The research only partially confirms the accepted statement that corn puffs produced with a large share of starchy raw materials in the recipe show the greatest ability to absorb and retain water (WÓJTOWICZ and BALTYN 2006). A confirmation of this thesis is only a variant with 5% matcha green tea powder additive, where the share of corn grits (as a source of starch) account for 95% in whole product and ensures lower value of the water absorption capacity index than corn puffs (100% of corn grits in whole products).

Geometric characteristics of corn puffs were highly varied (Table 2). The largest mass (1.28 g) was obtained for corn puffs with 3% and 5% matcha green tea powder additions. This mass was more than 2-fold higher than for the control corn puffs and puffs with 1% matcha green tea powder additive. Different results were observed in the volume analysis, the largest volume was characterized by the control corn puffs and puffs with 1% and 3% matcha green tea powder additions (5.16–5.40 cm^3). Puffs with 5% addition had the smallest volume, which was only 1.68 cm^3 and was 3-fold lower than the control corn puffs (significant difference, $p < 0.05$). Densities of products, which were estimated as the quotient of mass and volume, showed a rising tendency (higher share of additive means higher density of products). According to this statement, the highest specific density was characterized by corn puffs with 5% matcha green tea powder additive (0.77 g cm^{-3}).

The use of 5% matcha green tea powder resulted in a significant increase in length (4.64 cm) and a simultaneous reduction in the width (0.60 cm) of products. Similar observations were noted for 3% matcha green tea powder addition, where the length was 4.29 cm and the width was 1.42 cm. The dimensions for the control corn puffs and puffs with 1% additive of matcha green tea powder slightly differed from each other ($p > 0.05$).

Analysis of parameters such as elongation and expansion ratio, which estimated on the basis of length and width, showed that the additives used in the research also influenced their values. In the case of elongation, matcha

green tea powder addition of 3% and 5% significantly increased its value ($p < 0.05$), which was 2.4-(3% additive) and 6.2-fold (5% additive) higher than for the control corn puffs (1.25). However, the same additions resulted in a reduction of expansion ratio by almost 30 (3% additive) and 70% (5% additive), while expansion ratio was the highest for the control corn puffs and amounted to 4.07.

Table 2

Mass and geometrical characteristics of obtained corn puffs

Geometrical characteristics	Corn puffs			
	Control	1% MP	3% MP	5% MP
Weight [g]	0.53^{b}±0.01	0.57^{b}±0.01	1.28^{a}±0.12	1.28^{a}±0.06
Volume [cm^3]	5.40^{a}±0.34	5.16^{a}±0.30	5.38^{a}±1.70	1.68^{b}±0.25
Specific density [g cm^{-3}]	0.10^{c}±0.01	0.11^{c}±0.00	0.26^{b}±0.10	0.77^{a}±0.10
Length [cm]	2.27^{d}±0.08	2.52^{c}±0.07	4.29^{b}±0.11	4.64^{a}±0.13
Width [cm]	1.83^{a}±0.02	1.64^{b}±0.04	1.42^{c}±0.08	0.60^{d}±0.00
Elongation [-]	1.25^{d}±0.04	1.54^{c}±0.06	3.00^{b}±0.12	7.77^{a}±0.16
Expansion ratio [-]	4.07^{a}±0.00	3.64^{b}±0.03	3.14^{c}±0.02	1.33^{d}±0.00

MP – matcha green tea powder addition; a,b,c… – average values in lines with the same letter are not significantly different at $p > 0.05$ Table 2

To sum up, the addition of matcha green tea powder increases the mass and density of corn puffs, and at the same time reduces their volume. The obtained corn puffs are also longer and thinner than the control corn puffs.

The expansion index indicates the degree of starch degradation in the product. The value of the expansion index depends on the type and amount of gelatinized starch, on the type and amount of additional raw materials, as well as on the extrusion conditions. The use of additives may contribute to a change in the temperature of starch gelatinization, which directly affects its expansion. The higher starch gelatinization index causes a higher value of the expansion index and contributes to a product with higher porosity and thinner pore walls (BISHARAT et al. 2013).

The addition of the largest amount (5%) of matcha green tea powder causes a reduction in the value of the expansion index. Literature data show that this effect may be caused by a higher share of protein and fiber fractions in the obtained product (STOJESKA et al. 2008).

The use of matcha green tea powder additions influenced the hardness parameters of corn puffs (Table 3). Analysis of the results showed a relation between the share of matcha green tea powder addition and the individual elements of hardness (a higher addition of matcha green tea powder resulted in higher values of individual parameters, $p < 0.05$).

Table 3

Strength characteristics of obtained corn puffs

Strength characteristics	Corn puffs			
	control	1% MP	3% MP	5% MP
Displacement [mm]	3.48^{b}±0.54	2.83^{c}±0.61	2.21^{d}±0.36	4.16^{a}±0.06
Hardness [N]	41.10^{d}±1.66	62.00^{c}±1.85	126.30^{b}±2.39	1005.10^{a}±2.96
Energy compression [mJ]	108.50^{c}±3.24	150.40^{c}±1.21	245.60^{b}±1.34	1105.22^{a}±6.45
Breaking stress [MPa]	9.74^{d}±0.34	13.93^{c}±0.96	20.13^{b}±1.42	354.82^{a}±1.88
Elasticity index [N mm]	153.45^{c}±3.44	181.63^{b}±1.07	162.96^{c}±2.45	4111.46^{a}±3.06

MP – matcha green tea powder addition; *a*, *b*, *c*… – average values in lines with the same letter are not significantly different at $p > 0.05$

Parameters such as hardness (41.10 N), energy compression (108.50 mJ), breaking stress (9.74 MPa) and elasticity index (153.45 N mm) were the lowest for the control corn puffs. The use of matcha green tea powder in an amount of 1% and 3% resulted in an increase in the value of individual parameters ($p < 0.05$), from 43% (1% addition for breaking stress) to even 126% (3% addition for energy compression). The most visible changes were observed for the highest matcha green tea powder addition in the amount of 5%. In this case, energy compression increased 10-fold, hardness and elasticity index 25–27-fold, and breaking stress up to 36-fold compared to control corn puffs (significant differences, $p < 0.05$). Slightly different results were obtained with regard to displacement. The addition of matcha green tea powder in the amount of 1% and 3% reduced the value of displacement by almost 20% for 1% additive and almost 38% for 3% additive in comparison to the control sample (3.48 mm). The significant increase ($p < 0.05$) was found only for corn puffs with 5% share of matcha green tea powder (4.16 mm).

Hardness is a parameter very often determined during the examination of food products. In the evaluation of extruded products, this is one of the most important decisive factors in their consumption suitability. The value of this parameter in extruded products should be as small as possible, which affects the high brittleness of products (SURÓWKA 2000).

The use of matcha green tea powder additive in the extrusion process results in an increase the hardness of the products, the significant differences at level of $p < 0.05$ are only present at the highest addition (5%). The literature data show the impact of production parameters and the composition of raw materials on the hardness of products. For example, FORNAL (1998) reports the hardness of extrudates with 10% of casein addition from 47 N to 86 N. Whereas RZEDZICKI (1999) states that extrusion with oat components up to 20% allows obtaining a high quality product with

increased hardness of extrudates. A similar effect was observed by other authors who used various types of fiber (KITA et al. 2002) and protein preparations (PĘKSA et al. 2007).

Corn puffs without matcha green tea powder were the most flexible, which indicates their high ability to return to their original shape after stopping the deforming force. Matcha green tea powder contributed to the lower elasticity and flexibility of obtained corn puffs. The products with the highest addition (in amount of 5%) were characterized by very high values of these parameters, which results in the destruction of their structure during the compression test (no ability to return to the origin form), additionally they have a solid and hard structure (high values for hardness, breaking stress, energy compression).

One of the most important distinguishing features of the product, which are decisive for acceptance by the consumer, is color. Different systems and color models are used to define it, but the CIE L*a*b* model is the most popular. Parameter L* denotes brightness [%], parameter a* defines green (negative values) or red (positive values) color and b* defines blue (negative values) or yellow (positive values) color. The constituent values of the CIE L*a*b* model for the obtained corn puffs are presented in Table 4.

Negative values obtained for the a* parameter indicated the green color of the tested puffs. The highest share of greenness was observed for corn puffs with 5% matcha green tea powder addition (-6.46). The values for other variants were similar ($p > 0.05$) to each other ((-8.74) (-8.28)). The b* parameter was characterized by positive values, which was directly related to the occurrence of yellow color in the corn puffs. The most yellow color was observed for the control corn puffs (29.38) and puffs with 1% matcha green tea powder addition (29.46). The lowest value of this parameter was found for corn puffs with matcha green tea powder in the amount of 5% (almost 33% compared to the control sample). Analysis of the brightness parameter (L*) did not show significant differences between the control corn puffs and puffs with 1% matcha green tea powder additive. The least light were puffs with 3% matcha green tea powder (78.29%) and with 5% matcha green tea powder (78.38%) additions ($p > 0.05\%$).

Based on the constituent values of the CIE L*a*b* model, the difference in colors (ΔE) was determined by comparing the color of the control corn puffs with particular puffs variants with the addition of matcha green tea powder. In the discussion of results, the criteria given in RÓJ and PRZYBYŁOWSKI (2012) were used. Estimated values were in the ranges 2.44–11.78, which indicated the difference in color between the obtained corn puffs and control corn puffs. The determined difference in color

between the control corn puffs and corn puffs with 1% matcha green tea powder was 2.44, which indicates a noticeable difference in color even for an inexperienced observer ($2 < \Delta E < 3.5$, according to RÓJ and PRZYBYŁOWSKI 2012). However, the significant differences ($p < 0.05$) was recorded for the control corn puffs and corn puffs with 3% and 5% matcha green tea powder additive (7.50 and 11.78, respectively), these values show a large deviation of color ($\Delta E > 5$, according to RÓJ and PRZYBYŁOWSKI 2012). Changes of color were also noticeable in the visual assessment of the obtained products (Figure 1).

Color saturation (ΔC) is characterized by the distance from the center of the system, the most saturated colors are on the outside, and the least saturated ones are in the middle of the system (CZAPSKI 1997). The estimated values of the color saturation parameter showed that the addition of matcha green tea powder causes a gradual increase in the color saturation of the tested extrudates (Table 4). The highest parameter value was observed for corn puffs with 5% matcha green tea powder addition (10.08), which indicates that the color of these corn puffs was more saturated (and thus more vivid) compared to the color of the control sample (the distance from the center of the system is large, the color directed towards the outside of the system). Whereas, 1% matcha green tea powder addition showed a small impact on the value of color saturation parameter, the estimated value for this variant was only 0.47 compared to the control sample. In addition, the color of these corn puffs was close to the center of the system (white color), which clearly indicated a light color (close to gray) and low saturated (shades of gray characterized by lack of saturation).

Table 4

Color attribute of obtained corn puffs

Color attribute	Corn puffs			
	control	1% MP	3% MP	5% MP
L* [%]	84.46^{a}±0.23	82.07^{b}±0.28	78.29^{c}±0.16	78.38^{c}±1.02
a* [-]	-8.28^{b}±0.26	-8.74^{c}±0.82	$-8.39^{b,c}$±0.57	-6.46^{a}±0.57
b* [-]	29.38^{a}±1.10	29.46^{a}±1.98	25.10^{b}±1.17	19.46^{c}±0.88
ΔE [-]	–	2.44	7.50	11.78
ΔC [-]	–	0.47	4.28	10.08
SI [-]	30.53	30.73	26.47	20.50

MP – matcha green tea powder addition; ΔE – total color difference between color attributes of sample with matcha green tea powder and control sample; ΔC – color saturation parameter between color attributes of sample with matcha green tea powder and control sample; SI – saturation index between color attributes of sample with matcha green tea powder and control sample; *a*, *b*, *c*… – average values in lines with the same letter are not significantly different at $p > 0.05$

The value of the saturation index (SI) for the control sample was 30.52 (Table 4). The use of matcha green tea powder addition during the production of corn puffs resulted in a decrease in the value of saturation index, excluding corn puffs with 1% matcha green tea powder additive (values comparable to the control sample). In the other two variants, the decrease in the saturation index value was noted by 13.30% (3% matcha green tea powder additive) and 32.85% (5% matcha green tea powder additive).

Color is an important physical feature of the product, which has a significant impact on the choice of the product by consumers. It can be a source of information about the chemical composition of the product, its suitability for consumption and the storage process (ŁUKASIEWICZ and ZAPOTOCZNY 2012). It was shown that the color parameters (in extrudates) change significantly even with small changes in the formula of mixtures and may be an indicator of their composition (ZIELIŃSKI 2013).

The addition of matcha green tea powder influences the color of the obtained products, which is mainly related to the dark and green color of additive. These changes were visible primarily with the addition of 3% and 5%, these results in a darker and greener color, as well as a lowering the share of yellow color. PĘKSA et al. (2015) studied the color of corn puffs with various additives. They found that brightness of the corn puffs after adding a mixture of flours containing powdered pumpkin resulted also in a decrease in the brightness of the color from level L* = 78.77% to L* = 67.95% compared to the control sample. Research conducted by LUCAS et al. (2018) showed that the addition of *Spirulina* sp. LEB 18 (in amount of 2.6%) had a significant effect ($p < 0.05$) on the color of the extrudates. The additive used by the authors resulted in a decrease in the values of the L* and b* parameters, which determined the darker color of the snacks. Similar tendencies were also found by TAŃSKA et al. (2017) during the analysis of corn extrudates properties (including color) enriched with spirulina.

