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CAMELINA SATIVA AS A SUSTAINABLE AND FEASIBLE FEEDSTUFF FOR LAYING POULTRY: A REVIEW*

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Review paper

Abstract: *Camelina sativa* is a promising oilseed crop with unique characteristics, including rapid growth, drought and frost tolerance, low input requirements, and resistance to pests and diseases. It offers diverse applications in both feed and non-feed sectors, primarily due to its high levels of *n*-3 polyunsaturated fatty acids (PUFA) and antioxidants. However, the presence of secondary plant metabolites in camelina restricts its use in poultry nutrition. These compounds may inhibit some digestive enzymes, increase digesta viscosity, and affect nutrients absorption, potentially compromising bird health and product quality. Various techniques, such as heat treatment, multi-enzyme supplementation, and copper supplementation, have been employed to mitigate the negative effects of these antinutritional compounds. Inclusion at high levels (>10%) of camelina by-products in poultry diets has been found to decrease nutrients digestibility and laying performance. Nonetheless, the inclusion of camelina by-products, particularly oil, in the diets resulted in comparable or improved egg quality. The egg yolk fatty acid profile exhibited a higher content of PUFA, reducing the *n*-6/*n*-3 ratio, thereby enhancing the nutritional value of eggs. Sensory evaluations showed no significant differences in product quality among diet groups. This review highlights the feeding value of camelina by-products and provides a comprehensive overview of the existing literature, focusing on digestibility, performance, and egg quality evaluation in laying poultry diets.

Key words: false flax, feeding, inclusion level, live performance, egg quality, fatty acids, sensory analysis

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Introduction

In recent years, there has been a growing emphasis on identifying sustainable and economically viable alternatives to conventional feed sources in poultry production. The conventional feed sources, predominantly composed of soybean and corn, encounter challenges such as environmental impacts, price volatility, and limited availability. Consequently, in the last 20 years, researchers have explored alternative feed ingredients with the aim of maintaining good performance levels *(Rokka et al., 2002; Cherian et al., 2009; Kakani et al., 2012; Aziza et al., 2013; Lolli et al., 2020; Orczewski-Dudek et al., 2020; Cullere et al., 2023; Singh et al., 2023)*. One promising candidate in this regard is *Camelina sativa* (L.) Crantz, commonly known as camelina or false flax, an oilseed crop that has gained attention for its potential as a sustainable and feasible feedstuff for laying poultry.

Camelina sativa belongs to the Brassicaceae family and is recognised for its high oil content, ranging from 30% to 45%. This oil content is particularly rich in essential omega-3 fatty acids (FA) (*Singh et al., 2023*). Additionally, the cultivation of camelina requires fewer resources, such as water and fertilisers, in comparison to other traditional oilseed crops, rendering it an environmentally friendly option (*Zanetti et al., 2021*). As a hardy crop, camelina can thrive in marginal lands, thereby reducing the strain on prime agricultural areas. Additionally, camelina cultivation entails a low carbon footprint due to reduced pesticide and fertiliser applications, which contributes to sustainable farming practices (*Zanetti et al., 2021; Mondor and Hernández-Álvarez, 2022*). Moreover, incorporating camelina in poultry feed has the potential to decrease reliance on traditional feed ingredients, thereby fostering greater economic viability in poultry production.

These characteristics have generated increased interest in exploring the utilisation of camelina as a feed ingredient for laying poultry. The nutritional composition of camelina as is (seed) or as derived products (meal and oil) makes them highly valuable for their inclusion in poultry diets. They possess a favourable balance of amino acids, including essential amino acids that play a crucial role in optimising egg production and quality. Furthermore, by-products derived from camelina serve as a good source of energy, vitamins, and minerals, augmenting their potential as a feedstuff for laying poultry (*Pietras et al., 2012; Orczewska-Dudek et al., 2020*). The high presence of omega-3 fatty acids in camelina by-products can also have a positive influence on egg quality by enriching the eggs with these beneficial nutrients (*Pietras et al., 2012; Orczewska-Dudek et al., 2020*). The incorporation of camelina in laying poultry diets has demonstrated improvements in egg production, egg weight, feed conversion ratio, and shell quality (*Cherian et al., 2009; Kakani et al., 2012; Pietras et al., 2012; Bulbul and*

Ulutas, 2015; Aziza et al., 2013; Cherian and Quezada, 2016; Lolli et al., 2020; Orczewska-Dudek et al., 2020). The oil of camelina is rich in omega-3 FA, thus camelina inclusion in layers diet increases the omega-3 FA in eggs. Moreover, its ingredients have been shown an overall improvement in the egg's nutritional profile (*Rokka et al., 2002; Cherian et al., 2009; Pietras et al., 2012; Aziza et al., 2013; Cherian and Quezada, 2016; Panaite et al., 2016; Orczewska-Dudek et al., 2020*).

