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Composition, digestibility, feeding value estimation of forage pea (*Pisum sativum* L.) varieties and hybrids in breeding process and genetic advance evaluation

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Abstract. *Composition and digestibility are studied and potential forage feeding value is estimated of eight breeding spring and winter forms – two Bulgarian and two Russian varieties and their four hybrids, presented as Component A – winter forms and Component B – spring forms. Forage quality is evaluated in three vegetative stages of plants, grown in Competitive variety trial (2011 –2013) on the Second Experimental Field of the Institute of Forage Crops, Pleven: budding stage, beginning of flowering stage and full pod formation stage. The parameters of principal composition (Weende analysis), plant cell walls fiber components content (Van Soest detergent analysis), enzyme in vitro digestibility of dry and organic matter (method Aufrere), potential energy and protein feeding value by different systems are analyzed, determined and estimated. The correlation relations between parameters are established. The highest values for the crude protein content breeding forms appear in budding stage: CP 23.55±7.75% and the highest average digestibility in the full pod formation stage, which is a stage of technological maturity of varieties and hybrids: Digestibility of dry matter (IVDMD) 70.00±3.32% and Digestibility of organic matter (IVOMD 71.28±3.06%). Potential energy feeding value of the forage biomass average for all breeding accessions is the highest at full pod formation stage and protein feeding value – at budding stage. Genetic advance is evaluated by Principal Component Analysis, Cluster analysis and heritability (broad sense) is established.*

Keywords: *pea (Pisum sativum* L.), protein, enzyme *in vitro* digestibility, energy and protein feeding value, genetic advance

Abbreviations: RFV – Relative feeding value, DDM – Digestible dry matter, DMI – Dry matter intake, NDF – Neutral-detergent fiber, ADF – Acid-detergent fiber, ADL – Acid-detergent lignin, CP – Crude protein, CF – Crude fiber, GE – Gross energy, ME – Metabolic energy, IVDMD – *in vitro* dry matter digestibility, UFL, FUM, VEM – Feed units for milk by French, Bulgarian, Dutch systems, respectively, UFV, FUG, VEVI – Feed units for growth by French, Bulgarian, Dutch systems, respectively, TDP/PBD – Total Digestible Protein/Protein Brute Digestible, PDIN – Protein digestible in intestine, depending on nitrogen, PDIE – Protein digestible in intestine depending on energy, F1, F2, F3, F4 – principal components

Introduction

Pea is an important annual legume grown and consumed extensively as both human and animal feed. Forage pea is widely grown for hay, pasturage or silage production either alone or mixed with cereals. Both seeds and forages of pea are rich in protein and mineral content (Bilgili et al., 2010). Because of its high protein level (23 – 33%) (Cousin, 1997) and the increasing demand for protein-rich raw materials for animal feed or intermediary products for human nutrition there is rising interest in this crop as a protein source (Santalla et al., 2001). The legume cultures represent the second most important family of crop plants after *Poaceae* (grass family), accounting for approximately 27% of the world's crop production (Graham and Vance, 2003). Dry pea currently ranks second only to common bean as the most widely grown grain legume in the world with primary production in temperate regions and global production of 10.4 M tonnes in 2009 (FAOSTAT, 2011). Pea is also used as alternative protein source in animal feed industry in Europe. On the other hand, forage pea is a very suitable crop in annual crop rotation because it provides biological nitrogen for plants sown after them (Tan et al., 2012). Pea seeds are rich in protein (23–25%), slowly digestible starch (50%), soluble sugars (5%), fibers, minerals and vitamins (Graham et al., 2003; Georgieva and Nikolova, 2011; Smýkal et al., 2012). It has a prominent place among vegetables due to its high nutritive value, particularly proteins and other health building substances like carbohydrates, vitamin A, vitamin C, calcium and phosphorus (Sharma, 2010; Sharma et al., 2013). The success of genetic improvement is attributed to the magnitude and nature of variability present for a specific trait and the effectiveness of a breeding program largely depends on the existence of genetic variability, so a diverse genetic background provides desirable allelic variation among parental lines to produce new and valuable combinations (Tar'an et al., 2005; Gatti et al., 2011; Tiwari and Lavanya, 2012). In the process of creation of new forage plant cultivars the attention is directed to yield and quality (Čupić et al., 2013; Kirilov et al., 2010; Naydenova and Todorova, 2009; Naydenova et al., 2008). Between the separate varieties exists the determined difference in the content of chemical compounds – protein and fibers, determining forage feeding value (Naydenova et al., 2010, 2011, 2012, 2014).

The aim of this study is forage quality evaluation – composition, digestibility, energy and protein feeding value of breeding winter and spring grown forms of forage pea (*Pisum sativum* L.) and evaluation of genetic variability and the heritability of different forage quality characters of pea which may help to select suitable genotypes for future breeding programs.

Material and methods

The experimental study is conducted during the 2011 – 2013 period on the Second experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Eight breeding spring and winter forms – two Bulgarian and two Russian varieties and their four hybrids,

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Table 1. Origin and description of forage pea hybrids/genotypes

Variety	Pleven 10	Pleven 4	Shtambovyi	Rosacrono
Origin	Bulgaria	Bulgaria	Russia	Russia
Vine type	long-prostrate	long semi-erect	short semi-erect	long semi-erect
Flower position	axial	axial	terminal (with fasciation – Fa, fac)	terminal (with fasciation – fa, Fac)
Stipule type	normal	normal	double	double
Leaf type	Normal	Normal	Normal	Normal
Flower color	purple	white	white	pink

presented as Component A (Rosacrono and Pleven 10) – winter forms (*Pisum sativum ssp. arvense*) and Component B (Shtambovyi and Pleven 4) – spring forms (*Pisum sativum ssp.sativum*) are studied (Table 1). These forms are sown on a row spacing 70 cm and distance in a row 5 cm. Hand planting is applied with depth of sowing 5 cm. The forage pea is grown by approved technology of the Institute of Forage Crops – Pleven. The plants are harvested at three phenological stages of plant development – budding stage, beginning of flowering stage and full pod formation stage.