The structure of corn puffs was porous, but its appearance depended on the amount of the additive (Figure 1). The control corn puffs were characterized by a small number of pores with thin walls (*a*). The use of matcha green tea powder additive resulted in an increase in the number of pores (*b*, *c*), and was also observed the presence of empty spaces filled with air (*c*). A change in the thickness of pore walls was also visible (*c*, *d*). The 5% addition of matcha green tea powder caused a compact structure with small pores (*d*).

Conclusions

The results obtained in this research indicate the possibility of using matcha green tea powder as the additive in the extrusion process. However, the use of a larger amount of the additive (in this case 5%) resulted in a dense and hard structure of corn puffs, which may mean the necessity of using additional treatments, e.g. grinding, which will allow the product to be used in direct consumption. Matcha green tea powder also changes the taste of corn puffs causing a noticeable bitterness during the consumption of the product which may result in a negative approach by consumers with a rather sweet taste preference.

Matcha green tea powder addition affects the quality features of obtained corn puffs:

1. The use of a small amount of additive (1% and 3%) affects the lower water content, while ensuring greater water absorption capacity of the final product.
2. Matcha green tea powder additions increase the mass and specific density of corn puffs (a larger share of matcha results in a higher value of these parameters), at the same time reduces the product volume (which the most visible changes are at 5% additive).
3. The addition affects the geometric characteristics of corn puffs. The obtained products are characterized by a gradual reduction in width, with simultaneous effect on product elongation.
4. The obtained products have other strength characteristics than control corn puffs. The addition of 5% matcha green tea powder significantly increases the hardness of the product, as well as reduces its elasticity and flexibility.
5. Matcha green tea powder additive affects the color of the final product, with increasing the share of matcha green tea powder, the color of the product becomes mimic and greener.

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References

Barry R.J., Rushby J.A., Wallace M.J., Clarke A.R., Johnstone S.J., Zlojutro I. 2005. *Caffeine effects on resting-state arousal*. Clin. Neurophysiol., 116(11): 2693–2700.

Bisharat G.I., Oikonomopoulou V.P., Panagiotou N.M., Krokida M.K., Maroulis Z.B. 2013. *Effect of extrusion conditions on the structural properties of corn extrudates enriched with dehydrated vegetables*. Food Res. Int., 53: 1–14.

Chanvrier H., Jakubczyk E., Gondek E., Gumy J. C. 2014. *Insights into the texture of extruded cereals. Structure and acoustic properties*. Innov. Food. Sci. Emerg., 24: 61–68.

Chávez-Jáuregui R.N., Cardoso-Santiago R.A., Pinto e Silva M.E.M., Arêas J.A.G. 2003. *Acceptability of snacks produced by the extrusion of amaranth and blends of chickpea and bovine lung*. Int. J. Food. Sci. Tech., 38: 795–798.

Chrupki – metody badań. PN-A-88034:1998P.

Cisneros F.H., Kokini J.L. 2002. *A generalized theory linking barrel fill length and air bubble entrapment during extrusion of starch*. J. Food. Eng., 51: 139–149.

Cooper R., Morré D.J., Morré D.M. 2005. *Medicinal benefits of green tea.* Part I. *Review of noncancer health benefits*. J. Altern. Complem. Med., 11(3): 521–528.

Czapski J., Limanówka-Jacygrad D., Miller A. 1997. *Przewidywanie barwy mieszanin roztworów czerwonych barwników buraka ćwikłowego i karmelu*. Zywn-Nauk. Technol. Ja., 4(13): 26–35.

Dietz C., Dekkser M., Piqueras-Fiszman B. 2017. *An intervention study on the effect of matcha tea, in drink and snack bar formats, on mood and cognitive performance*. Food. Res. Int., 99: 72–83.

Einöther S.J., Martens V.E., Rycroft J.A., De Bruin E.A. 2010. *L-theanine and caffeine improve task switching but not intersensory attention or subjective alertness*. Appetite., 54(2): 406–409.

Ekielski A., Żelaziński T., Florczak I. 2013. *Wpływ rozdrobnienia ekstrudatu na wskaźniki wodochłonności i rozpuszczalności w wodzie*. Inż. Rol., 4: 79–89.

Filli K.B., Nkama I. 2007. *Hydration properties of extruded fura from millet and legumes*. Food. J., 109(1): 68–80.

Fornal Ł. 1998. *Ekstruzja produktów skrobiowych – nowe wyroby*. Pasze Przemysłowe, 3: 7–14.

Gambuś H., Gołachowski A., Bała-Piasek A., Nowotna A., Surówka K., Mikulec A., Bania M. 2000. *Ocena jakości ekstrudowanych chrupek z otrąb zbożowych*. Zywn-Nauk. Technol. Ja., 4(25): 55–63.

Galus S., Lenart A. 2012. *Wpływ emulsji tłuszczowej na wartości optyczne filmów serwatkowych*. Acta Agroph., 19(1): 29–36.

Gomez-Ramirez M., Kelly S. P., Montesi J. L., Foxe J. J. 2009. *The effects of L-theanine on alpha-band oscillatory brain activity during a visuo-spatial attention task*. Brain Topogr., 22(1): 44–51.

Gondek E., Gauze J., Jakubczyk E., Janczar-Smuga M., Nowak D., Stasiak M., Kamińska-Dwórznicka A., Samborska K. 2017. *Wysokobłonnikowe przekąski zbożowo-warzywne – analiza wybranych właściwości fizycznych i funkcjonalnych*. Zesz. Probl. Post. Nauk. Roln., 590: 15–27.

Gondek E., Jakubczyk E., Herremans E., Verlinden B., Hertog M., Vandendriessche T., Verboven P., Antoniuk A., Bongaers E., Estrade P., Nicolai B. 2013. *Acoustic, mechanical and structural properties of extruded crisp bread*. J. Cereal Sci., 58: 132–139.

Guang-Jian D., Zhiyu Z., Xiao-Dong W., Chunhao Y., Tyler C., Chun-Su Y., Chong-Zhi W. 2012. *Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea*. Nutrients., 4: 1679–1691.

Jakubczyk E., Linde M., Gondek E., Kamińska-Dwórznicka A., Samborska K., Antoniuk A. 2015. *The effect of phytosterols addition on the textural properties of extruded crisp bread*. J. Food Eng., 167: 156–161.

Kita A., Pęksa A., Zięba T., Figiel A. 2002. *Influence of pellets moisture and dietary fibre addition on some potato snack properties*. Acta Agroph., 77: 33–43.

KITA A., POPIELA-KUKUŚ K. 2010. *Wpływ dodatku wytłoków lnianych na wybrane właściwości smażonych chrupek ziemniaczanych*. Acta Agroph., 16(1): 69–77.

KOO S. I., NOH S. K. 2007. *Green tea as inhibitor of the intestinal absorption of lipids: Potential mechanism for its lipid-lowering effect*. J. Nutr. Biochem., 18(3): 179–183.

LEE E.Y., RYU G.H., LIM S.T. 1999. *Effects of processing parameters on physical properties of corn starch extrudates expanded using supercritical CO_2 injection*. Cereal. Chem., 76: 63–69.

LUCAS B.F., DE MORAIS M.G., SANTOS T.D., COSTA J.A. V. 2018. *Spirulina for snack enrichment: Nutritional, physical and sensory evaluations*. LWT., 90: 270–276.

ŁUKASZEWICZ N., ZAPOTOCZNY P. 2012. *Pomiar barwy w żywności*. Laboratorium – Przegl. Ogólnopolski., 7–8: 58–65.

MIKOŁAJCZAK N. 2016. *Wpływ wybranych dodatków na cechy jakościowe chrupek kukurydzianych*. Ekologia i Technika, 24(3): 112–118.

MITRUS M., WÓJTOWICZ A. 2011. *Wybrane cechy jakościowe przekąsek ekstrudowanych z dodatkiem skrobi modyfikowanych*. Acta Agroph., 18(2): 335–345.

MORSY O.M., SHAROBA A.M., EL-DESOUKY A.I., BAHLOL H.E.M., ABD EL MAWLA E.M. 2014. *Production and evaluation of some extruded food products using spirulina alga*. Ann. Agric. Sci. Moshtohor., 52: 329–342.

MRUK J., TELEŻYŃSKA K. 2009. *Raport – rynek słodyczy i słonych przekąsek*. Poradnik Handlowca, http://www.poradnikhandlowca.com.pl/archiwum/10-2009,Rok-2009,29.html.

PASTOR-CAVADA E., DRAGO S.R., GONZÁLEZ R.J., JUAN R., PASTOR J.E., ALAIZ M., VIOQUE J. 2011. *Effects of the addition of wild legumes (Latyhyrus annuus and Lathyrus clymenum) on the physical and nutritional properties of extruded products based on whole corn and brown rice*. Food. Chem., 128: 961–967.

PAULUS R., ROTH A., TITUS L., CHEN R., BRIDGES M. C., WOODYARD S. 2015. *Impact of various caffeine vehicles on mood and cognitive, neurological and physiological functions over five hours*. Ohio. J. Sci., 115(2): 12–23.

PĘKSA A. 2006. *Ocena jakości preparatów białka ziemniaczanego otrzymanych w różnych warunkach technologicznych i ich przydatności w produkcji wyrobów ekstradowanych*. Rozprawy CCXXXVIII Akademii Rolniczej we Wrocławiu, 533: 1–101.

PĘKSA A., RYTEL E., KAWA-RYGIELSKA J., GRYSZKIN A., ZIĘBA T. 2007. *Effect of protein prepaeations addition on prpoperties of potato snacks obtained from extruded semi-products*. Pol. J. Food. Nutr. Sci., 57: 429–435.

PĘKSA A., RYTEL E., TAJNER-CZOPEK A., KITA A., DANILCENKO H., JARENIE E., FIGIEL A., LECH K., MIEDZIANKA J., DROŻDŻ W. 2015. *Wpływ surowca i parametrów procesu na cechy sensoryczne i fizyczne ekstrudowanych przekąsek*. Zywn-Nauk. Technol. Ja., 3(100): 176–189.

ROGERS P. J., SMITH J. E., HEATHERLEY S. V., PLEYDELL-PEARCE C. 2008. *Time for tea. Mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together*. Psychopharmacology, 195(4): 569–577.

RÓJ A., PRZYBYŁOWSKI P. 2012. *Ocena barwy jogurtów naturalnych*. Brom. Chem. Toksykol., XLV(3): 813–816.

RZEDZICKI Z. 1999. *Badania możliwości zastosowania surowców owsianych do produkcji ekstrudatów spożywczych*. Zywn-Nauk. Technol. Ja., 1(18): 214–223.

SCHOLEY A., DOWNEY L.A., CIORCIARI J., PIPINGAS A., NOLIDIN K., FINN M., WINES M., CATCHLOVE S., TERRENES A., BARLOW E., GORDON L., STOUGH C. 2012. *Acute neurocognitive effects of epigallocatechin gallate (EGCG)*. Appetite., 58(2): 767–770.

STOJESKA V., AINSWORTH P., PLUNKETT A., IBANOĞLU E. 2008. *Cauliflower by products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks*. J. Food. Eng., 87: 554–563.

SURÓWKA K. 2000. *Tekstura żywności i metody jej badania*. Przem. Spoż., 10:12–17.

TAŃSKA M., KONOPKA I., KORZENIEWSKA E., ROTKIEWICZ D. 2011. *Colour of rapeseed (Brassica napus) surface and contamination by fungi during storage of dry and wet seeds*. Int. J. Food. Sci. Technol., 46: 2265–2273.

TAŃSKA M., KONOPKA I., RUSZKOWSKA M. 2017. *Sensory, physico-chemical and water sorption properties of corn extrudates enriched with spirulina*. Plant. Foods. Hum. Nutr., 2(3): 250–257.

TOWNSEND D., MAITIN V., CHESNUT T., VATTEM D. 2011. *Anti-retroviral activity of phytochemical rich dietary ingredients: Herbs, spices, fruits and matcha (green tea)*. Faseb. J., 25(1 suppl.): 350–352.

WEISS D.J., ANDERTON C.R. 2003. *Determination of catechins in matcha green tea by micellar electrokinetic chromatography*. J. Chromatogr. A., 1011(1): 173–180.

WIGHTMAN E.L., HASKELL C.F., FORSTER J.S., VEASEY R.C., KENNEDY D.O. 2012. *Epigallocatechin gallate, cerebral blood flow parameters, cognitive performance and mood in healthy humans: A double-blind, placebo-controlled, crossover investigation*. Hum. Psychopharm. Clin., 27(2): 177–186.

WÓJTOWICZ A., BALTYN P. 2006. *Ocena wybranych cech jakościowych popularnych przekąsek ziemniaczanych*. Zywn-Nauk. Technol. Ja., 2(47): 112–123.

ZIELIŃSKI T. 2013. *Wpływ dodatku gryki na zmiany barwy ekstrudatu kukurydzianego*. Post. Techn. Przetw. Spoż., 2: 25–30.

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THE IMPACT OF POLLEN ON THE HEALTH STATUS OF ANIMALS AND HUMANS

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Key words: pollen, health, bee, animals, humans.