Despite its potential, the utilisation of camelina in laying poultry diets does present certain challenges. Antinutritional factors, such as glucosinolates and erucic acid, necessitate attention to ensure the safe inclusion of camelina in feed formulations (*Russo and Reggiani, 2012*). Strategies, including genetic modification and processing techniques, have been employed to mitigate the presence of these undesirable components. Genetic engineering techniques have been utilised to develop camelina varieties with reduced levels of undesirable components. By targeting specific genes involved in the synthesis or regulation of glucosinolates and erucic acid, researchers have successfully developed genetically modified camelina plants with altered profiles of these compounds. Through the use of gene editing tools or the introduction of genes from other plant species, the expression of key enzymes involved in the synthesis of undesirable components can be suppressed or modified, leading to reduced levels in the resulting crops (*Vollmann and Eynck, 2015; Zanetti et al., 2021*).

Processing methods play a crucial role in reducing or eliminating undesirable components in camelina. Various techniques have been employed to detoxify or modify the composition of seed and meal. These techniques include heat treatment (*Zajac et al., 2021*), and enzymatic treatments (*Woyengo et al., 2016*). For instance, heat treatment can effectively reduce glucosinolates content. The use of these strategies, whether individually or in combination, aims to mitigate the presence of glucosinolates and erucic acid in camelina, thereby improving the safety and suitability of camelina as a feedstuff for livestock.

Apart from being a potential feed ingredient, offers a range of non-feed uses. These non-feed uses of camelina have gained attention due to its unique properties and potential environmental benefits. Some of the notable non-feed uses of camelina are: *Soil amendment and phytoremediation*: camelina plants have deep root systems that can help improve soil structure and reduce soil erosion. Additionally, camelina has the ability to extract heavy metals from contaminated soils, making it suitable for phytoremediation purposes to restore polluted sites (*Berti et al., 2017*). *Nutraceuticals and functional foods*: camelina oil contains omega-3 fatty acids, including alpha-linolenic acid, which is beneficial for cardiovascular health and inflammation reduction. It can be used as an ingredient in nutraceuticals and functional foods, such as supplements, health drinks, and fortified food products, to provide the health benefits associated with omega-3 FA (*Zubr, 2009; Faustino et al., 2016*). *Cosmetics and personal care products*: camelina oil is rich not only in omega-3 FA, but also in antioxidants, and vitamin

E, which are beneficial for healthy skin and hair. It can be incorporated into cosmetic formulations, including creams, lotions, serums, and hair care products, to provide moisturising, nourishing, and protective properties (*Arshad et al., 2022*). *Biofuel production*: camelina oil is suitable for biofuel production. The oil can be processed into biodiesel, which is a renewable and environmentally friendly alternative to fossil fuels. Camelina-based biodiesel has shown promising results in terms of lower greenhouse gas emissions (*Lebedevas et al., 2012*). *Green chemicals and bioplastics*: camelina oil can serve as a renewable feedstock for the production of green chemicals and bioplastics. The oil can be transformed into various chemical intermediates used in the manufacturing of biodegradable plastics, resins, coatings, etc… (*Balanuca et al., 2015; Kim et al., 2015*). These non-feed uses of camelina highlight its versatility and potential as a sustainable crop with applications in various industries. However, this article provides a comprehensive and critical review of research conducted on camelina and its by-products for utilisation in laying poultry feeding. The review presents valuable insights into the feeding value of camelina by-products and encompasses an extensive survey of the existing literature pertaining to their incorporation in laying poultry diets, focusing on aspects such as digestibility, performance, and egg quality.

Layer Digestibility

Most studies have revealed that nutrients and energy digestibility is reduced if poultry diets contain camelina seeds or cake, with some exception. *Varzaru et al. (2014)* included camelina seeds (2%) together with other oilseeds (linseed and fenugreek seeds at different inclusion levels) in laying hens' diets. The outcomes of the study revealed that the diets supplemented with 2% camelina seed and 5% linseed did not affect the digestibility coefficient of amino acids, except phenylalanine and arginine. However, digestibility coefficients of the amino acids decreased in the group supplemented with 2% camelina seed, 2% linseed, and 1% fenugreek seed.

As regards the use of camelina cake, when low inclusion level (1% or 2%) was used, crude protein and amino acids (except tyrosine, cysteine, glycine, and serine) digestibility did not vary in laying hens (*Peñagaritano et al., 2019*). Such low inclusion levels were not able to highlight the known negative effects of glucosinolates (5.73 μmoles/g) and erucic acid (4.01%) present in the cake. Using the same camelina cake inclusion level as in the previous work (2%), but supplementing the diet with exogenous enzymes (12.5 g/100 g feed) and copper (150 mg/kg feed), the apparent faecal digestibility of amino acids was significantly improved, however, showing an effect not entirely attributable to camelina cake (*Varzaru et al., 2019*). In contrast, increased inclusion level of camelina cake (up to 10%) in the diets of laying hens not only decreased the digestibility of crude protein, but also reduced the apparent metabolisable energy content of the diet (*Aziza et al., 2013*). The reason relates not only to the presence of glucosinolates but also to that of non-starch polysaccharides, which interfere with available nutrients by forming complexes and inactivating digestive enzymes (*Acamovic et al., 1999*).