Plant sample preparation from the above-ground part of the plants is effectuated by air ventilation at 65^{0}C till crumbly at previous fixing for 20 min at 105$^°$C and grinding till particle size 1.0 mm. Weende systematic analytic procedure (AOAC 2010) is applied and the parameters Dry matter; Crude ash (Mineral matter, MM); Crude protein (CP) by Kjeldhal method; Crude fiber (CF) – by Heneberg&Stoman method are established. Detergent analysis of Goering&Van Soest (1970) (EN ISO13906 2008) is performed as a standard systematic chemical analysis of plant cell walls fiber components. The following fiber fractions are determined: Neutral-detergent fiber (NDF); Acid-detergent fiber (ADF), Acid-detergent lignin (ADL). Polyosides hemicellulose and cellulose as cell wall components contained in fiber fraction are presented as Hemicellulose = NDF – ADF; Cellulose = ADF – ADL. The degree of lignification is presented as relation of ADL and NDF/100. The enzymatic *in vitro* digestibility of dry (IVDMD) and organic (IVOMD) matter is determined by the two-stage pepsin-cellulase enzyme method of Aufrere (Todorov et al., 2010).

The feeding value estimation – energy and protein – is performed as: 1. Evaluation of feeding value on the basis of fiber components – Relative feeding value RFV; potential intake of digestible dry matter (Linn and Martin, 1991). The Digestible Dry Matter (DDM% = 88.9-(0.779 x ADF%); Dry Matter Intake (DMI /% body weight/=120/NDF%) and Relative Feeding Value (RFV = DDM x DMI/1.29) are estimated. The plant cell walls fiber components content is discussed previously (Nikolova et al., 2014). 2. The Energy feeding value is calculated by French system: UFL – UFV (INRA, 1988), recalculated in Bulgarian by coefficients, followed by Todorov (1997). The following parameters are estimated: Gross energy (GE), Metabolic energy (ME) on the basis of equations according to experimental values of CP, CF (AOAC, 2010) and IVDMD. The coefficient of digestibility of organic matter $DMO_{in\ vivo}$ (Andrieu and Demarquilly, 1987) is received by relashionship on the basis of *in vitro* organic matter digestibility determined experimentally by the method of Aufrere (Todorov et al., 2010). Net energy is determined according to French (UFL-UFV), Bulgarian – Feed units for milk and Feed units for growth (FUM-FUG) and Dutch (VEM-VEVI) systems. The Protein feeding value is evaluated by French system (INRA, 1988). The parameters: TDP/PBD – Total Digestible Protein/Protein Brute Digestible and a really digestible protein in ruminant small intestine – PDIN (Protein digestible in intestine, depending on nitrogen) PDIN = PDIA + PDIMN and PDIE (Protein digestible in intestine depending on energy) PDIE = PDIA + PDIMN in g kg^{-1} dry matter are established. In comparative analysis in three vegetative stages and organic active products the individual and mean values and coefficients of variation of the parameters of forage feeding value are evaluated.

The following statistical methods are used to process the experimental data: factor analysis by the method of principal components (Vandev, 2003); hierarchical cluster analysis by the method of Ward (1963) – for the grouping of genotypes by similarity as a measure for the difference (the genetic distance), the Euclidean distance between them was used, having previously carried out data standardization. Broad-sense heritability (H2) for the traits is estimated using the formula adopted by Allard (1960). Correlation coefficient analysis according to the procedure given by Dimova and Marinkov (1999) is calculated. Genetic advance in absolute unit (GA) and Genetic Gain (GG), assuming selection of the superior 5% of the genotypes, are estimated in accordance with the methods illustrated by Johnson et al. (1995). The statistical processing of the experimental data was performed by the programmes Excel of Microsoft Office 2003 for Windows.

Results and discussion

Chemical composition and digestibility

Principal composition – crude protein and crude fiber content, plant cell walls fiber components content and digestibility of forage pea component A (winter varieties as parental forms: A1 Shtambovyi and A2 Pleven 10 and their hybrids/crosses in A4 F1 generation and A3 F2 generation) and component B (spring varieties as parental forms B1 Rosacrono and B2 Pleven 4 and their hybrids/crosses in first generation B4 F1 and second generation B3 F2) are presented in Table 2. The crude protein content of varieties and hybrids in budding stage ranged in close limits 23 – 25% of dry matter. Protein content higher than mean value show variety Pleven 10 (24.41%) and F1 generation of hybrid Rosacrono x Pleven 4 (24.92%). The protein content of each variety and hybrid decreases at the beginning of flowering stage (16.31 – 23.1%, mean value 21%) and in full pod formation stage (15.33 – 20.73%, mean value 17.84%). The variations in these stages are CV 9 – 10%. The crude fiber content of the varieties and hybrids in the three vegetative stages mentioned is more stable with mean values 23.30% in budding stage, 25,10% (CV 6.7%) at the beginning of flowering stage and 20.82% (CV 8.9%) in full pod formation stage.

Concerning plant cell walls fiber components content in all fiber fractions: total fiber components/cell walls NDF, accounting for intake; lignocellulose ADF, accounting for forage digestibility; lignin ADL, factor limiting digestibility; Hemicellulose, completely digestible and Cellulose, not completely digestible by ruminants, the

Table 2. Principal composition, fiber components content and digestibility of forage pea, Component A and Component B breeding forms