Abstract

Pollen is collected by worker bees from different plant species. This is a special product, rich in biologically active substances, that comprises food for larvae in bee colony. Chemically, it consists of approx. 250 chemical compounds, especially A, B, C, D and E vitamins, proteins, amino acids, fatty acids, as well as flavonoids, sterols, simple and complex sugars, and micro- and macronutrients such as potassium, calcium, magnesium, phosphorus, iron. Due to very diverse chemical composition and properties, pollen has been used for many years in natural medicine, but may be an allergen due to its composition. Standardized extracts of pollen positively affect the immune system, which consists of the activity of white blood cells and other forms of transmitters functioning in systemic blood and lymph. However the cross intake with drugs for these diseases shoud be carried out with precautions to avoid serious interactions.

Introduction and objective

In botanical terms, pollen is composed of male reproductive cells, produced by the plant in anthers (part of the stamens). This product, in the form of pollen load (bee pollen) or bee bread (stored inside the hive and add with honey) is the base food for the offspring (larvae) in the bee colony. To use bee pollen as food or as a complement in the diet is most valuable when it comes from as many various plant species as possible given, like this, the major variety of compounds. Its chemical composition depends on the season, in connection with the flowering phases specific to various plant species as well as on the soil fertility, the soil moisture and the

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weather conditions during anthers' formation and maturation. While collecting pollen, worker bees mix it with a small amount of the secretion from their salivary glands. Then, pollen is put in baskets, located on the third pair of legs, and transported to the hive. Finally, pollen is placed in the comb cells (MALERBO-SOUZA 2011).

Pollen (bee pollen) in the form of the so-called pollen load or bee bread is successfully used in apitherapy and alternative medicine because of its diverse composition and chemical properties. As a dietary supplement it is an important alternative solution to standard treatments for many diseases (YILDIZ et al. 2013, DENISOW et al. 2016, KUMARI et al. 2016, BAZMANDEGAN et al 2017). However, until now the scientific data available is not enough to consider this product as safe to be considering a drug. Increasingly, doctors, patients, as well as animal owners, acknowledge diets based on natural products and bee pollen follows that tendency because the traditional use in certain countries. A number of positive effects of pollen have been proven so far, effective against fungal, bacterial and viral diseases, as well as anti–inflammatory and immune-stimulating properties (KROYER et al. 2001, ALMARAZ-ABARCA et al. 2004, KOCOT et al. 2018). Nevertheless, in the majority of the cases, the activity is dependent of the floral source and the quality control of the product wil be the only way to assure that correspondance with the potential bioactivity. Propolis and bee pollen extracts are used instead of the raw substance due to the fact that they contain higher amounts of bioactive components. The properties of the extract depend strongly not only on the solvent used but also on extraction conditions, that is, time and temperature as well (KIM et al. 2015, DENISOW et al. 2016, PASUULETTI et al. 2017) . Pollen is collected from various species of plants by worker bees and the selection of the species involved in the mixtures collected are difficult. This natural product is rich in biologically active substances and more research should be carried out to assure the efficacy, safety and quality control required for the World Health Organization. In apitherapy, a way of treat peoplewith bee products such as honey, propolis, pollen or bee venom are used in the treatment and prevention of various diseases. These products should be safe as all the others used in medicine. (KOMOSIŃSKA-VASSEV et al. 2015, OLCZYK et al. 2016). Many scientific studies have confirmed a wide spectrum of pollen properties, for example: nourishing, detoxification, antisclerotic, antibiotic and anti–inflammatory (MOREIRA et al. 2013, WEI et al. 2017, KOCOT et al. 2018). However, to complete a full analysis a scarce essays were carried out to assure the safety of the product despite various countries have already legislation to assure the quality control of the product in the market

Description of the state of knowledge

In chemical terms, pollen contains approx. 250 substances and chemical compounds, especially B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, inositol, cyanocobalamin) and pro-vitamin A in the form of *β*-carotene, ascorbic acid (vit. C), calciferol (vit. D) and tocopherol (vit. E), 22.7% protein (albumin, globulin, glutelin, prolamin) and protein enzymes, 10.4% amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, cystine and tryptophan), fatty acids (saturated acids: myristic, palmitic, stearic, arachidic and unsaturated fatty acids: oleic acid, linoleic and α-linolenic acid) and flavonoids 1.4% (mainly kaempferol, quercetin and isorhamnetin), sterols, simple and complex sugars and micro- elements such as potassium, calcium, magnesium, phosphorus, iron, sodium and zinc (Rzepecka-Stojko et al. 2015, Panche et al. 2016). Phospholipids represent 1.5%, phytosterols 1.1% and phenolic compounds 1.6%. The detailed chemical composition of pollen depends on the plant type, geographical origin, climate conditions, soil type and even the breed of bees (Nougeira et al. 2012, Silva et al. 2014). Furthermore, the color, shape (round, cylindrical, triangular) and weight of grains of pollen depend on the plant species from which it is collected (Almeida-Muradian et al. 2005, Arraez et al. 2007, Campos et al. 2003, 2008, Kędzia et al. 2005, Khalil et al. 2010, Leja et al. 2012, Saric et al. 2009, Shubharani et al. 2013, Xu et al. 2009). Some studies have shown that the content and properties of bee pollen are dependent on the kind of its plant source and the conditions of the plants growing for example like soil and climate (Feas et al. 2012, Pascoal et al. 2014).

Research has confirmed a broad spectrum of pollen properties. For example, pollen as a potential as analgesic agent thanks to the content of ethanol extracts obtained from a pine species growing in Korea (*Pinus densiflora*), Choi et al. (2007), have demonstrated its antinociceptive effects. They administered intragastrically to mice, these extracts in a dose of 100 and 200 mg/kg b.w. an hour before testing for analgesic activity. They have proved that these extracts were characterized by potent antinociceptive properties comparable with aminopyrine (a standard painkiller).

Moreover, anti-angiogenic properties of pollen were study. The angiogenesis is a process of formation of blood vessels from a primary endothelialand increases the supply of nutrients, growth factors and molecular oxygen to sites damaged or renewed during such processes as pregnancy, menses, wound healing and revascularization of ischemic tissues. How-

ever, an excessive angiogenesis (neovascularization) is characteristic of rheumatoid arthritis, atherosclerosis or retinal vascularization (IZUTA et al. 2009). IZUTA et al. (2009) considered the effect of the pollen of two plants: *Cistus ladanifer* and *C. albidus* (grey-leaved cistus) on angiogenesis of endothelial cells of human umbilical vein (HUVEC). They found that the ethanolic extract is characterized by potent anti-angiogenic properties, i.e. inhibiting angiogenesis. They also found that humans receiving the pollen showed a 30% reduction of the ability of blood platelets to aggregate and a reduction of the level of lipids and cholesterol from 30 to 20%. This therapeutic potential of *C, ladanifer* and *C. albidus* bee pollen should be study in further research, however the hemorrhagic side effect due to the reducing of platelets aggregation need to be controlled.

SIAFAKA-KAPADAI et al. (1986) also demonstrated that a glyceryl ether fraction obtained from the *Pinus halepensis* pollen by chromatography showed anti-platelet aggregation properties. The fraction at a concentration of 4.5 µmol l^{-1} inhibited in vitro aggregation of a rabbit's blood platelets induced with an activating factor (PAF) at a concentration of 1.5 nmol l^{-1}. This bioactivity as a potential for prevent atherosclerotic events but, as mentioned above, the precaution with the hemorrhagic side effect is crucial for the success of further investigatios.

Antiatherosclerotic properties preventing ischemic myocardial disease and strokes in humans receiving the pollen were carried out by KĘDZIA et al.(1994) and SZCZĘSNA et al. (1999) among others. Patients with multiple atherosclerotic arteries and advanced myopia and partial optic nerve atrophy showed a reduction of the levels of serum cholesterol as well as an increased field of view and stabilization of visual acuity after administration of the pollen (MACHOY-MOKRZYŃSKA et al. 1992). In several studies on animals, pollen bioactive substances improved liver function (UZBEKOVA et al. 2003). KLARIC et al (2018) determined the influence of dietary supplementation with propolis and bee pollen on liver pathology in broiler chickens. The control group of chickens received a basal diet, the experimental groups of chickens were fed with the same diet further supplemented with bee pollen and propolis. Researchers showed that the clusters of lymphocytes in the hepatocytes, the vacuolar degeneration and necrosis of the liver parenchyma, the bile ductile hyperplasia, and the various forms of pathological changes in the liver arteries and veins were more frequent in liver tissue samples of the control group compared to liver tissue samples of all the experimental groups. KLARIC et al. (2018) suggested that the supplementation of broiler chickens with bee pollen and propolis has a strong protective effect on liver pathology in broiler chickens.

The protective properties of pollen against ionizing radiation and its anti-platelet activity with regard to blood platelets as well as inhibiting the production of lipofuscin pigment in experimental animals have also been described. WANG et al. (1987) γ-irradiated with a dose of 7-8 Gy (grays) a group of mice fed with standard diet, and another group whose feed with a diet contained pollen. The experiment showed that in the group of mice whose feed contained pollen, the mortality rate reached 23.3%, while in the other group, with no pollen added to the diet, the mortality rate was in a range of 83.3% .

The consumption of pollen also has an effect on the production of lipofuscin. As an organism ages, a brown pigment (lipofuscin) accumulates in the cells. This accumulation is directly proportional to age. LIU and LI (1990) have demonstrated a significant reduction in concentration of lipofuscin in the myocardium, liver, brain and adrenal glands of mice fed with pollen as compared to the levels found in animals which were not receiving pollen. They have proved that the pollen slows down aging processes of animals (LIU et al. 1990).

Through the presence of phospholipids, pollen can protect the body against hepatosis (functional disorder of the liver), thus against the development of atherosclerosis. Phospholipids found in pollen, as lipotropic factors, inhibit the accumulation of lipids in hepatocytes, and protect the body against hepatosis. These compounds are part of cell membranes and they regulate selectively the penetration of substances into cells, thus they play a very important role in metabolism (PAUPIERE et al. 2014). Pollen supplementation also regulates the lipid-protein metabolism of the organism. Matuszewski and Drake's research proves that pollen normalizes the lipid-protein metabolism, as the level of liver enzymes in the blood serum has been significantly reduced. Served with toxic substances, pollen protected the liver cells from their harmful influence. The phenolic compounds contained in pollen exhibit a broad spectrum of biological activity in the body, i.e. they have an anti-inflammatory, antioxidant and anti-arteriosclerosis function, they strengthen capillaries and protect against ionizing radiation. Tikhonov et al. investigated the effect of pollen in the case of hypoxia in experimental animals – rats. The first group of animals had been receiving an intragastric preparation based on pollen preventively for 14 days, while the other group within the same period had been treated with Piracetam (a drug acting on cells of the central nervous system). Piracetam is a product which increases the use of oxygen and glucose, and improves blood circulation in the brain by reducing blood viscosity. Moreover, it improves microcirculation without broadening blood vessels or changes in blood pressure. Brain hypoxia was invoked in rats by ligature

of both artery blood vessels. The analysis of the results showed that after 7 days in the control group receiving no preparation or drug – 27% of animals survived, in the group receiving Piracetam it was 38% and in the group receiving the pollen preparation – 55% of animals (TRICHONOW et al. 2008). Thus, flavonoids and phenolic acids play an important role in the process of detoxification of liver tissue (ADRITOIU et al. 2014, FLOREK et al. 1995, JUŹWIAK et al 1992, KHALIL et al. 2010, PUT et al. 1994, WÓJCICKI et al. 1985, YILDIZ et al. 2013).

Pollen supplementation in the diet intends to increase the mental and physical efficiency of people. It has been demonstrated that pollen has an adaptogenic (adaptive) effect by increasing the resistance against harmful factors: physical, chemical and biological. The increase encompasses both physical efficiency of the organism in situations of increased physical effort and the improvement of brain functions such as memory, learning, thinking and the ability to concentrate, as well as an increase of the body's resistance to infection. Clinical studies were conducted with the participation of people suffering from mental illnesses and the elderly. Due to its nourishing and toning properties, pollen improves mental abilities and strengthens the nervous system weakened by stress or overwork. Particularly positive results were obtained in the elderly. The long-term use of pollen, even in small doses, results in a gradual improvement in the mood, restores interest in life and strengthens the body (WÓJCICIKI 1991). Both pollen and its extracts can be used in post-infarction states, as well as in peripheral circulatory disturbances and hypertension. In addition, in the case of the elderly the administration of small doses of this product helps inhibit cerebral atherosclerosis and improves cerebral circulation (HOWE et al. 1985).

TELESZUN et al. (1993) compared the effects of pollen in the form of bee pollen loads, and a lyophilised extract of pollen in the rehabilitation of workers eliminating the breakdown in the Chernobyl nuclear power plant, and suffering from complex neurological diseases. Bee pollen basket and the lyophilized extract of pollen were administered to patients with vegetative-vascular dystonia and organic damage of the central nervous system caused by ionizing radiation. It was proved that the neurological status of both groups of patients improved significantly after the treatment with pollen. They obtained better results using pollen loads compared to treatment with the lyophilised extract. The symptoms like headaches, pain of vegetative points, irritability, vascular lability and sleeping disorder disappeared after a few days. Also, it was observed that the administration of pollen results in a significant increase in phagocytic activity of peripheral blood. In the case of treatment using pollen loads, the number

of phagocytes capable of absorbing and disposing of pathogenic microorganisms has increased x 2.26, while using the lyophilized extract of pollen x 1.9 (TELESZUN et al. 1993).