Layer performance

The effect of the dietary inclusion of camelina by-products on layer poultry productive performance is presented in Table 1. The dietary inclusion of 10% camelina seeds improved hens egg production by 5.1%, and also increased the feed intake by 9.44 g/bird/day compared to the control group. The study did not report the feed conversion ratio (FCR) or feed efficiency, however average egg weight was lowered for group-fed camelina seeds compared to the control group (*Cherian and Quezada, 2016*).

Table 1. Effect of the dietary inclusion of camelina by-products on layer poultry productive performance

Species	Matrix	Bird age, wk	Feeding, wk	Inclusion, %	FI, g/d/bird	FCR	Egg production, %	Egg weight, g	Ref.
Hen	Control			0	116±7.04	2.20±0.32	87.6±4.62	61.4±2.31	1-9
	Seed	48	16	10	128**	-	92.8*	60.4*	1
	Oil	23	4	1.5, 3	119	2.68	83.7	60.2	2
		26	7	4	116	1.99	91.0	61.1	3
	Cake	26	7	10	118	2.02	89.8	61.3	
		24	12	10	-	-	95.6*	56.5	4
		58	11.4	5, 10, 15	102	-	77.1**	61.6	5
		29	12	5, 10	-	-	95.3	59.0*	6
		60	6	2	116*	2.05*	88.8*	64.7*	7
		21	30	10, 20	118	2.21	-	62.0	8
		58 & 60	6 & 4	2		1.98*	86.8	64.6	9
Quail	Control			0	38.1	3.81	86.8	11.5	10
	Cake	8	8	5, 10, 15, 20	35.2*	3.70	84.2**	11.3	

(Mean value of species-specific control diets ± Standard deviation)*;* wk: weeks; Ref.: references; 1: *Cherian and Quezada (2016);* 2: *Pietras et al. (2012);* 3: *Orczewska-Dudek et al. (2020);* 4: *Aziza et al. (2013);* 5: *Cherian et al. (2009);* 6: *Kakani et al. (2012);* 7: *Panaite et al. (2016);* 8: *Lolli et al. (2020);* 9: *Varzaru et al. (2019);* 10: *Bulbul and Ulutas (2015);* *P<0.05, **P<0.01 or P<0.001 vs control within study.

In studies conducted by *Pietras et al. (2012)* and *Orczewska-Dudek et al. (2020)*, the impact of incorporating camelina oil into the laying hen diet at levels of 1.5%, 3%, and 4% was investigated. As expected, the inclusion of camelina oil did not significantly affect performance and production parameters, which remained comparable to the control group. It should be noted that camelina oil does not contain antinutritional compounds that could negatively impact bird performance and production. In laying quails, a small amount of camelina oil (0.39% of the diet), in replacement of the soybean oil, significantly increased the egg weight after 6 weeks of administration (*Dalle Zotte et al., unpublished data*). These findings highlight the potential of camelina oil as a beneficial dietary component for layers.

As for the dietary inclusion of camelina cake, the results on laying hens generally revealed good egg production and quality. When the 5% or 10% inclusion level was applied, no differences in live performances were observed (*Cherian et al., 2009; Kakani et al., 2012; Orczewska-Dudek et al., 2020*), except for a lower egg weight ($P<0.05$) compared to the control diet (*Kakani et al., 2012*). However, literature results are nonuniform, as at 10% inclusion level the egg production increased by 8% compared to the control diet without affecting the eggs weight (*Aziza et al., 2013*).

The upper limit for including camelina cake is still unclear, as the limited studies conducted so far have presented conflicting data. Indeed, whereas the 15% inclusion level significantly reduced egg production (<19%; $P<0.01$) (*Cherian et al., 2009*), the 20% did not negatively affect feed intake, FCR and egg production (*Lolli et al., 2020*).

Considering that the supplementation of Cu in the laying hens' diet has been shown to reduce the concentration of antinutritional compounds, particularly glucosinolates, either through absorption from the intestinal lumen or through their transformation into other by-products (*Panaite et al., 2016; Varzaru et al., 2019*), further research has been conducted by enriching diets with Cu when camelina cake was included, without exceeding 200 ppm concentration, as higher levels can lead to gizzard degeneration, consequently reducing feed intake (*Kim et al., 1992*). Therefore, the result of two studies which tested the dietary inclusion of camelina cake at 2% showed promising results when Cu was supplemented. In the first study, the diets also contained linseed cake (5%) and 3 of them were supplemented with increasing Cu concentrations (75, 100, 150 mg/kg feed). As a result, FCR decreased linearly and egg production increased with Cu concentration (*Panaite et al., 2016*). In the second study (*Varzaru et al., 2019*), a control diet was compared with a diet containing camelina cake (2%) and linseed cake (5%), supplemented with Cu (150 mg/kg feed) and cellulolytic enzyme (12.5 kg/100 g feed) fed to laying hens. The findings revealed that the inclusion of these supplements also led to improvements in FCR, confirming the enhancement of the nutrient utilisation by the hens. however egg production and egg weight were comparable to those in the control group.