% DM	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
BUDDING STAGE										
A1-I	23.02	24.82	36.03	32.48	5.71	3.55	26.77	15.8	73.19	72.85
A2-I	24.41	23.63	35.37	26.98	4.20	8.39	22.78	11.9	72.23	71.80
A3-I	23.72	21.03	37.51	30.08	5.20	7.43	24.88	13.9	70.32	70.45
A4-I	23.23	23.14	34.86	29.74	5.01	5.12	24.73	14.4	67.99	68.49
B1-I	23.40	24.31	36.05	30.63	5.12	5.42	25.51	14.2	69.78	68.88
B2-I	23.13	22.65	33.73	28.04	4.65	5.69	23.39	13.8	70.87	72.00
B3-I	22.56	23.54	33.59	31.90	5.36	1.69	26.54	16.0	66.00	65.68
B4-I	24.92	23.27	37.68	29.20	4.91	8.48	24.29	13.0	68.35	67.55
Min	22.56	21.03	33.59	26.98	4.20	1.69	22.78	11.9	66.00	65.68
Max	24.92	24.82	37.68	32.48	5.71	8.48	26.77	16.0	73.19	72.85
Mean	23.55	23.30	35.60	29.88	5.04	5.72	24.86	14.1	69.84	69.71
SD	7.75	1.14	1.54	1.84	0.49	2.36	1.40	1.35	2.35	2.48
CV	3.3	4.9	4.3	6.2	9.7	41.3	5.6	9.5	3.4	3.6
BEGINNING OF FLOWERING STAGE										
A1-II	21.60	23.31	37.70	36.36	5.95	5.34	26.41	15.8	71.00	71.16
A2-II	16.31	27.03	42.54	35.15	6.36	7.39	28.79	15.0	62.56	63.51
A3-II	21.81	24.40	38.25	29.18	6.05	9.08	23.12	15.8	71.46	73.08
A4-II	23.11	23.82	37.59	30.57	5.25	7.02	25.32	13.9	68.56	69.10
B1-II	21.18	27.36	40.43	33.54	5.90	6.89	27.64	14.6	65.81	66.17
B2-II	22.49	23.07	39.93	29.60	5.48	10.3	24.12	13.7	72.37	72.28
B3-II	21.30	25.60	36.82	31.42	5.55	5.40	25.87	15.0	67.63	67.22
B4-II	20.17	26.17	40.56	33.20	6.20	7.36	26.99	15.3	67.86	67.22
Min	16.31	23.07	36.82	29.18	5.25	5.34	23.12	13.7	62.56	63.51
Max	23.11	27.36	42.54	36.36	6.36	10.33	28.79	15.8	72.37	73.08
Mean	21.00	25.10	39.23	32.38	5.84	7.35	26.03	14.9	68.41	68.72
SD	2.08	1.67	1.94	2.61	0.38	1.69	1.85	0.78	3.24	3.29
CV	10.0	6.7	5.0	8.1	6.5	23.0	7.1	5.3	4.7	4.8
FULL POD FORMATION STAGE										
A1-III	17.56	20.00	36.76	27.71	4.71	9.05	23.00	12.8	71.71	73.00
A2-III	16.36	20.58	36.02	28.73	4.88	7.29	23.85	13.5	71.55	73.66
A3-III	17.70	18.22	35.29	26.32	4.58	8.97	21.74	13.0	63.96	66.17
A4-III	18.80	23.43	36.82	29.96	5.47	6.86	24.49	14.8	67.28	68.30
B1-III	18.30	19.52	36.45	26.49	4.71	9.96	21.78	12.9	75.12	75.40
B2-III	17.98	21.60	37.87	30.15	5.13	7.72	25.02	13.5	69.30	70.12
B3-III	15.33	19.88	37.64	27.23	4.38	10.41	22.85	11.6	71.00	73.05
B4-III	20.73	23.35	36.78	31.92	5.08	4.86	26.84	13.8	70.21	70.60
Min	15.33	18.22	35.29	26.32	4.38	4.86	21.74	11.6	63.96	66.17
Max	20.73	23.43	37.87	31.92	5.47	10.41	26.84	14.8	75.12	75.40
Mean	17.84	20.82	36.70	28.56	4.87	8.14	23.70	13.2	70.00	71.28
SD	1.61	1.85	0.82	1.98	0.35	1.82	1.73	0.92	3.32	3.06
CV	9.0	8.9	2.2	7.0	7.1	22.4	7.3	7.0	4.7	4.3

classical difference between parameters at vegetative stages is obviously shown – simultaneously increasing plant maturity from budding to beginning of flowering stage, fiber components content increases, by 4%-units on average for NDF, by 2%-units on average for ADF, by nearly 1%-unit on average for ADL. Then the fiber content decreases at full pod formation stage for the reason of filling pods.

The digestibility of dry and organic matter of parental variety forms is high – over 70% at the three vegetative stages studied with the exception of winter variety Pleven 10 at the beginning of flowering stage 62 – 63%. The most digestible parental form is variety Shtambovyi 71 – 73% at all vegetative stages. At the budding stage winter hybrid Shtambovyi x Pleven 10 in generation F2 is more digestible by 2%-units (70.3 – 70.4%) compared to generation F1 (68.0 – 68.5%) but higher F1 nor F2 generations do exceed the digestibility of parental forms. The same tendency is valid also for spring hybrid forms (F1 and F2) for which the digestibility is approximately 3% lower than that of parental forms. At the begining of flowering stage the parental form variety Pleven 4 demonstrates decreasing of digestibility to 62 – 63%, which is probably due to the presented increase of fiber components content at this vegetative stage. The hybrid form Shtambovyi x Pleven 10 F2 generation is as digestible as its parental form variety Shtambovyi. The spring hybrids Shtambovyi x Pleven 4 in F1 and F2 generations are equally digestible (67.6 – 67.8%). At the full pod formation stage digestibility of both spring and winter parental forms is higher than 70% and close for the winter varieties Shtambovyi and Pleven 10: 71 –73% and divergent for the spring varieties Rosacrono and Pleven 4. The tendencies in digestibility of hybrids in F1 and F2 generations are stable and demonstrative. The winter hybrids Shtambovyi x Pleven 10 in F1 and F2 generations are digestible 64 – 68%, which values are 5 –7% lower than those of the parental forms. However, the spring hybrids Rosacrono x Pleven 4 in F1 and F2 generations demonstrate close digestibility values of dry and organic matter (70 – 73%) and do not exceed parental forms, either. It is obvious that the spring hybrid forms in F1 and F2 generations have higher digestibility than winter hybrids in the same generation.

Energy feeding value and Protein feeding value

The relative feeding value is the highest at budding stage 171.7 rel. %, decreases slowly by 20 rel. % at the beginning of the flowering stage and then at full pod formation stage increases by 17 rel. % (Table 3). The same is the tendency in changing the net energy feeding values estimasted as feed units for milk and feed units for growth by French (UFL-UFV), Bulgarian (FUM-FUG) and Dutch (VEM-VEVI) systems. The highest feeding value belongs to spring parental forms – variety Pleven 4(B2-I) at budding stage: UFL 0.839 and spring variety Rosacrono (B1-III) at full pod formation stage: UFL 0.848. The highest energy feeding hybrids are hybrid Shtambovyi x Pleven 10 in F2 generation at budding stage: UFL 0.800; Shtambovyi x Pleven 10 in F2 generation at the beginnig of flowering stage UFL: 0.826 and Rosacrono x Pleven 4 in F2 generations at full pod formation stage UFL: 0.835. Consequently, the hybrids in F2 generation have higher energy feeding value than hybrids in F1 generation.