GULHAN (2018) investigated therapeutic and protective effects propolis and pollen on reproductive functions of L-NAME-induced hypertensive male rats. Animals were indiscriminately separated into four groups: control, L-NAME, L-NAME+ propolis, L-NAME+ pollen. He showed that the levels of parameters (TOS, NF-kB, MDA) in L-NAME+ propolis and L-NAME+ pollen groups compared to the L-NAME group have decreased. TAS levels, PON1 and CAT activities were significantly decreased in testis tissue samples in the L-NAME-induced group. He thought that propolis or pollen is thought to help regulate reproductive function by inhibiting the functioning of inflammatory pathways leading to hypertension.

The impact of pollen on the physical condition of climbers was also evaluated. It was noticed that after pollen supplementation the number of various forms of white blood cells, including lymphocytes, monocytes and eosinophils, significantly increased. The level of eosinophils in climbers' blood serum at the end of the experiment was 2x higher, monocytes 2.5x higher than in serum prior to the experiment (DROŻDŹ et al. 1986). MALMSTRÖM and CEDERLÖF (1983) used the properties of pollen in the treatment of the upper respiratory tract. For 14 days soldier trainees received pollen preparation to prevent a cold. It was demonstrated that only 35% of them developed various kinds of infections of the upper respiratory tract. Those who did not receive the pollen, had a cold of various intensity. There are many reports on the antibacterial and antifungal effect of pollen. It affects both human pathogenic bacteria (*Staphylococcus aureus* and Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeurgionsa*) and the yeast-like fungi (*Candida albicans*). Mainly flavonoids and phenolic acids are responsible for this effect (BALTRUSYAT et al. 2007, ERKMEN 2008, LIEBELT et al. 1994, MATEESCU et al. 1997).

Pollen also has a positive impact on curing prostate inflammation. In the research, approximately 87% of patients with nonbacterial inflammation of prostate felt better after pollen treatment. What is more, a positive effect was observed in 50% of patients with the benign prostatic hypertrophy (DOBROVODA 1986, KRIWCZANSKIJ 1987). Pollen can also be used in curing iron deficiency anemia. It was noticed that in people treated with pollen, the number of red blood cells and the level of hemoglobin and Fe increased. Clinicians have proved that pollen is very effective in curing duodenal ulcer disease. The radiological and endoscopic research has proved that after administration of pollen the duodenal ulcers began to cicatrize. Satisfying results were also obtained in the treatment of bleed-

ing duodenal ulcers not qualified for surgery. The hemorrhages disappeared after 3–4 days of pollen treatment, while in the traditional way it takes 10 days to stop duodenal ulcers bleeding (KEDZIA 1994). Pollen showed good effects in treating: gout, kidney inflammation, urinary tract disorders, climacteric and vegetative dystonia. There is an increasing amount of data on supplementation of pollen to animal diets, for example, the research on its influence on the condition of pigs. Pollen is characterized by a high anti-inflammatory effect confirmed in animal studies. Its properties can be compared to anti-inflammatory drugs such as naproxen or indomethacin. The mechanism is based on inhibiting the activity of enzymes responsible for forming inflammatory mediators in tissues. Concentrated extract of the pollen fed to a rat eliminated paw edema associated with the administration of carrageenan. Flavonoids, phenolic acids, fatty acids and phytosterols which are present in pollen are responsible for the antioedematous effect. It has been demonstrated in experimental studies that the administration of pollen in a dose of 50 mg/kg b.w. eliminates 75% of paw edema (CHOI 2007, LOSCHEN et al. 1991).

It has been proved that pollen has a high nutritional value. Mice and rats fed with pollen were characterized by a higher content of vitamin C and Mg in tissues and a higher content of hemoglobin, as well as a bigger number of red blood cells compared to animals given a standard feed. Feeding with pollen caused a rapid increase in the body weight compared to an ordinary diet served to starved animals or animals on a diet without vitamins. OLIVEIRA et al. proved that a crucial role in this process is played by exogenous amino acids, vitamins and bioelements. Moreover, studies in rodents showed that the contents of vitamin C and Mg in the thymus, heart muscle and skeletal muscles increase after pollen supplementation. Both the level of hemoglobin and the number of red blood cells was higher compared to the results obtained in animals fed with standard feed (OLIVEIRA et al. 2009, LIEBELT et al. 1994, MATEESCU et al. 1997).

A hypolipidemic effect of the pollen has also been reported. Scientists conducting research on rats and rabbits have discovered that pollen reduces the content of total lipids, triglycerides, total cholesterol, LDL cholesterol fraction and β-lipoproteins in blood serum (JUŹWIAK et al. 1989). Similar studies were conducted in humans. They confirmed that in the blood serum of people with impaired lipid metabolism, there was a reduction in the content of lipids after the administration of pollen (MANNING 2001, KASSYANENKO et al. 2010).

Due to high contents of phytosterols, pollen shows an estrogenic effect by stimulating the process of producing and maturation of ova in animals and humans (TRICHONOW et al. 2006). Pollen contains 0.1–1.6% of phytos-

terols. Phytoestrogens are structurally similar to endogenous estrogens and therefore they connect with estrogen receptors. Also, a relationship has been detected between the activity of estrogen receptors and cardiac physiology, which influences the regulatory systems of oxytocin. The estrogenic activity of these compounds has a beneficial effect on the maturation of oocytes in animals. Researchers conducted tests on rabbits fed with pollen which checked:

a) the functioning of ovaries;
b) therapeutic effects on blood profiles and parameters;
c) the number of offspring.

They discovered that a dose of 200 mg/kg b.w. in a rabbit caused an increase in the quality of sperm, increased fertility, as well as improved blood and biochemical parameters (ATTIA et al. 2011).

Many publications are devoted to the detoxifying properties of pollen (FLOREKET al. 1995, JUŹWIAK et al. 1992, WÓJCICKI et al. 1989). Polyphenols, mainly flavonoids and phenolic acids present in pollen play an important role in the process of body detoxification. Studies were conducted in rats which were administered organic solvents, such as carbon tetrachloride and trichloroethylene. These substances caused deep damages of the rats' liver cells. The animals were also given ethanol and allyl alcohol which caused fatty liver cirrhosis, as well as drugs: acetaminophen and hydrocortisone. After feeding rats with pollen the level of liver enzymes and bilirubin in the blood serum was reduced to physiological values. There was a very high concentration of alanine, aspartate transaminase, acid phosphatase and bilirubin in the blood serum. Hence, pollen is recommended in acute and chronic inflammation, in the early stages of degenerative diseases, congestive liver diseases, as well as toxic and traumatic injuries of this organ.

Metabolizing properties of Fe, Ca and Mg in rats with anemia were examined, as well as the antiallergic properties of myricetin present in pollen given to mice. The impact of the pollen and propolis on the organism was studied. It was found that the addition of these products to the diet resulted in body weight gain. An increased level of hemoglobin in animals with anemia was observed, and a positive effect of these products on the level of Mg in the organism (HARO et al. 2008). The therapeutic effects of the pollen present in Apiter product were observed in dogs and cats infected with rickettsiales. The pollen was administered to animals at the age of 2–8 months with bone and articular diseases and ascertained rickettsiosis. Patient selection was based on the analysis of X-ray images and biochemical tests. Research has shown that in animals supplemented with pollen, biochemical parameters have reached a physiological level: Ca level

increased from 4 mg dl^{-1} to 9.2–11.5 mg dl^{-1}, P from 1.6–3.81 mg dl^{-1} to 4.1–5.7 mg dl^{-1}, Mg level normalized (2.5–3 mg dl^{-1}). The reduction in alkaline phosphatase 116-443 UI l^{-1} to 77–187 Ul l^{-1} has been reported, with a simultaneous normalization of liver enzymes (ALT, GGT, AP) (SAPCALIU et al. 2009). The influence of pollen on the length of the villi of the small intestine in broilers was studied. It was found that the supplementation of pollen in the feed has a beneficial effect on the intestinal villi, increasing their length (in the 1st and 2nd week of age in chickens) by 37.1% and 29.4% in duodenum, 28.1% and 33.7% in jejunum and 18.6% and 16.2% in ileum (Wang et al. 2009).

On the basis of the above mentioned research results it can be concluded that flower pollen (bee pollen) is characterized by a number of favorable biological properties which are often used in medicine to improve health (ALYANE et al. 2008, ARCT et al. 2008, PASUPULETI et al. 2017). For many years we have been observing an increased interest of supplements based on bee products which are often used in alternative medicine or as a supplement to conventional medicine.

The major problem is that bee-collected pollen may constitute risk factor concerning the presence of contaminants like a heavy metals, pesticides, bacteria, antibiotics). Therefore standardized pollen tablets are recommended. Moreover, allergic reactions including anaphylaxis have been recognised after intake of bee-collected pollen. Patients who are prone to allergies or atopic individuals should avoid any type of bee pollen, bee-collected and extracts. The application of bee pollen should be discussed with doctors in order to avoid complications (DENISOW et al. 2016).

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References

ALMARAZ-ABARCA N., CAMPOS M.G., ÁVILA-REYES J.A., NARANJO-JIMÉNEZ N., HERRERA-CORRAL J., GONZÁLEZ-VALDEZ L.S. 2004. *Variability of antioxidant activity among honeybee-collected pollen of different botanical origin*. Interciencia, 29: 574–578.

ALMEIDA-MURADIAN L.B., PAMLONA L.C., COIMBRA S., BARTH O.M. 2005. *Chemical composition and botanical evaluation of dried bee pollen pellets*. J. Food. Compos. Anal., 18: 105–111.

ALYANE M., KEBSA L.B., BOUSSENANE H., ROUIBAH H., LAHOUEL H. 2008. *Cardioprotective effects and mechanism of action of polyphenols extracted from propolis against doxorubicin toxicity*. Pak. J. Pharm. Sci., 3: 201–209.

ANDRITOIU C.V. OCHIUZ L., ANDRITOIU V., POPA M. 2014. *Effect of apitherapy products against carbon tetrachloride-inducted toxicity in Wistar rats*. Rom. J. Morphol. Embryol., 55: 835–847.

ARCT J., PYTKOWSKA K. 2008. *Flavonoids as components of biologically active cosmeceuticals*. Clin. Dermatol., 4: 347–357.

ARRAEZ ROMAN D., ZUREK G, BABMANN C., ALMARAZ-ABARCA N., QUIRANTES R., SEGURA-CARRETERO A., FERNANDEZ-GUTIERREZ A. 2007. *Identification of phenolic compounds from pollen*

extracts using capillary electrophoresis-electrospray time of flight mass spectrometry. Anal. Bioanal. Chem., 389: 1909–1917.

Attia Y.A., Al-Hanoun A., Tag El-Din A.E., Bovera F., Shewika E. 2011. *Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months*. J. Anim. Physiol. Anim. Nutr. (Berl)., 95(1): 17–26.

Baltrusayt V., Venskmonis P.R., Ceksteryte V. 2007. *Antibacterial activity of honey and bee-bread of different origin against Saureus and S-epidermidis*. Food Technol. Biotech., 2: 201–208.

Bazmandegan G., Boroushaki M.T., Shamsizadeh A., Ayoobi F., Hakimizadeh E., Allahtavakoli M. 2017. *Brown propolis attenuates cerebral ischemia-induced oxidative damage via affecting antioxidant enzyme system in mice*. Biomed. Pharmacother., 85: 503–510.

Campos M.G.R., Bogdanov S., Almeida-Muradian L.B., Szczesna T., Mancebo Y., Frigerio C., Ferreira F. 2008. *Pollen composition and standardization of analytical methods*. J. Apicult. Res. Bee World, 47: 156–163.

Campos M.G., Webby R.F., Markham K.R., Mitchell K.A., Da Cunha A.P. 2003. *Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids*. J. Agric. Food. Chem., 51: 742–745.

Choi E.M. 2007. *Antinociceptive and antiinflammatory activities of pine (Pinus densiflora) pollen extract*. Phytother. Res., 21: 471–5.

Denisow B., Denisow-Pietrzyk M. 2016. *Biological and therapeutic properties of bee pollen*. J. Sci. Food. Agri., 96: 4303–4309.

Dobrovoda I. 1986. *Včelie produkty a zdravie*. Priroda, Bratislava.

Drożdż E., Gwizdek E. 1986. *Pyłek kwiatowy jako odżywka regeneracyjna*. V Międzynarodowe Sympozjum Apiterapii. Zagadnienia wybrane. Wydawnictwo Polskiego Związku Pszczelarskiego, Kraków-Kamianna, pp. 24–27.

Erkmen O., Ozcan M.M. 2008. *Antimicrobial effects of Turkishpropolis pollen, and laurel on spoilage and pathogenic foodrelated microorganisms*. J. Med. Food., 11: 587–592.

Feás X., Vázquez-Tato M.P., Estevinho L., Seijas J.A., Iglesias A. 2012. *Organic bee pollen: botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality*. Molecules., 7: 8359–8377.

Florek E., Leciejewska A. 1995. *Próba zastosowania preparatów pszczelarskich w profilaktyce zatruć trichloroetylenem*. Herba. Pol., 41(2): 70–76.

Gulhan M.F. 2018. *Therapeutic potentials of propolis and pollen on biochemical changes in reproductive function of L-NAME induced hypertensive male rats*. Clin. Exp. Hypertens., 17: 1–7.

Haro A., Lopez-Aliaga F., Lisbona M., Barrionuevo M.J., Alferez M., Campo M.S. 2000. *Beneficial effect of pollen and propolis on the metabolism of iron, calcium, phosphorous, and magnesium in rats with nutritional ferropenic anemia*. J. Agric. Food. Chem., 48: 5715–5722.