A study investigated the inclusion of camelina cake (5%, 10%, 15%, or 20% inclusion level) in diets for laying quails (*Bulbul and Ulutas, 2015*), whose eggs are commercially sold as table eggs in many countries. Quails layers fed with a 15% or 20% inclusion level reduced their feed intake and lowered their egg production, but the lower inclusion levels did not affect their performances, resulting comparable to the group fed a control diet. Significantly lower digestibility of ether extract and energy, and lower egg weight, were recently observed when a 15% camelina cake inclusion level was tested in laying quails, which were fed the camelina-containing diet from their pullet stage; however, the same inclusion level did not affect feed intake, FCR, egg production, and egg weight when camelina cake inclusion only covered the laying period (*Dalle Zotte et al., unpublished data*), thus suggesting the possibility of considering the inclusion of camelina cake in diets for laying quails as a promising alternative ingredient to conventional raw materials.

Egg quality

The inclusion of camelina by-products into layers diet can positively modify the various egg quality traits, playing an important role on consumer satisfaction and on profitability of the production chain (Tables 2-5).

The effect of the dietary inclusion of camelina in layers on the egg physical traits is depicted in Table 2. The inclusion of camelina seeds at 10% in laying diets seems too high, as it negatively affected albumen weight and yolk weight and colour (*Cherian and Quezada, 2016*). On the contrary, the use of camelina oil gave good results, since at 1.5% and 3% inclusion levels it was able to increase the yolk proportion (*Pietras et al., 2012*), and at 4% inclusion it also intensified the yolk colour (*Orczewska-Dudek et al., 2020*).

Table 2. Effect of the dietary inclusion of camelina by-products on egg physical traits of layer poultry

Species	Matrix	Bird age, wk	Feeding, wk	Inclusion, %	Yolk weight, g	Yolk, %	Yolk colour†	Albumen weight, g	Albumen, %	Shell weight, g	Shell thickness, mm	Haugh unit	Shape index, %	Ref.
Hen	Control			0	15.5±1.75	25.6±2.22	4.97±2.11	38.2±1.30	63.2±2.18	7.24±1.41	0.39±0.03	74.4±10.1	-	1-7
	Seed	48	16	10	15.3	25.4	5.40	38.4*	63.5	6.76	0.43	68.7	-	1
	Oil	23	4	1.5, 3	13.8*	22.9	-	40.2	66.8	6.28	0.36	87.0	-	2
		26	7	4	16.6	27.2	2.90**	37.1	60.7	7.44	0.38	-	-	3
	Cake	26	7	10	16.6	27.0	2.93**	37.6	61.4	7.13	0.38	-	-	
		24	12	10	12.8*	22.7*	5.63*	37.0	65.5	6.56	0.41	77.1	-	4
		58	11.4	5, 10, 15	16.3*	26.4*	6.50**	39.1	63.4*	6.23	0.42	-	-	5
		29	12	5, 10	-	-	-	-	-	-	0.40	75.0	-	6
		21	30	10, 20	-	-	-	-	-	6.21	0.37	-	-	7
Quail				Control	5.80	51.38	4.30	4.45	38.7	1.25	0.21	65.1	78.0	8
	Cake	8	8	5, 10, 15, 20	5.50	53.1	6.73	4.54	40.2	1.25	0.21	62.29	77.5	

(Mean value of species-specific control diets ± Standard deviation); wk: weeks; Ref.: references; †Roche yolk colour fan; 1: *Cherian and Quezada (2016);* 2: *Pietras et al. (2012);* 3: *Orczewska-Dudek et al. (2020);* 4: *Aziza et al. (2013);* 5: *Cherian et al. (2009);* 6: *Kakani et al. (2012);* 7: *Lolli et al. (2020);* 8: *Bulbul and Ulutas (2015);* *P<0.05, **P<0.01 or P<0.001 vs control within study.