The protein feeding value at the vegetative stages ranged TDP/PBD 181 – 203, mean 190; 120 – 186, mean 165; 110 – 162, mean 135 g kg^{-1} dry matter; PDIN 142 – 156, mean 148;102 –145, mean 132; 110 – 162, mean 135 g kg^{-1} dry matter; PDIE 104 – 112, mean 108; 90 – 108, mean 103; 94 – 103, mean 98 g kg^{-1} dry matter. Among the winter varieties the Bulgarian Pleven 10 has significantly higher protein feeding value than the Russian variety Shtambovyi TDP/PBD 198; PDIN153 and PDIE 112 g kg^{-1} dry matter at budding stage. The hybrid Rosacrono x Pleven 4 in F1 generation has the highest protein feeding value, exceeding parent forms: TDP/PBD 203, PDIN 156 and PDIE 110 g kg^{-1} dry matter at budding stage as well as at full pod formation stage: TDP/PBD 162, PDIN 130 and PDIE 110 g kg^{-1} dry matter.

Correlation analysis

Applying rank correlation analysis on values of all parameters of forage chemical composition, digestibility and feeding value of pea varieties and hybrids, the rank correlation coefficients of Spearman and their statistical reliability are established and presented in a correlation matrix (Table 4). The crude protein content correlates strong positively with GE and PDIE, correlation coefficients: 0.997 and 0.993, respectively, and more stronger with TDP/PBD and PDIN 1,000 at $P \leq 0.01$. The crude fiber content correlate also positively with fiber components ADF and ADL, correlation coefficients 0.737 and 0.744 and negatively with DDM: -0.821, and more weakly with RFV: -0.648 and UFV: -0.524; FUG: -0.523. It is obviously proved that IVDMD and IVOMD are strongly positively correlated with all parameters of net energy UFL-UFV, FUM-FUG, VEM-VEVI, correlation coefficients 0.976-0.971 as well as ME 0.886; 0.837, respectively, as correlation coefficients for IVDMD have higher values UFL-UFV; FUM-FUG but not for VEM-VEVI. Fiber components NDF and ADF correlate highly positively with ADL and strongly negatively with DDM (ADF -0.953). The higher correlation is between NDF and DMI: - 0.997 and RFV: -0.951; ADF – DDM: -0.953 and ADF – ADL: -0.836. The ADL influences DDM: - 0.849; RFV: - 0.801 and DMI – RFV: 0.945. Metabolizable energy is strongly influenced by net energy, measured by French, Bulgarian and Dutch systems: 0.811 – 0.996 as the influence of VEM – VEVI is stronger 0.995–0.996, respectively. Concerning protein feeding value, TDP/PBD is influenced strongly positively except for CP content by GE: correlation coefficient 0.998. The PDIN also is strongly positively influenced by GE and TDP/PBD. The PDIE strongly positively depends on GE, TDP/PBD and PDIN 0.927; 0.938 and 0.939, respectively, at $P \leq 0.01$ and less on VEM-VEVI 0.645 – 0.646.

Principal component analysis

The genetic diversity between hybrids and source parent forms is shown by applying factorial analysis by the method of principal components (PCA – Principal component analysis) on the basis of 21 forage quality parameters studied (Table 5). There are four eigen values higher than 1 which determines the choice of four principal components. The first of them accounts for 44.58%, the second – for 26.81%, the third – for 18.06% and the fourth – for 7.38% of all variations. The principal components (F1 and F2) account for 71.39 % of all variations of genotype x characteristics. The first component is related mainly to performance of the parameters UFL, UFV, FUM, FUG, VEM, VEVI, ME, DMD and DMO. Parameters GE, PBD, PDIN, CP, PDIN and PDIE also participate in the formation of the second component. Characteristics of significance in shaping the third component are DDM, DMI, RFV. The fourth component is related mainly to the parameters NDF and DDM. Russian varieties Rosacrono and Shtambovyi are characterized by positive values of the two principal components. Bulgarian spring variety Pleven 4 is phenotypically different from winter variety Pleven 10, as well as from their hybrids in the second generation, Rosacrono x Pleven 4 and Shtambovyi x Pleven 10, respectively, and refer to the second and third quadrant, respectively. Hybrids located in the upper left

Table 3. Energy and protein feeding value of forage pea, Component A and Component B breeding forms

	DDM	DMI	RFV	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE
BUDDING STAGE												
A1-I	63.60	3.33	164.2	0.823	0.726	0.682	0.593	978	2006	184	145	110
A2-I	67.88	3.39	178.5	0.800	0.698	0.663	0.570	966	1987	198	153	112
A3-I	65.47	3.20	162.4	0.800	0.699	0.663	0.571	962	1981	191	149	109
A4-I	65.73	3.44	175.4	0.761	0.654	0.631	0.534	931	1932	187	146	107
B1-I	65.04	3.33	167.8	0.770	0.664	0.638	0.543	939	1945	188	147	107
B2-I	67.06	3.56	184.9	0.839	0.745	0.695	0.608	989	2024	185	145	109
B3-I	64.05	3.57	177.4	0.738	0.629	0.612	0.514	910	1900	181	142	104
B4-I	66.15	3.18	163.3	0.756	0.647	0.626	0.529	935	1938	203	156	110
Min	63.60	3.18	162.4	0.756	0.629	0.612	0.514	910	1900	181	142	104
Max	67.88	3.57	184.9	0.839	0.745	0.695	0.608	989	2024	203	156	112
Mean	65.62	3.38	171.7	0.785	0.683	0.651	0.558	951	1964	190	148	108
SD	1.43	0.15	8.41	0.04	0.04	0.03	0.03	27	42	7.6	4.8	2.0
CV	2.2	4.3	4.9	4.5	6.0	4.5	5.9	2.8	2.1	4.0	3.3	1.9
BEGINNING OF FLOWERING STAGE												
A1-II	63.69	3.18	157.2	0.815	0.718	0.675	0.586	964	1984	170	135	106
A2-II	61.52	2.82	134.5	0.721	0.615	0.598	0.503	870	1838	120	102	90
A3-II	66.18	3.14	160.9	0.826	0.730	0.685	0.597	974	2000	173	137	107
A4-II	65.09	3.19	161.1	0.772	0.667	0.640	0.545	939	1944	186	145	107
B1-II	62.77	2.97	144.4	0.751	0.645	0.622	0.527	916	1909	167	133	101
B2-II	65.84	3.01	153.4	0.815	0.717	0.676	0.586	969	1991	180	141	108
B3-II	64.42	3.26	162.8	0.815	0.717	0.676	0.586	969	1991	168	134	102
B4-II	63.04	2.96	144.6	0.754	0.649	0.625	0.530	912	1903	157	127	100
Min	61.52	2.82	134.5	0.721	0.615	0.598	0.503	870	1838	120	102	90
Max	66.18	3.26	162.8	0.826	0.730	0.685	0.597	974	2000	186	145	108
Mean	64.07	3.06	152.4	0.784	0.682	0.650	0.558	939	1945	165	132	103
SD	1.61	0.15	10.1	0.04	0.04	0.03	0.036	37	58	20.1	13.1	6.0
CV	2.5	4.9	6.7	5.0	6.4	5.0	6.4	4.0	3.0	12.1	9.9	5.8
FULL POD FORMATION STAGE												
A1-III	67.31	3.26	170.3	0.826	0.734	0.685	0,600	952	1965	132	110	99
A2-III	66.52	3.33	171.8	0.829	0.738	0.687	0,603	949	1960	121	103	97
A3-III	68.40	3.40	180.3	0.751	0.648	0.623	0,529	895	1876	134	111	94
A4-III	65.56	3.26	165.6	0.788	0.689	0.653	0.563	931	1933	144	118	98
B1-III	68.26	3.29	174.2	0.848	0.759	0.703	0.620	972	1996	139	115	102
B2-III	65.41	3.17	160.7	0.792	0.695	0.657	0.568	929	1929	136	113	98
B3-III	67.69	3.19	167.3	0.835	0.746	0.692	0.610	948	1959	110	96	94
B4-III	64.03	3.26	161.9	0.804	0.706	0.667	0.577	952	1965	162	130	103
Min	64.03	3.17	160.7	0.751	0.648	0.623	0.529	895	1876	110	96	94
Max	68.40	3.40	180.3	0.848	0.759	0.703	0.620	972	1996	162	118	103
Mean	66.65	3.27	168.8	0.809	0.714	0.670	0.584	940	1947	135	112	98
SD	1.55	0.07	6.3	0.03	0.04	0.03	0.03	23	35	15	10.1	3.2
CV	2.3	2.2	3.7	3.9	5.1	3.9	5.1	2.4	1.8	11.4	9.0	3.3