Howe S.R., Dimick P.S., Benton A.W. 1985. *Composition of freshly harvested and commercial royal jelly*. J. Apic. Res., 24: 52–61.

Izuta H., Shimazawa M., Tsuruma K., Araki Y., Mishima S., Hara H . 2009. *Bee products prevent VEGF-induced angiogenesis in human umbilical vein endothelial cells. BMC Complement Alternat*. Med., 9: 45–55.

Juźwiak S., Raińska T., Dutkiewicz T., Cioch U., Olenderek B., Krasowska B., Różewicka L., Juzyszyn Z., Wójcicki J., Samochowiec L. 1992. *Pollen extracts reduce the hepatotoxicity of paracetamol in mice*. Phytother. Res., 6: 141.

Juźwiak S., Samochowiec L., Wojcicki J. 1989. *The influence of pollen extracts on serum triglyceride lipase activity in rabbits on a high-fat diet*. Herba Pol., 35: 43–48.

Kassyanenko V., Komisarenko I., Dubtsova E. 2010. *Influence of honey, pollen and bee bread on serum cholesterin of patients with pathological lipid metabolism, in beekeeping, apitherapy and life quality*. International Industrial Acadamy, Moscow Russia, pp. 81–82.

Kędzia B., Hołderna E. 1994. *Leczenie produktami pszczelimi*. PWRiL, Warszawa.

Kędzia B., Hołderna-Kędzia E. 2005. *Produkty pszczół w terapii. Zagadnienia wybrane*. Wydawnictwo Fundacji Humana Divinis, Toruń.

Khalil F.A., El-Sheikh N.M. 2010. *The effects of Dietary Egyptian Propolis and Bee pollen Supplementation against toxicity if sodium fluoride in rats*. J. Am. Sci., 6: 310–316.

Kim S.B., Jo Y.H., Liu Q., Ahn J.H., Hong I.P., Han S.M., Hwang B.Y., Lee M.K. 2015. *Optimization of extraction condition of bee pollen using response surface methodology: correlation between anti-melanogenesis, antioxidant activity, and phenolic content*. Molecules., 11: 19764–19774.

Klaric I., Pavic M., Miskulin I., Blazicevic V., Dumic A., Miskulin M. 2018. *Influence of dietary supplementation of propolis and bee pollen on liver pathology in broiler chickens*. 8: 1–10, doi: 10.3390/ani8040054.

Kocot J., Kiełczykowska M., Luchowska-Kocot D., Kurzepa J., Musik I. 2018. *Antioxidant potential of propolis,bee pollen and royal jelly: possible medicalapplication*. Oxid. Med. Cell. Longev, 1–29.

Komosinska-Vassev K., Olczyk P., Kamierczak J., Mencner L., Olczyk K. 2015. *Bee pollen. chemical composition and therapeutic application*. Evidence-Based Complementary and Alternative Medicin, article ID 297425, http://dx.doi.org/10.1155/2015/297425.

Kriwczanskij I.E., Indriczanu W.N. 1987. *Ljubitelskoje pczełowodstwo*. Kartja Mołdowenjaske, Kisziniew.

Kroyer G., Hegedus N. 2001. *Evaluation of bioactive properties of pollen extracts as functional dietary food supplement*. Innov. Food Sci. Emerg., 2: 171–174.

Kumari S., Naik P., Vishma B.L., Sujith R.S., Raviraj A.D., Saleemulla K., Srinivas M., Guruprasad K., Satish K.A. 2016. *Mitigating effect of Indian propolis against mitomycin C induced bone marrow toxicity*. Cytotechnol., 68: 1789–1800.

Leja M., Mareczek A., Wyzgolik G., Klepacz-Baniak J., Czekońska K. 2007. *Antioxidative properties of bee pollen in selected plant species*. Food Chem., 100: 237–240.

Liebelt R.A., Lyle D., Walker J. 1994. *Effects of a Bee Pollen diet on survival and growth of inbred strains of mice*. Am. Bee. J., 134: 615–620.

Liu X.L., Li L.M. 1990. *Morphological observation of effect of bee pollen on intercellular lipofuscin in NIH mice*. J. Chin. Mater. Med., 15: 561–3.

Loschen G., Ebeling L. 1991. *Hemmung der Arachidonsäure-Kaskade durch einen Extrakt aus Roggenpollen*. Arzneim.- Forsch./Drug Res., 41: 162.

Machoy-Mokrzynska A., Loniewski I., Wojcicki J., Samochowiec L. 1992. *Influence of pollen extracts on the central nervous system*. Herba Pol., 38: 189–194.

Malerbo-Souza D.T. 2011. *The corn pollen as a food source for honeybees*. Acta. Sci. Agron., 33: 701–704.

Malmström S., Cederlöf R. 1983. *Pollen as a prophylactic against the common cold*. Herba Pol., 29: 229–35.

Manning R. 2001. *Fatty acids in pollen: a review of their importance for honey bees*. Bee World, 82: 60–75.

Mateescu C., Barbulescu D., Cristea A. 1997. *Pollen extracts – nutritive-functional and therapeutical potentialities*. XXXVth Int. Congr. Apimondia, Antwerp (Belgia), 122.

Moreira F., de Souza G.H.B., Rodrigues J.V., Lopes N.P., de Oliveira A.R.M. 2013. *A non-Michaelian behavior of the in vitro metabolism of the pentacyclic triterpene alfa and beta amyrins by employing rat liver microsomes*. J. Pharm. Biomed. Anal., 84: 14–19.

Nogueira C., Iglesias A., Feás X., Estevinho L.M. 2012. *Commercial Bee Pollen with different geographical origins: a comprehensive approach*. J. Mol. Sci., 13: 11173–11187.

Olczyk P., Koprowski P., Kafmierczak J., Mencner L., Wojtyczka R., Stojko J., Olczyk K., Komosinska-Vassev K. 2016. *Bee Pollen as a promising agent in the burn wounds treatment*. Evidence-Based Complementary and Alternative Medicine. Doi:10.1155/2016/8473937.

Oliveira F.P.M., Absy M.L., Miranda I.S. 2009. *Recurso polínico coletado por abelhas sem ferrão (Apidae, Meliponinae) em um fragmento de floresta na região de Manaus, Amazonas*. Acta Amaz., 39: 505–518.

Panche A.N., Diwan A.D., Chandra S.R. 2016. *Flavonoids: an overview*. J. Nutr. Sci., 5: 47.

Pascoal A., Rodrigues S., Teixeira A., Feás X., Estevinho L.M. 2014. *Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory*. Food Chem. Toxicol., 63: 233–239.

Pasupuleti V.R., Sammugam L., Ramesh N., Gan S.H. 2017. *Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits*. Oxid. Med. Cell. Longe., 1–21.

Paupière M.J., van Heusden, A.W., Bovy A.G. 2014. *The metabolic basis of pollen thermo-tolerance. Perspectives for breeding*. Metabolites, 4: 889–920.

Put A., Myśliwiec Z. 1994. *Der Einfluss von Pollenextrakten auf biochemische Indikatoren der Leberfunktion im Verlauf einer chronischen Intoxikation mit Ammonium fluorid bei Ratten*. Teil II. Arztezeit Nanurheil., 35: 351.

Rzepecka-Stojko A., Stojko J., Kurek-Górecka A., Górecki M., Kabała-Dzik A., Kubina R. Moździerz A., Buszman E. 2015. *Polyphenols from bee pollen: structure, absorption, metabolism and biological activity*. Molecules, 12: 21732–21749.

Sapcaliu A., Radoli I., Condur D., Cauia E., Guresoaiei I., Radoi C., Siceanu A., Antonescu C., Butu A., Crengu P. 2007. *Effects of therapeutic nutrition with the apitherapeutic product Apiter in dogs and cats in rickets disease*. Lucrări ştiinţifice Zootehnie şi Biotehnologii, 40(2): 118–125.

Sarić A., Balog T., Soboćanec S., Kušic B., Šverko V., Rusak G., Likic S., Bubalo D., Pinto B., Reali D., Marotti T. 2009. *Antioxidant effects of flavonoid from Croatian Cystus incanus L rich bee pollen*. Food. Chem. Toxicol., 47: 547–554.

Shubharani R., Roopa P., Sivaram V. 2013. *Pollen morphology of selected bee forage plants*. Global J. Bio. Sci. Biotechnol., 2: 82–90.

Siafaka-Kapadai A., Demopulos C.A., Andriokopulo N.K. 1986. *Biological activity of lipids of pine pollen on platelet aggregation in correlation with the platelet activating factor*. Biochem. Int., 12: 33–41.

Silva G.R., da Natividade T.B., Camara C.A., da Silva E.M.S., de Assis Ribeiro dos Santos S., Silva T.M.S. 2014. *Identification of sugar, amino acids and minerals from the Pollen of jandaíra stingless bees (Melipona subnitida)*. Food. Nutr. Sci., 11: 1015–1021.

Szczęsna T., Rybak-Chmielewska H., Chmielewski W. 1999. *Pyłek kwiatowy (obnóża) – naturalna odżywka i surowiec farmaceutyczny*. Wyd. Oddz. ISK, Skierniewice, p. 37.

Teleszun O.P., Drobjazina I.S., Tonakowa G.W. 1993. *Słożnyje lipidy cwietocznoj pylcy w profilaktikie naruszenij obmiena wieszczestw*. Apiterapia i Pczełowodstwo, 19: 49–56.

Tichonow A.L., Sodzawicznyj K., Tichonowa S.A. 2006. *Pylca cwietocznaja (obnożka pczelinaja) w farmacii i medicinie*. Charkow, Izd. Originał.

Tichonow A.I., Sodzawiczn K., Tichonow S.A., Jarnych T.G., Bondarczuk L.I., Kotenko A.M. 2008. *Pyłek kwiatowy – obnóże pszczele w farmacji i medycynie*. Wydawnictwo Apipol-Farma, Kraków.

Uzbekova D.G., Makarova V., Khvoynitskaya L.G., Slepnev A.A. 2003. *Evaluation of bee-collected pollen influence on lipid peroxidation, antioxidant system and liver function in old animals*. J. Hepatol., 38: 203.

Wang W., Hu J., Cheng J. 1987. *Biological effect of pollen from beehives radioprotective effect on hematopoietic tissues of irradiated mice*. XXXI Int. Apicult. Congr. Apimondia, Warsaw, p. 176.

Wang J., Li S., Wang Q., Xin B., Wang H. 2007. Trophic effect of bee pollen on small intestine in broiler chickens. J. Med. Food., 10: 276–280.

Wei C.C., Chang C.H., Liao V.H.C. 2017. *Anti-parkinsonian effects of β-amyrin are regulated via LGG-1 involved autophagy pathway in Caenorhabditis elegans*. Phytomedicine, 36: 118–125.

Wójcicki J. 1991. *The effect of pollen extracts on the endocrine function in rabbits*. Herba Pol., 27: 151.

Wójcicki J., Samochowiec L., Hinek A. 1985. *The effect of Cernitins on galactosamine-induced hepatic injury in rat*. Arch. Immunol. Ther. Exper., 33: 361–70.

WÓJCICKI, J., SAMOCHOWIEC L., KADLUBOWSKA D. 1989. *The influence of pollen extracts on biochemical disturbances in rats exposed to prolonged ethyl alcohol intake*. Herba Pol., 35: 201.

XU X., SUM L., DONG J., ZHANG H. 2009. *Breaking the cells of rape bee pollen and consecutive exraction of functional oil with supercritical carbon oxide*. Inno. Food Sci. Emerg., 10: 42–46.

YILDIZ O., CA Z., SARAL Ö., YULUL E., ÖZTÜRK F., ALIYAZICIOGLU R., CANPOLAT S., KOLAYLI S. 2013. *Hepatoprotective potential of chestnut Bee Pollen on carbon, tetrachloride-induced hepatic damages in rats*. Evidence-Based Complementary and Alternative Medicine, article ID 461478, 1–9.

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MICROBIOME OF THE DIGESTIVE TRACT AND PROBIOTIC THERAPY IN CYPRINIDS

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Key words: C*yprinidae*, gut microbiota, probiotic therapy.

Abstract

Bacteria play an extremely important role in the digestive processes occurring in the digestive tract of all vertebrates. A disturbance of microbial balance may lead to disorders in digestive processes. Due to the high demand for fish from aquaculture, many studies have focused on the microbiome of the digestive tract in these animals, especially synbiotic *Lactobacillus bacteria*, which play an extremely important and beneficial role in digestive processes. Research conducted in recent years has shown that many factors influence the microflora in fish, such as the surrounding environment, oxygenation, water temperature, food intake, antibiotics, chromium oxide, linoleic acid, and finally the development stage of the fish. The authors of the publication provide an overview of the current knowledge on the gastrointestinal microflora of *Cyprinidae* and its effect on their digestive processes. In this context information on the probiotic therapy in *Cyprinidae* was also presented.