The camelina cake inclusion level resulted in contradictory results. Some authors observed no significant differences in the physical traits of hen eggs with 5% (*Cherian et al., 2009*) or 10% (*Kakani et al., 2012; Lolli et al., 2020*) camelina cake supplementation. Others, observed that a 10% dietary inclusion increased (*Orczewska-Dudek et al., 2020*) or reduced the yolk colour, together with the yolk weight (*Aziza et al., 2013*). The worst results were found by *Cherian et al. (2009)* who observed that the inclusion of camelina cake at 10% or 15% significantly reduced the yolk weight, yolk colour, yolk and albumen proportions compared to the control group. It is likely that the contrasting data on yolk colour depend on the defatting level of the camelina cakes, since the higher the oil content, the higher the pigments in the diet. Quail eggs are also affected by the inclusion of the camelina cake, and the 15% inclusion level (with cake containing 12.7% oil) showed a significant increase in yolk a* and b* values (*Dalle Zotte et al., unpublished data*).

Research on the impact of camelina dietary inclusion in laying hens on egg chemical composition is limited (Table 3). However, two notable studies conducted by *Pietras et al. (2012)* and *Orczewska-Dudek et al. (2020)*, which tested 1.5%, 3% camelina oil, and 10% camelina cake or 4% camelina oil, respectively, provided insights into this aspect. Both studies did not observe significant differences in the chemical composition of the eggs, compared to the hens receiving the control diet. This result is expected, as negligible changes in egg proximate composition are observed when the laying hen's diet is nutritionally balanced.

Table 3. Effect of the dietary inclusion of camelina by-products on chemical composition (%) and cholesterol of laying hen egg yolk

Species	Matrix	Bird age, wk	Feeding, wk	Inclusion, %	Water, %	Protein, %	Lipids, %	Cholesterol, g/kg	Ref.
Hen	Control			0	52.6±0.36	15.8±0.01	32.1±0.01	9.63±0.01	1-2
	Cake	26	7	10	52.9	16.0	33.2	9.76	1
	Oil	26	7	4	53.7	15.9	33.3	9.34	
		23	4	1.5, 3	51.1	15.9	33.2		2

(Mean value of species-specific control diets ± Standard deviation); wk: weeks; Ref.: references; 1: *Orczewska-Dudek et al. (2020);* 2: *Pietras et al. (2012).*

Considering the FA profile of camelina, whose seeds contain an average of 54% PUFA, of which 36% are *n*-3 FA, and the *n*-6/*n*-3 ratio is approximately 0.5 (*Dalle Zotte et al., unpublished data*), studies have focused on the extent to which eggs are enriched with *n*-3 fatty acids as a result of the dietary inclusion of camelina (Tables 4 and 5). Only one study addressed the effect of the seed inclusion (10%) on laying hens and, as expected, laid eggs were significantly higher in *n*-3 PUFA and lower in *n*-6/*n*-3 ratio (*Cherian and Quezada, 2016*).

Table 4. Effect of the dietary inclusion of camelina by-products on main fatty acid classes (% fatty acid methyl esters) of laying hen egg yolk

Species	Matrix	Bird age, wk	Feeding trial, wk	Inclusion, %	SFA	MUFA	PUFA	*n*-6	*n*-3	*n*-6/*n*-3	Ref.
Hen	Control			0	35.6±2.14	49.8±3.39	16.7±6.24	14.2±5.64	1.61±0.72	8.79±4.03	1-7
	Seed	48	16	10	34.1	52.0	-	10.8	3.12*	2.45*	1
	Oil	52	3	5	-	-	21.4	14.6	6.83	2.14	2
		23	4	1.5, 3	39.8**	49.2*	10.5**	8.43**	2.08**	4.27**	3
		26	7	4	35.7	45.1**	19.0**	13.6	4.65**	2.92**	4
	Cake	26	7	10	35.8	45.3**	18.9**	13.4	4.14**	3.23**	
		24	12	10	33.0*	43.4*	23.6**	19.2	4.39*	4.37*	5
		58	11.4	5, 10, 15	33.3**	50.9**	-	13.3**	2.74**	4.85**	6
		60	6	2	32.6*	-	30.4*	25.7	5.11*	5.02*	7

(Mean value of species-specific control diets ± Standard deviation); wk: weeks; Ref.: references; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; 1: *Cherian and Quezada (2016);* 2: *Rokka et al. (2002);* 3: *Pietras et al. (2012);* 4: *Orczewska-Dudek et al. (2020);* 5: *Aziza et al. (2013);* 6: *Cherian et al. (2009);* 7: *Panaite et al. (2016);* *P<0.05, **P<0.01 or P<0.001 vs control within study

Table 5. Effect of the dietary inclusion of camelina by-products on fatty acid profile (% FAME) of laying hen egg yolk