Table 4. Rank correlation coefficients of Spearman between parameters of chemical composition, digestibility and feeding value for evaluation of forage pea, Component A, Component B breeding forms

	CP	CF	DMD	DMO	NDF	ADF	ADL	DDM	DMI	RFV	UFL	UFV	FUM	FUG	VEM	VEVI	OE_MJ	ME_MJ	PBD	PDIN
CF	0.369																			
DMD	0.151	-0.356																		
DMO	-0.065	-0.509	**0.953**																	
NDF	-0.266	0.477	-0.264	-0.274																
ADF	0.123	**0.737**	-0.372	-0.481	0.493															
ADL	0.043	**0.744**	-0.350	-0.415	**0.652**	**0.836**														
DDM	-0.118	**-0.821**	0.457	**0.572**	**-0.536**	**-0.953**	**-0.849**													
DMI	0.265	-0.449	0.225	0.236	**-0.997**	-0.475	-0.634	0.510												
RFV	0.149	**-0.648**	0.333	0.386	**-0.951**	**-0.718**	**-0.801**	**0.763**	**0.945**											
UFL	-0.129	-0.507	**0.895**	**0.970**	-0.267	-0.437	-0.381	**0.542**	0.235	0.374										
UFV	-0.188	**-0.524**	**0.876**	**0.964**	-0.249	-0.439	-0.380	**0.542**	0.217	0.362	**0.998**									
FUM	-0.134	-0.511	**0.894**	**0.971**	-0.266	-0.439	-0.382	**0.543**	0.234	0.374	**1.000**	**0.998**								
FUG	-0.190	**-0.523**	**0.876**	**0.964**	-0.245	-0.439	-0.379	**0.541**	0.213	0.358	**0.998**	**1.000**	**0.998**							
VEM	0.356	-0.275	**0.916**	**0.881**	-0.359	-0.333	-0.316	0.432	0.329	0.401	**0.880**	**0.851**	**0.878**	**0.849**						
VEVI	0.355	-0.272	**0.914**	**0.880**	-0.359	-0.330	-0.313	0.429	0.329	0.400	**0.880**	**0.851**	**0.878**	**0.849**	**1.000**					
OE_MJ	**0.997**	0.362	0.128	-0.092	-0.269	0.111	0.029	-0.112	0.268	0.153	-0.169	-0.227	-0.173	-0.229	0.317	0.315				
ME_MJ	0.411	-0.195	**0.886**	**0.837**	-0.340	-0.268	-0.256	0.366	0.312	0.364	**0.845**	**0.813**	**0.842**	**0.811**	**0.995**	**0.996**	0.368			
PBD	**1.000**	0.368	0.149	-0.069	-0.266	0.121	0.041	-0.116	0.265	0.150	-0.135	-0.194	-0.140	-0.196	0.350	0.349	**0.998**	0.404		
PDIN	**1.000**	0.369	0.151	-0.066	-0.267	0.123	0.043	-0.118	0.266	0.150	-0.130	-0.188	-0.134	-0.191	0.355	0.355	**0.997**	0.410	**1.000**	
PDIE	**0.939**	0.179	0.474	0.282	-0.352	-0.047	-0.101	0.083	0.338	0.277	0.210	0.152	0.206	0.150	**0.646**	**0.645**	**0.927**	**0.684**	**0.938**	**0.939**

* $p \leq 0.05$; ** $\mathbf{p \leq 0.01}$

Table 5. The Eigen values and vectors for forage pea hybrids/genotype parameters

Parameters	F1	F2	F3	F4
CP	0.231	0.839	0.488	-0.012
CF	-0.520	0.428	-0.559	-0.182
IVDMD	0.890	0.061	-0.402	-0.056
IVDMO	0.944	-0.179	-0.251	0.042
NDF	-0.114	0.491	-0.575	0.643
ADF	0.086	0.398	-0.682	-0.602
ADL	0.269	0.670	-0.239	-0.283
DDM	0.235	-0.493	0.668	0.485
DMI	0.090	-0.562	0.562	-0.595
RFV	0.155	-0.655	0.693	-0.253
UFL	0.965	-0.217	-0.141	0.017
UFV	0.954	-0.251	-0.160	0.021
FUM	0.966	-0.218	-0.136	0.023
FUG	0.953	-0.254	-0.162	0.022
VEM	0.996	0.067	0.003	0.010
VEVI	0.996	0.063	0.002	0.012
GE	0.130	0.857	0.496	-0.018
ME	0.993	0.098	0.007	0.001
PBD	0.216	0.844	0.487	-0.009
PDIN	0.230	0.840	0.487	-0.014
PDIE	0.636	0.692	0.338	0.006
Eigen value	9.362	5.630	3.794	1.551
Variability (%)	44.581	26.812	18.067	7.385

quadrant of the coordinate system Rosacrono x Pleven 4 (F1) and Shtambovyi x Pleven 10 (F1) have positive values of the component F2. The space available between them implies a similar phenotype in a relatively small number of quality parameters. The location of the varieties and hybrids in bipolar plane found that from all qualitative characteristics, the defining ones being GE, PBD, CP and PDIN. Correlation of DMI and RFV with principal component F1 is negative. Vectors with the greatest length and determinants of the level of diversity are VEVI, VEM, ME and DMD.