Introduction

In terms of their taxonomic diversity fish constitute the greatest group among all the vertebrates found on Earth (NAYAK 2010, RAY et al. 2012). They inhabit both small freshwater watercourses and vast saltwater oceans. They have adapted to life under diverse environmental conditions, which affect the composition of their bacterial microflora, both external and internal. The microbiome in fish may be divided into autochthonous and allochthonous (RINGØ and BIRKBECK 1999, SYVOKIENI 1989). The former is a natural, endogenous element, while the latter is exogenous in character. In these vertebrates aerobic bacteria predominate along with

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facultative anaerobic bacteria (GHOST et al. 2002). The bacterial count in the digestive tract of fish is estimated at approx.10^8 aerobes and 10^5 anaerobes per 1 gram (TRUST and SPARROW 1974, KAMEI et al. 1985). The variation in the quantitative composition of the bacterial population in the surrounding environment exceeds that of the bacteria found in the digestive tract of fish (CLEMENTS et al. 2014). The common bacterial species colonizing the digestive tract of both freshwater and marine fish include *Vibrio, Aeromonas, Flavobacterium, Plesiomonas, Pseudomonas, Enterobacteriaceae, Micrococcus, Acinetobacter, Clostridium, Fusarium* and *Bacteroides*, which occurrence may differ depending on the species as well as environmental conditions. Moreover, several previously unknown bacteria, i.e. *Mycoplasma, Arthrobacter, Brochothrix, Jeotgailbacillus, Ochrobactrum, Psychrobacter* and *Sejongia*, have been identified in the digestive tract of various fish species (NAYAK 1997).

Aeromonas, Pseudomonas as well as *Flavobacterium* are dominant bacteria in freshwater fish species. However, feeds have a crucial effect on the composition of the intestinal microbiome (BAIRAGI et al. 2002, SAVAŞ et al. 2005). In the case of farmed fish *Enterobacteriaceae* account for approx. 50% bacterial population in their alimentary canal, which is directly related with the feeds used in fish farming, while in the case of species living in natural water bodies bacteria from genera *Aeromonas* and *Lactobacillus* predominate in their microflora (GANGULY et al. 2013). Recent research indicates that percentage shares of individual bacteria in the species composition depends on the position of the fish in individual trophic classes. In the case of herbivorous fish *Proteobacteria* account for 45.52%, while in predatory, omnivorous and planktivorous fish they constitute 32.82%, 37.32% and 38.13%, respectively. Bacteria *Firmicutes* rank second in terms of their numbers, with their shares in these groups of fish ranging from 21.16% to 27.13%. For example, in predatory fish bacteria from the genus *Fusobacterium* account for a considerable share 21.91% in the bacterial population, in comparison to 9.41% in planktivorous fish. An opposite situation is observed in the case of *Acidobacteria*, accounting for 5.21% bacterial population in planktivorous fish, whereas in predatory fish it is only 1.08%. The shares of the other taxonomic groups are smaller (LIU et al. 2016). In the case of cyprinids *Firmicutes* and *Bacterioides* are dominant, while when consider the quantitative composition of bacteria *Proteobacterie* and *Firmicutes* are most numerous. Abundance of *Bacterioides* is relatively limited (WU et al. 2012).

Cyprinids are dominant fish in Polish aquaculture. The most numerous representatives of that family in fish farming is the common carp (*Cyprinus carpio*). Production of common carp for human consumption in

2016 in Poland was 18.55 thousand tons, which constituted 49% fish farming production in this country (LIRSKI and MYSZKOWSKI 2017). The other cyprinid species raised in Poland include the grass carp (*Ctenopharyngodon idella*), the crucian carp (*Carassius carassius*), the silver crucian carp (*Carassius gibelio*), the tench (*Tinca tinca*) as well as two planktivorous species: the bighead carp (*Hypophthalmichthys nobilis*) and the silver carp (*Hypophthalmichthys molitrix*).

Functions of intestinal microflora in fish

An appropriate composition of the intestinal microflora plays numerous roles required for the physiological function of the entire organism. This microflora produces antibiotics against pathogenic bacterial strains constantly penetrating the animal's body from the external environment. It participates in the host's digestive processes and supplies the host with active nutrients, such as digestive enzymes, vitamins and amino acids (SHCHERBINA and KAZLAWLENE 1971). It needs to be stressed here that in herbivorous fish the degradation of crude fibre, i.e. practically cellulose, takes place, among others, with the participation of aerobic bacteria or facultative anaerobic bacteria (SAVAŞ 2005). However, not all fish (*Carassius auratus*, *C. idella*) predominantly utilize cellulolytic bacteria to degrade crude fibre (GANGADHARA et al. 2004). Apart from microbial enzymes fish also have endogenous enzymes at their disposal. These enzymes have been described by numerous authors (DHAGE 1968, KAWAI 1972, SHCHERBINA et al. 1976, FAGBENRO 1990, DAS and TRIPATHI 1991, FAGBENRO et al. 2000). A good example in this respect may be provided by α-amylase, produced practically over the entire length of the intestinal section of fish both endogenously and by intestinal microflora (FAGBENRO et al. 2000, TENGJAROENKUL et al. 2000, ALARCÓN et al. 2001, FERNANDEZ et al. 2001). Thanks to the colonization rate of the digestive tract by enzymatically active intestinal bacteria, certain fish species digest some nutrients more readily than even closely related other fish species. Conducted studies have shown that intestinal bacteria are capable of producing various bioactive substances such as tetrodotoxin, vitamin B12, biotin, eicosapentaenoic acid or antibiotics, which through their action exert a positive effect on the host's organism (GANGULY and PRASAD 2012). Studies have shown that in common carps bacteria from the genera *Firmicutes* and *Bacterioides* promote a more rapid growth rate thanks to improved feed digestion and nutrient absorption (LI et al. 2013).

Gut microbiota and digestive processes in cyprinids

The microbiome of the digestive tract is not identical in all fish species. Differences are observed between various species from the family *Cyprinidae* or even individual representatives of a given species. A considerable variation is found in cellulose degrading microorganisms. The silver crucian carp (*Carassius auratus gibelio*) does not have its autochthonous cellulolytic microflora. In turn, the cellulolytic microbiota content in the grass carp is below 103 b.b./g (LESEL et al. 1986). Its nutrition is based on the consumption of large amounts of aquatic biomass and recovery of disaccharides and protein from that biomass (GANGADHARA et al. 2004). In contrast, the cellulolytic activity of the intestinal microbiota in the common carp is high (BAIRAGI et al. 2002). Two theories have been proposed on the origin of cellulase. One refers to the participation of the endosymbiotic flora in the production of this enzyme. This hypothesis is supported by the inhibition of cellulase secretion after fish have been administered antibiotics (SAHA et al. 2006). Common carps are capable of endogenous production of cellulase by the autochthonous microflora (SHCHERBINA and KAZLAWLENE 1971). This process takes place in the proximal section of the gut, where a portion of crude fibre is digested. The rest of this nutrient is digested in the distal sections of the intestines. In view of cellulose digestion, the diet of herbivorous cyprinids has to contain crude fibre (DAS and TRIPATHI 1991). Another theory proposes that fish collect cellulolytic flora with the consumed plant food. Conducted studies have shown a positive correlation between the amount of cellulase and the consumed food (PREJS and BŁASZCZYK 1997). Those authors confirmed that bacteria subsequently found in digestive tracts of fish were isolated on aquatic plants. These bacteria exhibited cellulolytic activity. Cellulase seems to be of both exogenous and endogenous origin. This would explain digestion of cellulose by certain fish species, having no autochthonous cellulolytic microflora, e.g. the above-mentioned *C. auratus*, or those having small amounts of this microflora, e.g. the grass carp. In the case of cellulase producers in common carps the most important bacteria included strains from the genera *Vibrio* spp., *Aeromonas* spp. and *Bacillus* spp. as well as the species *Bacillus megaterium* and *Enterobacter asburiae* (FENG et al. 2008). The most specific as well as the most numerously represented cellulolytic bacteria found in the digestive tract of the grass carp included *Anoxybacillus, Leuconostoc, Clostridium, Actinomyces* and *Citrobacter* (WU et al. 2012). The greatest cellulolytic activity is observed for such species as *Bacillus circulans* or *B. megaterium*. However, the composition of the cellulolytic microbiota varies both between individual fish specimens and depending on the

feed it consumes (KOIKE and KOBAYASHI 2001). Starch is a major nutrient for freshwater fish, including also cyprinids (TAKEUCHI 1991). It is degraded by amylase, an enzyme abundant in the digestive tract of cyprinids (SUGITA et al. 1997). Similarly, as in the case of cellulase, amylase activity depends mainly on the environmental conditions and feed quality. It also varies in individual fish species (SILVA et al. 1995). A considerable role in the production of both amylase as well as other enzymes is played by bacterial colonies inhabiting the gut (GANGULY and PRASAD 2012). However, it also needs to be stressed that fish utilize sugars ingested with food only to a relatively limited extent. Availability of certain types of carbohydrates for fish may be limited (FURUICHI and YONE 1982). In the common carp an increased α-amylase activity is observed when administering diets rich in carbohydrates (AL-TAMEEMI et al. 2010). For this reason, in the case of fish from aquaculture it is necessary to include animal origin feeds into their diet, e.g. fish meal (DAS and TRIPATHI 1991). There is no consensus among researchers concerning factors having a major effect on the activity of individual enzymes. On the one hand it was stated that α-amylase activity is closely related with the phylogenesis and plays a markedly greater role than diet (CHAN et al. 2004). In contrast, other researchers concluded that diet has a much greater role in the modification of enzyme activity (FERNANDEZ et al. 2001). However, the latest studies confirm that diet has a more important effect on enzymes (KUZ'MINA 1996, HOOPER and MACPHERSON 2010). The activity of α-amylase is greatest in herbivorous fish. Its level decreases in omnivorous fish, while it is lowest in predatory fish (COWEY 1989, GERMAN et al. 2004, GERMAN et al. 2009, AL-TAMEEMI et al. 2010). It depends on their nutrition (GERMAN et al. 2009). Amylolytic bacterial populations are mainly composed of *Bacillus* spp. Amylolytic bacteria composed of *Bacillus* spp. predominate in the digestive tract of the common carp and the grass carp (SASHA et al. 2006, GANGULY and PRASAD 2012). In 2002 three species from the genus *Bacillus* were isolated from the digestive tract of the common carp and they were classified as, *Bacillus pumilus* and *Bacillus cereus, B. circulans*. Also *B. megaterium* has a amylolytic activity (SASHA et al. 2006). These microorganisms are characterized by an extensive adaptability to changes in temperature and pH (GHOSH et al. 2002). Studies show that they are a permanent element in the digestive tract of common carps. They may be classified as autochthonous flora. They play an advantageous role in the digestive processes in common carps (GHOSH et al. 2010). Studies have shown the degradation of bacteriocenosis over the length of the gut. Strains of autochthonous bacteria were isolated from the proximal (PI) and the distal sections (DI) of the intestines in three cyprinid species: *Catla catla*,

Cirrhinus mrigala and *Labeo rohita*. The populations of amylolytic strains were the most numerous in PI of *C. catla* and the least numerous in DI of *L. rohita*. The greatest viable populations of bacteria producing cellulase and protease were recorded in DI and PI, respectively, of *C. mrigala*. More active cellulase producing bacterial strains were isolated from distal sections of the gut (RAY et al. 2010). The certain cyprinids are characterized by a relatively high activity of this enzyme. Such an example is *Labeo bata*, a species belonging to the family *Cyprinidae*. In this case, exogenous amylase activity is greater in proximal sections of the intestine (MONDAL et al. 2008). It is an opposite situation to that in cellulase, which generally is exogenously found in the distal sections of the gut. Proteases in common carp, and grass carp originate from two sources. They are synthesized endogenously by gland cells and by the intestinal microbiota. Generally, the distal sections of the gut exhibit the greatest proteolytic activity (MONDAL et al. 2008). The study indicated that greatest cellulolytic activity is observed for such species as *B. circulans* and *B. megaterium* have a proteolytic activity (SASHA et al. 2006). The intestinal microflora in the common carp is capable of producing vitamin B12. This concerns obligate anaerobic bacteria from the genus *Bacterioides* type A, to a lesser extent other representatives of the family *Bacterioidaceae*, as well as the genus Clostridium (SUGITA et al. 1991). Aerobes play a much lesser role in its production. However, it needs to be stressed that the process of vitamin B12 synthesis involves also bacteria from the family *Enterobacteriaceae*, as well as genera *Pseudomonas* and *Plesiomona*s. Thanks to their specific microflora common carps do not require a share of vitamin B12 in the diet (SYVOKIENI 1989). It needs to be stressed that bacteria from the genus *Bacterioides* type B, as anaerobes, do not efficiently produce cobalamin. It is useful from the point of veterinary practice that the supply of sulphonamides does not always reduce the level of synthesized vitamin B12 (KASHIWADA et al. 1970).

Probiotic therapy

The development of aquaculture as a branch of economy supplying fish to the food market has rapidly accelerated in the last decades. Considerable needs in terms of increased disease resistance, growth of aquatic organisms and feed conversion rates have resulted in the application of probiotics in fish farming practice. Probiotics were first used in 1986 to verify their capacity to promote growth of hydrobionts (organisms living in the aquatic environments). Later probiotics were used in order to improve water quality and control bacterial infections (MARTÍNEZ CRUZ et al. 2012).