Species	Matrix	Bird age, wk	Feeding, wk	Inclusion, %	C16:0	C18:0	C16:1	C18:1 *n*-9	C18:2 *n*-6	C18:3 *n*-3	C20:4 *n*-6	C22:5 *n*-3	C22:6 *n*-3	Ref.
Hen	Control			0	27.5± 2.14	8.07± 1.20	4.16± 1.04	43.7± 4.53	11.8± 4.44	0.31± 0.23	1.68± 0.16	0.05± 0.04	0.98± 0.45	1-7
	Seed	48	16	10	25.8	7.78	5.99	45.5**	9.38	1.53*	1.31*	0.27*	1.25*	1
	Oil	52	3	5	23.8	9.32	1.93	39.6	13.8	5.02	-	-	1.81	2
		23	4	1.5, 3	31.4**	7.81	4.17*	45.0**	6.75**	0.72**	1.62**	-	1.35**	3
		26	7	4	27.9	7.24**	2.99	42.2	11.9	2.86**	1.58	0.05	1.75*	4
	Cake	26	7	10	28.0	7.22**	3.80	42.2	11.7	2.65**	1.55	0.05	1.63*	
		24	12	10	23.9*	8.15	3.71*	39.7*	17.2*	3.05*	1.48*	0.16	1.17	5
		58	11.4	5, 10, 15	25.3**	7.80	3.68**	46.7**	12.1**	1.42**	1.16**	-	1.32**	6
		60	6	2	-	10.5	-	33.8	21.7*	1.90*	-	0.21*	2.79*	7

(Mean value of species-specific control diets ± Standard deviation); wk: weeks; Ref.: references; 1: *Cherian and Quezada (2016);* 2: *Rokka et al. (2002);* 3: *Pietras et al. (2012);* 4: *Orczewska-Dudek et al. (2020);* 5: *Aziza et al. (2013);* 6: *Cherian et al. (2009);* 7: *Panaite et al. (2016);* *P<0.05, **P<0.01 or P<0.001 vs control within study

Since the seed is currently used for the extraction of oil for non-livestock purposes, researchers have focused more their study on the use of the cake, which is a by-product (of the oil) and therefore a more sustainable foodstuff. However, camelina oil could be an interesting dietary ingredient for poultry layers, since low amounts (1.5% or 3%, *Pietras et al., 2012*; 4%, *Orczewska-Dudek et al., 2020*) are able to significantly decrease SFA and MUFA contents in yolk lipids, in favour of the PUFA content, enriching eggs with C18:3 *n*-3 and C22:6 *n*-3 FA.

When using camelina cake instead of its oil, similar results on the FA profile of eggs were found, however closely dependent on the dietary inclusion level, and for the cake, depending on its lipid content. Thus, PUFA and *n*-3 FA increased in eggs of hens fed diet containing 2% (*Panaite et al., 2016*), 5% (*Cherian et al., 2009*) 10% (*Cherian et al., 2009; Kakani et al., 2012; Aziza et al., 2013*), 15% (*Cherian et al., 2009*) camelina cake, indicating that camelina products are a viable dietary source of *n*-3 FA for poultry, able to enrich eggs with *n*-3 FA. Furthermore, the *n*-6/*n*-3 PUFA ratio was halved or even reduced by 4 times in egg yolk lipids from hens fed camelina seeds or oil or cake compared to the control group.

Camelina has a mustard-like taste, and hens fed cruciferous plants could eggs presenting off-odours or off-flavours, often described as fishy. However, none of the three studies which performed sensory tests or consumer tests on eggs coming from hens fed 5% camelina oil (*Rokka et al., 2002*), either 4% camelina oil or 10% camelina cake (*Orczewska-Dudek et al., 2020*), or 5 or 10% extruded and defatted camelina cake (*Kakani et al., 2012*) reported such adverse effects.

Conclusions

Camelina exhibits potential as a valuable feed ingredient for layer poultry due to its rich content of antioxidants, favourable amino acids, and desirable fatty acid profiles, which contribute to high-quality egg production. However, the presence of antinutritional compounds in camelina limits its utilisation in poultry nutrition, particularly at higher inclusion levels (>10%), as it hinders nutrient absorption and utilisation. Inclusion of camelina (seed or cake) in diets with reduced glucosinolates levels, or supplemented with exogenous enzymes and copper has shown improved nutrient digestibility and productive performance in laying poultry. The 10% inclusion level of camelina cake could represent a compromise, to have unaltered FRC, oviposition rate and egg quality traits, and to provide eggs with excellent fatty acid profile. Further research is however needed to fix the optimal camelina cake inclusion level, the optimum lipids content in camelina cake (degree of defatting) and to optimise the processing methods in poultry diets to improve sustainability and product quality.