Cluster analysis

Based on the values of quality parameters studied, hierarchical cluster analysis of the varieties of forage pea and their hybrids is performed. Euclidean distance is used as a measure of genetic distance. The results presented as a dendrogram (Figure 1) show different groupings of varieties by similarity and difference. Samples were clustered into two groups (A and B). The data shows that there is significant genetic distance between spring varieties of subspecies *Pisum sativum* Shambovii and Pleven 4 and their hybrids in both generations. These varieties are divided in an independent group "A". The other main group "B" includes varieties Rosacrono and Pleven 10, which are subspecies Pisum arvense, as well as two hybrid generations (F1 and F2). This group is divided into two sub-groups "B1" and "B2". In subgroup "B1" variety Pleven 10 and second generation of hybrid Rosacrono x Pleven 4 (F2) are included. Variety Rosacrono, Rosacrono x Pleven 4 (F1) can be characterized as genetically closer. Rosacrono and Shtambovyi x Pleven 10 (F2) are grouped separately to subgroup "B2". In the same subgroup, hybrids Rosacrono x Pleven 4 (F1) and Shtambovyi x Pleven 10 (F1) are also forming the second branch.

To obtain a more prominent transgressive forms in hybrid combinations, genotypes from different groups must be included so as to expect a good combination of favorable genes into one