Fish are exposed to many external factors affecting negatively their homeostasis, such as e.g. inappropriate environmental conditions, stress, inadequate diet or antibiotic therapy. They are also exposed to the action of pathogenic bacterial strains. *Aeromonas hydrophila*, a gram-negative bacterium from the family *Aeromonadaceae*, is one of the most significant pathogens for fish (RAHMAN et al. 1997). It is found in freshwater lakes and rivers, under aerobic and anaerobic conditions (HANSON et al. 1977). Disturbance of fish homeostasis during fish growth in aquaculture is a highly disadvantageous phenomenon, since it results in a reduction of body mass increase. It is crucial to maintain an appropriate composition of the intestinal tract microflora, which facilitates minimization of the negative factors as well as boosts the immune system (HOOPER and MACPHERSON 2010). It has an advantageous effect on phagocytic properties of the host, on the activity of lysozyme and antibodies, while it also affects peroxide production. However, antibiotics, stress, poor diet, pollution of the surrounding environment or diseases may disturb the physiological structure of the microbiota (LARSEN and ARIAS 2014). The above-mentioned factors may cause excessive growth of bacterial strains, which leads to intestinal dysbacteriosis, and thus many pathological phenomena taking place in the host organism, such as e.g. diarrhea or reduced immunity, potentially resulting in disease (TAMBOLI et al. 2004).

Probiotics promote the maintenance of physiological microflora of the digestive tract. Evidence has shown that they may improve digestibility and absorption of nutrients and enhance the activity of digestive enzymes (FULLER 1989, TOVAR-RAMÍREZ et al. 2004), increase stress tolerance and encourage reproduction activity (MARTÍNEZ CRUZ et al. 2012). They also stimulate the immune system and warn the organism during pathogen penetration (HARIKRISHNAN et al. 2010). As a result, they act as alternative measures in relation to antibiotics or other bioactive substances (MAZURKIEWICZ and PRZYBYŁ 2010). Knowing the adhesive properties of pathogenic bacterial strains in relation to the host's mucous membrane it may be stated which probiotic may be most effective towards these microorganisms (GRZEŚKOWIAK et al. 2011). Microorganisms producing digestive enzymes may be collected from the fish digestive tract and used as probiotics. They may be successfully used in rearing of fish, particularly in the larval period, when feed production costs may be minimized (GANGULY and PRASAD 2012). Probiotics composed of such bacterial genera as *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacteri, Saccharomyces*, or even potentially pathogenic bacteria from the genera *Pseudomonas* or *Clostridium* have successfully been used in fish farming practice.

The most commonly used probiotics are spores of gram-positive bacteria from the genus *Bacillus* (WANG et al. 2008). They are characterized by exceptional stability as well as resistance to environmental conditions. The species of that genus most commonly used in the therapy of cyprinids include *Bacillus genus, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquifaciens* and *Paenibacillus polymyxa*. YANBO and ZIRONG (2006) indicate that the probiotics composite with Bacillus sp. highly increased the growth performances and digestive enzyme activities and decreased FCR of common carp. They act as excellent immunostimulators, also against *A. hydrophila (*VIJAYABASKAR and SOMASUNDARAM 2008). Studies have shown a positive effect of bacteria species *B. coagulans, P. polymyxa* and *B. licheniformis* on growth increments, feed uptake and survival rates of common carps (VERSCHUERE et al. 2000, GUPTA et al. 2014). According to literature reports, carp was observed increased survival after experimentation infection *A. hydrophila* bacteria and application of bacteria probiotic *Lactobacillus acidophilus* and also *B. coagulans, Bacillus licheniformis, Paeninibacillus polymyxa* (ALY et al. 2008, KAZUŃ 2018) However, it needs to be stressed that the action of probiotics varied depending on the species, age, nutrition, as well as the composition, manner and frequency of probiotic application (LARA-FLORES 2011). For example, studies conducted under laboratory conditions showed that the application of identical probiotics as those given to common carps (*B. coagulans, P. polymyxa, B. licheniformis*) has no effect on growth or feed uptake in the Nile tilapia (*Oreochromis niloticus*) (GANGADHARA et al. 2004). However, in other studies indicated that *B. subtillis* and *L. acidophilus* increased survival rate of Nile tilapia infected for *A. hydrophila* and *Pseudomonas fluorescens* (KAZUŃ 2018). Probiotic therapy has been shown to have a highly advantageous effect in younger fish. In contrast, fish with an anatomically and functionally developed digestive tract responded much less markedly to administered probiotics (MAZURKIEWICZ et al. 2007). A positive effect of *B. circulans* bacteria on digestive processes was observed in common carps. A probiotic preparation based on this bacterium enhances the secretion of endogenous digestive enzymes. This probiotic causes an increase in weight gains and reduce FCR rates in common carps (SIVANI et al. 2016). A positive effect on these parameters was also observed at the application of preparations from *Streptococcus faecium* bacteria. Moreover, a complete elimination of *Escherichia coli* bacteria from the intestinal microbiome was recorded during supplementation with this probiotic. Studies were conducted on common carp from India (GHOSH et al. 2003) and from Israel (BOGUT et al. 1998). Probiotics containing lactic acid bacteria by reducing pH in the gut

prove to be excellent growth inhibitors against pathogenic bacterial strains (FAYOL-MESSAOUDI et al. 2005). Lactic acid bacteria is the most frequent used probiotic, both in human and animals nutrition, including fish. They occur in sweet fish as one of components of natural intestinal flora of cold-blooded animals (HOFFMAN et al. 2017). They promote expulsion of pathogenic bacteria from the intestines. Apart from lowering pH, they are capable of producing various metabolites such as bacteriocines, hydrogen peroxide, acetic aldehyde and other substances, which also inhibit growth of pathogenic bacterial strains (PRZYBYŁ et al. 2006). *L. acidophilus* have a positive effect on increased body weight gains in grass carp (WANG 2011). In addiction, the study indicated that the isolation of *Lactobacillus* bacteria from the intestinal tract of adult fish can be used as an addition to food intended for young fish stages (HOFFMAN et al. 2017). The studies showed no positive effect on the growth increment in common carp during supplementation either with *Carnobacterium divergens* (MAZURKIEWICZ et al. 2007). However, probiotics for fish composed of bacteria *C. divergens* and its metabolites inhibit activity, growth and survival of the *A. hudrophila, Aeromonas salmonicida, Vibrio angillarum, Edwardsiella Tarda* (GRAJEK et al. 2015). *C. divergens* can stay in the gut for a long time and is also capable for producing bacteriocins, that inhibit bacterial growth (MAZURKIEWICZ and PRZYBYŁ 2010). The study indicated, that *Carnobacterium maltaromaticum* and *Carnobacterium mobile* have a bacteriostatic effect on bacterial strains of *V. anguillarum*, *Vibrio salmonicida*, *A. salmonicida*, *Vibrio splendidus* potentially pathogenic to fish (RINGØ 2008). In addiction, the study indicated that *Carnobacterium pisciola* inhibit the growth of *A. hydrophila (*MAZURKIEWICZ and PRZYBYŁ 2010*).* BALCÁZAR et al. (2008) indicated that probiotic with *Lactococcus lactis* reduced the adhesion to the intestines of *A. hydrophila, A. salmonicida, Yersinia ruckeri and V. anguillarum*,whereas *Lactobacillus plantarum* reduced the adhesion of *A. hydrophila* and *A. salmonicida.* In the same study found that *Lactobacillus fermentum* reduced adhesion to intestinal mucus of *A. hydrophila, A. salmonicida, Y. ruckeri.* The same results obtained in the case of *B. subtilis*. In the case of the silver crucian carp bacteria from the genera *Proterobacteria* and *Firmicutes* are dominant in its intestinal microflora. Analysis of 10 most numerous taxonomic units showed the predominance of *Firmicutes*. The isolated bacteria presented in the decreasing order depending on the population size include *Veilonella* spp., *Lachnospiraceae*, *Lactobacillales*, *Streptococcus* spp. and *Lactobacillus* spp. Potential probiotic bacteria (*Lactobacillus* spp., *Bacillus* spp.), as well as opportunistic bacteria (*Aeromonas* spp., *Acinetobacter* spp.) were isolated in relatively small numbers from the intestinal tract of the silver crucian

carp (WU et al. 2013). This indicates that supplementation with probiotics composed of lactic acid bacteria may not always bring satisfactory results, since the growing population after probiotic supplementation decreases rapidly within a period of several days, when probiotic supplementation ceases (WU et al. 2013). Due to the considerable shares of bacteria from the genera *Pseudomonas* and *Aeromonas* in the fish microbiota they may serve important biological functions and may be used as probiotics (WU et al. 2012). However, the bacteria from the genera *Pseudomonas* and *Aeromonas* may prove to be potentially pathogenic for fish. Further studies need to be conducted in order to precisely determine their role. The microbiome in the digestive tract of the silver crucian carp is strongly related with the benthic microflora, on which it feeds, as well as ingested food, since 75% taxonomic units were identical to those isolated from the used feed (HAN et al. 2010). This is valuable information in view of the oral administration of probiotics together with feeds. Additionally, a documented positive effect on body weight gains in common carp is also observed for probiotics containing yeast *Saccharomyces cerevisiae*, as well cyanobacteria *Spirulina maxima* (RAMAKRISHNAN et al. 2008). Determination of the physiological intestinal microflora and identification of properly selected probiotics is a key element of appropriate fish rearing. Probiotic therapy is a perfect form of veterinary prophylaxis, facilitating maintenance of homeostasis and immune equilibrium, and as a result promoting physiological digestive processes. It has an essential effect on good health and high productivity of fish (MAZURKIEWICZ and PRZYBYŁ 2010).

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References

ALARCÓN F.J., MARTÍNEZ T.F., DÍAZ M., MOYANO F.J. 2001. *Characterization of digestive carbohydrase activity in the gilthead seabream (Sparus aurata)*. Hydrobiologia, 445: 199–204.

AL-TAMEEMI R., ALDUBAIKUL A., SALMAN N.A. 2010. *Comparative study of α-amylase activity in three Cyprinid species of different feeding habits from Southern Iraq*. Turk. J. Fish. Aquat. Sci., 10(3): 41–414.

ALY S.M., AHMED Y.A.G., GHAREEB A.A.A., MOHAMED M.F. 2008. *Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (Oreochromis niloticus) to challenge infections*. Fish Shellfish Immunol., 25(1–2): 1281–136.

BAIRAGI A., GHOSH K.S., SEN S.K., RAY A.K. 2002. *Enzyme producing bacterial flora isolated from fish digestive tracts*. Aquacult. Int., 10(2): 109–121.

BALCÁZAR J.L., VENDRELL D., DE BLAS I., RUIZ-ZARZUELA I., MUZQUIZ J.L., GIRONES O. 2008. *Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish*. Aquaculture, 278(1–4): 188–191.

BOGUT I., MILAKOVIC Z., BUKVIC Z., BRKIC S., ZIMMER R. 1998. *Influence of probiotic Streptococcus faecium M74 on growth and content of intestinal microflora in carp Cyprinus carpio*. Czech J. Anim. Sci., 43: 231–235.

CHAN A.S., HORN M.H., DICKSON K.A., GAWLICKA A. 2004. *Digestive enzyme activities in carnivores and herbivores: comparisons among four closely related prickleback fishes (Teleostei: Stichaeidae) from a California rocky intertidal habitat*. J. Fish Biol., 65(3): 848–858.

CLEMENTS K.D., ANGERT E.R., MONTGOMERY W.L., CHOAT J.H. 2014. *Intestinal microbiota in fishes: What's known and what's not*. Mol. Ecol., 23(8): 1891–1898.

COWEY C.B. 1989. *Intermediary metabolism*. Fish Nutrition., 2: 259–329.

DAS K.M.,TRIPATHI S.D. 1991. *Studies on the digestive enzymes of grass carp, Ctenopharyngodon idella (Val.)*. Aquaculture, 92: 21–32.

DHAGE K.P. 1968. *Studies of the digestive enzymes in the three species of the major carps of India*. J. Biol. Sci., 11: 63–74.

FAGBENRO O.A. 1990. *Food composition and digestive enzymes in the gut of pond cultured Clarias isheriensis (Sydenham 1980), (Siluriformes: Clariidae)*. J. Appl. Ichthyol., 6(2): 91–98.

FAGBENRO O.A., ADEDIRE C.O., AYOTUNDE E.O.,FAMINU E.O. 2000. *Haematological profile, food composition and digestive enzyme assay in the gut of the African bony-tongue fish, Heterotis (Clupisudis) niloticus (Cuvier 1829) (Osteoglossidae)*. Trop. Zool., 13: 1–9.

FAYOL-MESSAOUDI D., BERGER C.N., COCONNIER-POLTER M.H., LIEVIN-LE MOAL V., SERVIN A.L. 2005. *pH-, Lactic acid-, and non-lactic acid-dependent activities of probiotic Lactobacilli against Salmonella enterica Serovar Typhimurium*. Appl. Environ. Microbiol., 71(10): 6008–6013.

FENG X., WU Z.X., ZHU D.M., WANG Y., PANG S.F., YU Y.M., CHEN X.X. 2008. *Study on digestive enzyme-producing bacteria from the digestive tract of Ctenopharyngodon idellus and Carassius auratus gibelio*. Fresh. Fish., 38(3): 51–57.

FERNANDEZ I., MOYANO F.J., DIAZ M., MARTÍNEZ T. 2001. *Characterization of alpha-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei)*. J. Exp. Mar. Biol. Ecol., 262: 1–12.

FULLER R. 1989. *A review. Probiotics in man and animals*. J. Appl. Bactenol., 66.

FURUICHI M., YONE Y. 1982. *Availability of carbohydrate in nutrition of carp and red sea bream*. Bull. Jpn. Soc. Sci. Fish., 48: 945–948.