Camelina sativa, održiva i upotrebljiva hrana za kokoši nosilje: pregled

Yazavinder Singh, Marco Cullere, Antonella Dalle Zotte

Rezime

Camelina sativa je perspektivna uljarica sa jedinstvenim karakteristikama, koje uključuju brzi porast, otpornost na sušu i mraz, niske zahteve u smislu input-a, kao i otpornost na štetočine i bolesti. Nudi raznovrsnu primenu u sektoru proizvodnje hrane za životinje, kao i u sektoru koji nije za životinje, prvenstveno zbog visokog nivoa *n*-3 polinezasićenih masnih kiselina (PUFA) i antioksidanata. Međutim, prisustvo sekundarnih biljnih metabolita u kamilini ograničava njenu upotrebu u ishrani živine. Ova jedinjenja mogu inhibirati neke digestivne enzime, povećati viskozitet digeste i uticati na apsorpciju hranljivih materija, potencijalno ugrožavajući zdravlje živine i kvalitet proizvoda. Za ublažavanje negativnih efekata ovih antinutritivnih jedinjenja koriste su različite tehnike, kao što su termička obrada, dodatak multi-enzimima i dodatak bakra. Utvrđeno je da uključivanje u visokim nivoima (>10%) nusproizvoda kamiline u ishranu živine smanjuje svarljivost hranljivih materija i učinak nosivosti. Bez obzira na to, uključivanje nusproizvoda kamiline, posebno ulja, u ishranu rezultiralo je istim ili poboljšanim kvalitetom jaja. Profil masnih kiselina u žumancu je pokazao veći sadržaj PUFA, smanjujući odnos *n*-6/*n*-3, čime se povećava nutritivna vrednost jaja. Senzorne procene nisu pokazale značajne razlike u kvalitetu proizvoda među grupama na ishrani. Ovaj pregledni rad naglašava hranidbenu vrednost nusproizvoda kamiline i pruža sveobuhvatan pregled postojeće literature, fokusirajući se na svarljivost, performanse i procenu kvaliteta jaja u ishrani kokoši nosilja.

Ključne reči: lažni lan, ishrana, nivo uključivanja, proizvodne performanse, kvalitet jaja, masne kiseline, senzorna analiza

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ASSESSMENT OF EGG AND EGGSHELL QUALITY: INSIGHTS FROM A THREE-YEAR STUDY ON ISA BROWN HYBRID LAYERS

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Abstract: The aim of this research was to examine the influence of the year (production cycle) and laying age on the quality of eggs for consumption. Examination of certain properties of egg and shell quality was carried out over a period of three years continuously and in four evenly spaced time intervals (24, 35, 46, and 57 weeks of age). Determining the external quality of eggs included determining the weight and shape index. Examination of the internal quality of eggs included the determination of albumen height and Haugh units. At the same time, the quality of the eggshell involved the determination of the following properties: (weight, thickness, deformation, breaking force, and color of the shell). The obtained results showed that during three production cycles, the genetic progress of this hybrid was confirmed, which is primarily reflected in a statistically significantly higher ($p<0.05$) value of egg weight, egg white height, and Haugh's units in the third year compared to the first year examinations. Also, under the influence of this factor, statistically significantly higher values of weight and shell thickness and a darker shell color were determined in the third compared to the first year of research. No statistically significant differences were found for other examined parameters under the influence of this factor. In the presence of statistically significant influence ($p<0.05$), various laying ages were systematically examined for internal and external egg quality attributes alongside shell quality properties, excluding shell deformation. Egg weight increased significantly with increasing laying age, while shape index values, contrary to egg weight, decreased. The parameters of internal egg quality were worse with increasing laying age. Regarding the shell quality properties, it was found that the shell weight and thickness, similar to the egg weight, increased with the age of the layers, while the breaking force was found to be the lowest in the oldest layers. The interaction of both tested factors did not cause a statistically significant effect on tested egg quality traits.

Key words: egg quality, laying age, shell quality, Isa Brown

Introduction

Breeding centers in the poultry industry have created easy-line hybrids characterized by annual egg production that increases year by year and is at the limit of the biological maximum of the species. The goal of the selection work is that such intensive production is accompanied by good animal health, low mortality, and as favorable a feed conversion as possible. Egg quality traits are also the focus of selection improvement and are important for producers and end consumers. Producers are particularly interested in improving shell quality characteristics, while consumer demands focus on eggs' internal quality.

The internal quality of eggs can be affected by many factors, the most important of which are age, diet, and genotype (*Tang et al., 2015*). Research by *Zita et al. (2009)* and *Kraus and Zita (2019)* confirm significant differences in egg quality characteristics under the influence of genotype and laying age. *Perić et al. (2017)* also investigated the influence of age and storage time on egg quality properties. They reported a significant decrease in the value of egg white height and Haugh units in older laying hens. *Johnston and Gous (2007)* and *Bozkurt and Thackerley (2009)* state that egg weight increases with age. In the research by *Vitorović et al. (2002)* and *Galea (2011)*, it is established that nutrition is also a factor that can influence egg and shell quality characteristics. The economic success of the production and the placement of the product on the market largely depend on the characteristics of the quality of the shell. Eggshell quality traits can be affected by age, diet, stress, disease, and rearing system *Roberts (2004)*.