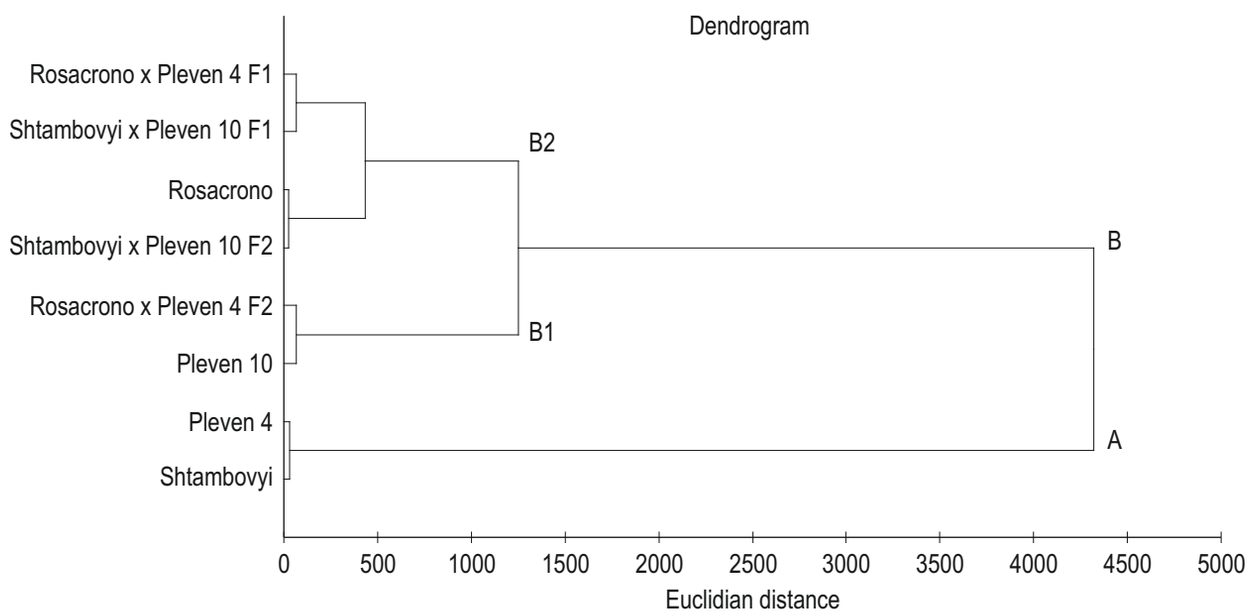

Figure 1. Dendrogram of forage pea varieties and hybrids

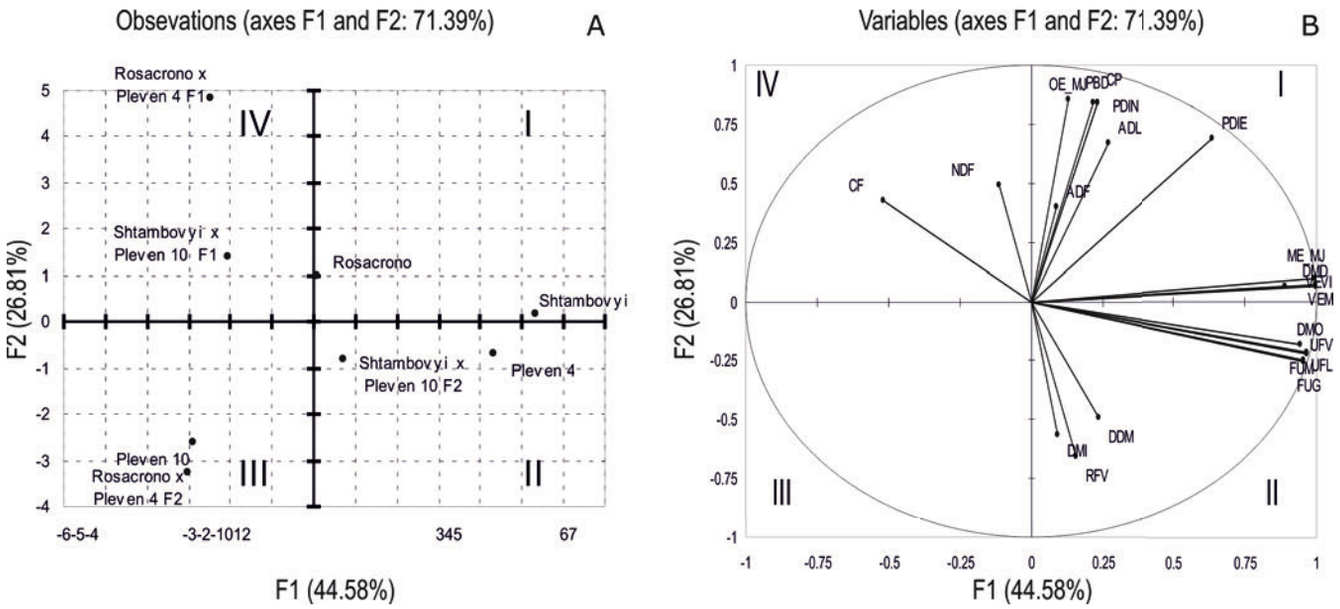

Figure 2. Principal Component Analysis of qualitative characteristics of *Pisum sativum* hybrids/genotypes

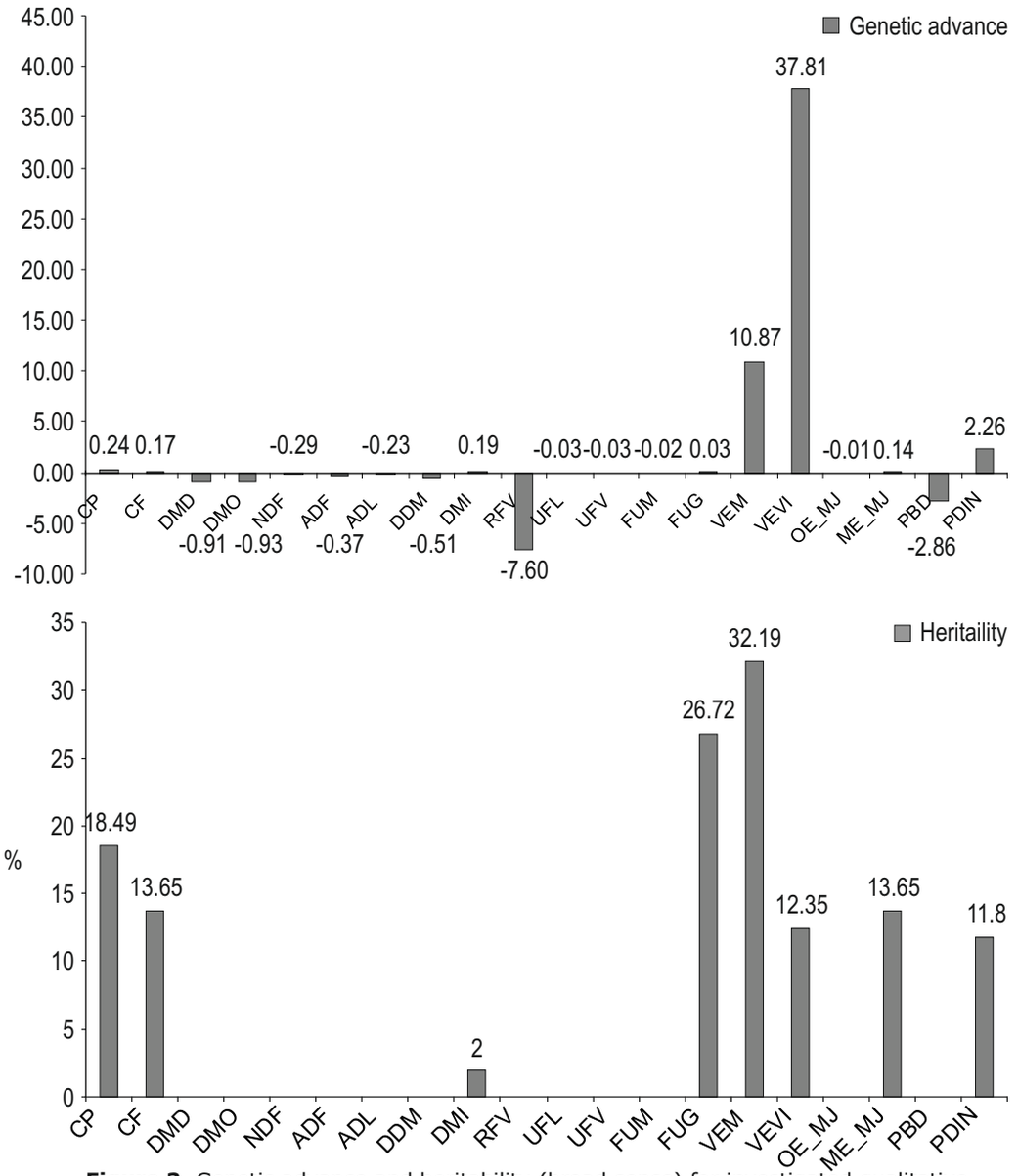

Figure 3. Genetic advance and heritability (broad sense) for investigated qualitative traits of Pisum sativum genotypes

genotype. Hierarchical cluster analysis can be used in the selection for planning initial parent combinations (Dragavtsev, Averyanova, 1983).

Estimation of genetic advance and heritability (broad sense)

The consequences of heritability coupled with genetic advance showed that VEM has high heritability as well as high genetic advance, indicating that this characteristic is the least affected by the environment. Thus, this characteristic may be improved through selection method. Almost all traits showed low heritability coupled with low genetic advance indicating that the involvement of non-additive gene action (dominance and epistasis) is exhibited due to the favorable influence of the environment. So selection would be ineffective for them, therefore other breeding methods should be taken for improvement of these traits.

Systematization of assessment information gathered from pea collection along with chemical characterization created possibilities for increasing the effectiveness of a breeder's programme (Angelova and Stoilova, 2009). According to Micek et al. (2014) and Nikolova et al. (2015) the net energy content of the bean seeds used in the study is related to their chemical composition and especially to CP, NDF and ADF content and IVTD absorption. Nikolova et al. (2014) reported that genotypes with high energy feeding value are characterized by low values of NDF and ADF and high protein content and digestibility.