GANGADHARA B., KESHAVANATH P., RAMESHA T.J., PRIYADARSHINI M. 2004. *Digestibility of bamboo-grown periphyton by carps (Catla catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carpio, Ctenopharyngodon idella, and Tor khudree) and hybrid red tilapia (Oreochromis mossambicus x O. niloticus)*. J. Appl. Aquacult., 15: 151–162.

GANGULY S., DORA K.C., SARKAR S., CHOWDHURY S. 2013. *Supplementation of prebiotics in fish feed: a review*. Rev. *Fish* Biol. Fisher., 23(2): 195–199.

GANGULY S., PRASAD A. 2012. *Microflora in fish digestive tract plays significant role in digestion and metabolism*. Rev. *Fish* Biol. Fisher., 22(1): 11–16.

GERMAN D.P., HORN M.H., GAWLICKA A. 2004. *Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects*. Physiol. Biochem. Zool., 77(5): 789–804.

GERMAN D.P., NAGLE B.C., VILLEDA J.M., RUIZ A.M., THOMSON A.W., CONTRERAS-BALDERAS S., EVANS D.H. 2009. *Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effect on gut size and digestive physiology*. Physiol. Biochem. Zool., 83: 1–18.

GHOSH K., SEN S.K., RAY A.K. 2002. *Characterization of bacilli isolated from gut of rohu, Labeo rohita, fingerlings and its significance in digestion*. J. Appl. Aquacult., 12: 33–42.

GHOSH K., SEN S.K., RAY A.K. 2003. *Supplementation of an isolated fish gut bacterium, Bacillus circulans, in formulated diets for rohu, Labeo rohita, fingerlings*. Israeli J. Aquacult. Bamigdeh., 55: 13–21.

GHOSH K., ROY M., KAR N., RINGØ E. 2010. *Gastrointestinal bacteria in rohu, Labeo rohita (Actinopterygii: Cypriniformes: Cyprinidae): scanning electron microscopy and bacteriological study*. Acta Ichthyol. Piscatoria, 40: 129–135.

Grajek W., Sip A., Przybył A., Mazurkiewicz J. 2015. *Probiotyk dla ryb słodkowodnych, mięczaków i skorupiaków zawierający szczep bakterii Carnobacterium divergens oraz jego metabolity*. PL399670-A1.

Grześkowiak Ł., Collado M.C., Vesterlund S., Mazurkiewicz J., Salminen S. 2011. *Adhesion abilities of commensal fish bacteria by use of mucus model system. Quantitative analysis.* Aquaculture, 318(1): 33–36.

Gupta A., Gupta P., Dhawan A. 2014. *Dietary supplementation of probiotics affects growth, immune response and disease resistance of Cyprinus carpio fry*. Fish Shellfish Immunol., 41(2): 113–119.

Han S., Liu Y., Zhou Z., He S., Cao Y., Shi P., Ringö E. 2010. *Analysis of bacterial diversity in the intestine of grass carp (Ctenopharyngodon idella) based on 16S rDNA gene sequences.* Aquacult. Res., 42(1): 47–56.

Hanson P.G., Standridge J., Jarrett F., Maki D.G. 1977. *Freshwater wound infection due to Aeromonas hydrophila.* JAMA, 238(10): 1053–1054.

Harikrishnan R., Balasundaram C., Heo M.S. 2010. *Lactobacillus sakei BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, Epinephelus bruneus.* Fish Shellfish Immunol., 29(6): 1037–1043.

Hooper L.V., Macpherson A.J. 2010. *Immune adaptations that maintain homeostasis with the intestinal microbiota*. Nat. Rev. Immunol., 10(3): 159–169.

Hoffmann L., Mazurkiewicz J., Florczyk K., Burchardt H. 2017. *Zastosowanie probiotycznego dodatku paszowego w chowie karpi*. Kom. Ryb., 2(157): 14–21.

Kamei Y., Sakata T., Kakimoto D. 1985. *Microflora in the alimentary tract of Tilapia: characterization and distribution of anaerobic bacteria*. J. Gen. Appl. Microbiol., 31: 115–124.

Kawai S. 1972. *Studies on digestive enzymes of* shes *fishes.* II. *Effect of dietary change on the activities of digestive enzymes in carp intestine.* Bul. Jpn. Soc. Sci. Fish., 38: 265–270.

Kashiwada K., Teshima S., Kanazawa A. 1970. *Studies on the production of B vitamins by intestinal bacteria of fish.* 5. *Evidence of the production of vitamin B_{12} by microorganisms in the intestinal canal of carp, Cyprinus carpio*. Bull. Jap. Soc. Scient. Fish., 36: 421–424.

Kazuń B. 2018. *Czy stosowanie probiotyków może stanowić alternatywę dla kuracji antybiotykowej?* Przegl. Ryb., 2: 25–26.

Koike S., Kobayashi Y. 2001. *Development and use of competitive PCR assays for the rumen cellulolytic bacteria: Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens*. FEMS Microbiol. Lett., 204(2): 361–366.

Kuz'mina V.V. 1996. *Influence of age on digestive enzyme activity in some freshwater teleosts.* Aquaculture, 148(1): 25–37.

Lara-Flores M. 2011. *The use of probiotic in aquaculture: an overview*. Int. Res. J. Microbiol., 2(12): 471–478.

Larsen A.M., Arias C.R. 2014. *More than mucus: the hidden world of the fish microbiota*. Fisheries., 39(4): 145–192.

Lesel R., Fromageot C., Lesel M. 1986. *Cellulose digestibility in grass carp*, *Ctenopharyngodon idella and in goldfish*, *Carassius auratus*. Aquaculture, 54(1–2): 11–17.

Lirski A., Myszkowski L. 2017. *Obraz polskiej akwakultury 2016 roku, czyli co wynika z analizy kwestionariuszy R-22*. Przegl. Ryb., 6: 14–18.

Liu H., Guo X., Gooneratne R., Lai R., Zeng C., Zhan F.,Wang W. 2016. *The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels.* Sci. Rep., 6: 24340.

Li X., Yan Q., Xie S., Hu W., Yu Y., Hu Z. 2013. *Gut microbiota contributes to the growth of fast-growing transgenic common carp (Cyprinus carpio L.).* PLoS One, 8(5): e64577.

Martínez Cruz P., Ibáñez A.L., Monroy Hermosillo O.A., Ramírez Saad H.C. 2012. *Use of probiotics in aquaculture*. Microbiol., 1–13.

Mazurkiewicz J., Przybył A., Sip A., Grajek W. 2007. *Effect of Carnobacterium divergens and Enterococcus hirae as probbiotic bacteria in feed for common carp, Cyprinus carpio L.* Arch. Pol. Fish., 15(2): 93.

MAZURKIEWICZ J., PRZYBYŁ A. 2010. *Probiotyki i prebiotyki w żywieniu karpi.* In: *Rozród, podchów, profilaktyka ryb rzadkich i chronionych*. Ed. Z. Zakęś. Wyd. IRS Olsztyn, pp. 110–121.

MONDAL S., ROY T., SEN S.K., RAY A.K. 2008. *Distribution of enzyme-producing bacteria in the digestive tracts of some freshwater fish*. Acta Ichthyol. Piscat., 1(38): 1–8.

NAYAK S.K. 1997. *Role of gastrointestinal microbiota in fish*. Aquacult. Res., 41(11): 1553–1573.

NAYAK S.K. 2010. *Probiotics and immunity: a fish perspective.* Fish Shellfish Immunol., 29(1): 2–14.

PREJS A., BLASZCZYK M. 1997. *Relationships between food and cellulase activity in freshwater fishes*. J. Fish Biol., 11: 447–452.

PRZYBYŁ A., MAZURKIEWICZ J., ROŻEK W. 2006. *Zastosowanie probiotyków i prebiotyków w żywieniu karpi*. Aparatura Badawcza i Dydaktyczna, 11(1): 65–70.

RAHMAN M. H., KAWAI K., KUSUDA R. 1997. *Virulence of starved Aeromonas hydrophila to cyprinid fish*. Fish Pathol., 32(3): 163–168.

RAMAKRISHNAN C.M., HANIFFA M.A., MANOHAR M., DHANARAJ M., AROCKIARAJ A.J., ARUNSINGH S.V. 2008. *Effecs of probiotics and spirulina an survival and growth of juvenile common carp (Cyprinus carpio) – Israeli*. J. Aquaculture, 60: 128–133.

RAY A.K., ROY T., MONDAL S., RINGØ E. 2010. *Identification of gut associated amylase, cellulase and protease producing bacteria in three species of Indian major carps*. Aquacult. Res., 41(10): 1462–1469.

RAY A.K., GHOSH K., RINGØ E. 2012. *Enzyme-producing bacteria isolated from fish gut: a review*. Aquac. Nutr., 18: 465–492.

RINGØ E., BIRKBECK T.H. 1999. *Intestinal microora of sh and fry: a review*. Aquac. Res., 30(2): 73–93.

RINGØ E. 2008. *The ability of carnobacteria isolated from fish intestine to inhibit growth of fish pathogenic bacteria: a screening study*. Aquac. Res., 39(2): 171–180.

SAHA S., ROY R.N, SEN S.K, RAY A.K. 2006. *Characterization of cellulase-producing bacteria from the digestive tract of tilapia, Oreochromis mossambica (Peters) and grass carp, Ctenopharyngodon idella (Valencinnes)*. Aquac. Res., 37: 380–388.

SAVAŞ S., KUBILAY A., BASMAZ N. 2005. *Effect of bacterial load in feeds on intestinal microflora of seabream (Sparus aurata) larvae and juveniles Israeli*. J. Aquacult. Bamigdeh., 57(1): 3–9.

SHCHERBINA M.A., TROFIMOVA L.N., KAZLAWLENE O.P. 1976. *The activity of protease and the intensity of protein absorption with the introduction of different quantities of fat into the food of the carp Cyprinus carpio*. J. Ichthyol., 16: 632–636.

SHCHERBINA M.A KAZLAWLENE O.P. 1971. *The reaction of the medium and the rate of absorption of nutrients in the intestine of carp*. J. Ichthyol., 11: 81–85.

SILVA S.D., ANDERSON T.A., SARGENT J.R. 1995. *Fish nutrition in aqua*culture. Rev. Fish Biol. Fish., 5(4): 472–473.

SIVANI G., BHASKAR M., SHARMA G.R.K. 2016. *Influence of probiotics on growth performance and digestive enzyme activities among common carps (Cyprinus carpio)*. Int. J. Environ. Sci. Technol., 5(2): 564–574.

SUGITA H., MIYAJIMA C., DEGUCHI Y. 1991. *The vitamin B12-producing ability of the intestinal microflora of freshwater fish*. Aquaculture, 92: 267–276.

SUGITA H., KAWASAKI J., DEGUCHI Y. 1997. *Production of amylase by the intestinal microflora in cultured freshwater fish*. Lett. Appl. Microbiol., 24(2): 105–108.

SYVOKIENI J. 1989. *Symbiotic digestion in hydrobionts and insects*. Mokslas, Vilnius.

TAKEUCHI T. 1991. *Digestion and nutrition of fish*. In: *Fish Physiology*. Ed. M.Y. Itazawa, I. Hanyu, 1: 67–101.

TAMBOLI C.P., NEUT C., DESREUMAUX P., COLOMBEL J.F. 2004. *Dysbiosis in inflammatory bowel disease*. Gut., 53(1): 1–4.

TENGJAROENKUL B., SMITH B.J., CACECI T., SMITH S.A. 2000. *Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, Oreochromis niloticus L.* Aquaculture, 182: 317–327.

TOVAR-RAMÍREZ D., INFANTE J.Z., CA C., GATESOUPE F.J., VÁZQUEZ-JUÁREZ R. 2004. *Influence of dietary live yeast on European sea bass (Dicentrarchus labrax) larval development*. Aquaculture, 234: 415–427.

TRUST T.J., SPARROW R.A.H. 1974. *The bacterial flora in the alimentary tract of freshwater salmonid fishe*s. Can. J. Microbiol., 20: 1219–1228.

VERSCHUERE L., ROMBAUT G., SORGELOOS P., VERSTRAETE W. 2000. *Probiotic bacteria as biological control agents in aquacultur*e. Microbiol. Mol. Biol. Rev., 64: 655–671.

VIJAYABASKAR P., SOMASUNDARAM S.T. 2008. *Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen Aeromonas hydrophila*. Biotechnol. J., 7(1): 124–128.

WANG Y.B, LI J.R, LIN J. 2008. *Probiotics in aquaculture: challenges and outlook*. Aquaculture, 28: 1–4.

WANG Y. 2011. *Use of probiotics Bacillus coagulans, Rhodopseudomonas palustris and Lactobacillus acidhilus as growth promoters in grass carp (Ctenopharyngodon idella) fingerlings*. Aquacult. Nutr., 17(2): e372–e378.

WU S., WANG G., ANGERT E.R., WANG W., LI W., ZOU H. 2012. *Composition, diversity, and origin of the bacterial community in grass carp intestine*. PloS one., 7(2): e30440.

WU S.G., TIAN J.Y., GATESOUPE F.J., LI W.X., ZOU H., YANG B.J., WANG G.T. 2013. *Intestinal microbiota of gibel carp (Carassius auratus gibelio) and its origin as revealed by 454 pyrosequencing*. World J. Microbiol. Biotechnol., 29(9): 1585–1595.

YANBO W., ZIRONG X. 2006. *Effect of probiotics for common carp (Cyprinus carpio) based on growth performance and digestive enzyme activities*. Anim. Feed Sci. Technol., 127(3–4); 283–292.