Kalia and Sood (2009) found both additive and non-additive gene action in the inheritance of the signs studied by them (number and weight of pods from one plant, days to full flowering, crude protein content). They assume the crossing of selected material in bialellic scheme, allows to occur additive and non-additive gene effects, which could be useful for genetic improvement of valuable traits and characteristics. Kumari et al. (2012) reported low heritability for total crude protein and ash content coupled with highly expected genetic advance in pea.

Conclusion

At full pod formation stage digestibility in both spring and winter parental forms is higher than 70% and close for the winter varieties Shtambovyi and Pleven 10: 71 – 73% and divergent for the spring varieties Rosacrono and Pleven 4. The tendencies in digestibility of hybrids in F1 and F2 generations are stable and demonstrative. The winter hybrids Shtambovyi x Pleven 10 in F1 and F2 generations are digestible 64 – 68% which values are 5 – 7% lower than those of the parental forms. However, the spring hybrids Rosacrono x Pleven 4 in F1 and F2 generations demonstrate close digestibility values of dry and organic matter (70 – 73%) and do not exceed parental forms either. The spring hybrid forms in F1 and F2 generations are more digestible than winter hybrids in the same generation.

The highest energy feeding hybrids are hybrid Shtambovyi x Pleven 10 in F2 generation at budding stage: UFL 0.800; Shtambovyi x Pleven 10 in F2 generation at the beginnig of the flowering stage UFL 0.826 and Rosacrono x Pleven 4 in F2 generations at full pod formation stage UFL 0.835. Consequently the hybrids in F2 generation have higher energy feeding value than hybrids in F1 generation.

Between the winter varieties the Bulgarian Pleven 10 has a significantly higher *protein feeding value* than the Russian variety Shtambovyi at budding stage. The hybrid Rosacrono x Pleven 4 in F1 generation has the highest protein feeding value, exceeding parent forms at budding stage as well as full pod formation stage:.TDP/PBD 162, PDIN 130 and PDIE 110 g kg^{-1} dry matter.

The highest correlation is between NDF and DMI: - 0.997 and RFV: -0.951; ADF – DDM: -0.953 and ADF – ADL: -0.836. The ADL influence DDM: - 0.849; RFV: - 0.801 and DMI – RFV: 0.945. Metabolizable energy is strongly influenced by net energy 0.811 – 0.996 and the influence of VEM – VEVI is stronger 0.995 – 0.996, respectively. The protein feeding value TDP/PBD is influenced strongly positively except CP content by GE correlation coefficient 0.998. The PDIN is also strongly positively influenced by GE and TDP/PBD. The PDIE strongly positively depends on GE, TDP/PBD and PDIN 0.927; 0.938 and 0.939, respectively at $P \leqslant 0.01$ and weaker on VEM-VEVI 0.645 – 0.646.

Genotypes in terms of genetic similarity and genetic remoteness on the basis of the characteristics studied are well grouped by cluster analysis. Moreover, it could be used in breeding process for planning of the original parental combinations. Ecologically remote hybrids/genotypes show similarity in the characteristics, can be effectively included in a crossing scheme.

Genetic advance is the highest for net energy feeding value VEVI and VEM. Thus, these characteristics may be improved through selection method. Other forage quality characteristics express moderate or low genetic advance. The parental components sort different genetic basis which enriches genetic plasm with significant for the breeding process biochemical characteristics.

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CONTENTS 1 / 2

Review

Achievements and problems in the weed control in common wheat (*Triticum Aestivum* L.) and durum wheat (*Triticum Durum* Desf.) 281
G. Delchev, M. Georgiev

Genetics and Breeding

Vokil – a new Bulgarian sunflower hybrid 287
G. Georgiev, V. Encheva, N. Nenova, Y. Encheva, D. Valkova, P. Peevska, G. Georgiev

Composition, digestibility, feeding value estimation of forage pea (*Pisum sativum* L.) varieties and hybrids in breeding process and genetic advance evaluation 293
Y. Naydenova, V. Kosev

Effect of age at first calving on conformation traits in Black-and-White cows 303
I. Marinov, Zh. Gergovska

Nutrition and Physiology

Stimulating feeding and development of hypopharyngeal and thoracic glands of honeybees (*Apis mellifera* L.) 309
R. Shumkova, I. Zhelyazkova

Histopathological changes in small intestines of broiler chickens with experimental aflatoxicosis 313
N. Grozeva, I. Valchev, Ts. Hristov, L. Lazarov, Y. Nikolov

Histopathological pancreatic changes in broiler chickens with experimental aflatoxicosis 319
I. Valchev, N. Grozeva, D. Kanakov, Y. Nikolov

Production Systems

Effect of foliar fertilization on sunflower (*Helianhtus annuus* L.) 324
G. Milev

Changes in the productivity and the yield structural elements in some spring crops sown on damaged by frost crops of winter oilseed canola 328
G. Delchev

Agriculture and Environment

Chromium level in water, sediment, aquatic plants and meat of common carp (*Cyprinus carpio* L.) from different water bodies in Bulgaria 334
E. Valkova, V. Atanasov, K. Velichkova, G. Kostadinova, M. Tzanova

CONTENTS 2 / 2

Visible – NIR reflectance for evaluation of luvisols and phaeozems 339
M. Todorova, M. Mihalache, L. Ilie, S. Atanassova

Groundwater quality as a source for irrigation in Strumica valley, Republic of Macedonia 344
B. Kovacevik, S. Mitrev, B. Boev, I. Karov, V. Zajkova Panova

Chemical and physical properties of the Albic luvisols from Albota-Pitești 350
M. Mihalache, L. Ilie, M. Todorova, N. Grozeva

Product Quality and Safety

Effects of salt-curing on physicochemical and technological properties of Japanese quail breast meat 354
S. Ribarski, H. Lukanov, I. Penchev, A. Genchev, E. Lyutskanova

Study of physicochemical parameters of lamb meat from North East Bulgarian fine fleece breed of sheep and its crossbreds from internal breeding 360
R. Slavov, G. Mihaylova, S. Ribarski, P. Slavova, D. Pamukova

Egg quality and shell colour characteristics of crosses between Araucana and Schijndelaar with highly productive White Leghorn and Rhode Island Red strains 366
H. Lukanov, A. Genchev, A. Pavlov

Influence of the sweet red papers extract on the quality and oxidative changes in fats of sausages 372
A. Kuzelov, V. Ilieva, N. Taskov, D. Saneva

Effect of dietary vitamin E supplementation on the oxidative stability of lipids and proteins in cooked pork 379
T. Popova, P. Marinova, M. Ignatova

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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