Sometimes, one-sided selection and improvement of certain traits can result in weaker results for other traits, so the goal of the research was to determine genetic progress and changes in egg quality traits over the course of three years in the light line hybrid Isa Brown, which is produced in significant numbers in the Republic of Serbia, as well as the effect of hen age on external and internal egg quality characteristics as well as shell quality.

Materials and Methods

Sample material

The research was carried out on the experimental farm of the Animal Husbandry Institute in Zemun on chickens of the light line hybrid Isa Brown. During three consecutive production cycles (years 2019-2021), 2000 birds at the age of 18 weeks were moved to a facility for exploitation, cage type. The initial body weights of layers were uniform and in accordance with the norms for the specified age. The animals originated from the same supplier, who is the general representative of the said hybrid for our country.

Diet and environmental conditions

The layers were provided with the same housing, environment, and healthcare conditions. Diet and nutrition were provided ad libitum. The hens were fed with complete mixtures for layers of table eggs, which were made according to the same recipes and which were adapted to the nutritional needs of the said hybrid: in the period of 18-30 weeks of age, a complete mixture containing 17% crude protein was used; in the period from 31-50 weeks of age a mixture with a content of 16.5% crude protein and after the 50 weeks of age a mixture with a 16% crude protein content. The light regime and lighting were controlled and in accordance with the technological recommendations of hybrids with an adequate ventilation system during the entire production cycle.

Determination of egg quality properties

During each production cycle, 30 eggs were randomly sampled at the end of the 24, 35, 46, and 57 weeks of age (total of 360 eggs). The quality of fresh eggs was determined immediately after collection, representing the initial quality of table eggs. Determining the external quality of eggs included determining the weight and shape index. Examination of the internal quality of eggs included the determination of egg white height and Haugh units (HU), while the quality of the eggshell involved the determination of the following properties: (weight, thickness, deformation, breaking force, and color of the shell). The properties of egg and shell quality were determined according to the methods mentioned in the research by *Petričević et al. (2017).*

Statistical data processing

The obtained data were processed using the program package "STATISTICA" (Stat Soft Inc, 2012). The data analysis from these studies was performed based on calculated mean values and their standard deviations for all investigated properties. Testing the significance of the differences in the average values of the tested traits between the experimental groups of laying hens was performed using the appropriate variance analysis model for two factors (year and age). If the analysis of variance and the applied F-test determined an influence of the investigated factors or their combination, the group comparison was switched to an individual, whereby the degree of statistical significance of the differences between the groups was determined using the Tukey test.

Results and Discussion

The average values of the external and internal characteristics of egg quality obtained during the test are shown in Table 1. A statistically significant influence ($p<0.05$) of the tested year on egg weight, egg white height, and Haugh's unit value was determined. Significantly higher values were found for the mentioned properties during the third year of the test compared to the first year. The egg shape index, which represents the ratio between egg width and length, did not differ under the influence of this factor. The second examined factor (laying age) had a statistically significant effect on all external and internal egg quality properties. Egg weight increased significantly ($p<0.05$) with increasing laying age, so at 57 and 46 weeks, a significantly higher weight was recorded compared to the 24th week. Contrary to egg weight with increasing laying age, a significant decrease in shape index was found (values in the 24 and 35 weeks were significantly higher compared to the 57 weeks). The best internal egg quality was recorded in the youngest laying age. Significant differences ($p<0.05$) in the 24 weeks compared to the 46 and 57 weeks were confirmed for the height of the egg white and Haugh's units. The interaction of both examined factors had no significant effect on eggs' external and internal qualities for consumption.

Egg weight is an important parameter that substantially affects the overall quality of eggs and the production economy (*Dikmen et al., 2017*). Higher egg weight values were found in the second and third year of testing compared to the first year. Also, *Škrbić et al. (2006)* found a significant increase in egg weight under the influence of the year of examination, which is probably the result of selection work aimed at increasing the genetic potential of hens. With increasing layer age in our study, egg weight increased while the value of Haugh units decreased, which was established by *Samiullah et al. (2014)* and *Vlčkova et al. (2018)*. In contrast to the previously mentioned studies, *Zemkova et al. (2007)*, in hens aged from 39 to 75 weeks, did not find significant differences in egg weight with increasing age. Examining the influence of genotype and age on egg quality traits such as egg weight and shape index, *Petričević et al. (2017)* determined that the genotype did not influence both investigated traits, while statistical significance was confirmed in the influence of age only on the egg shape index. The values for the egg shape index decreased with age (at 35 weeks, it was 78.08%, and at 65 weeks, 76.27%). *Ledvinka et al. (2012)* and *Kraus and Zita (2019)* affirm that a progressive increase in age is consistently linked to a significant decline in the egg shape index. *Rakonjac et al. (2018)* have found a decrease in egg shape index with the growing age of reared layers in different rearing systems. The height of the albumen and Haugh units significantly decreased with the age of the layer, which is in accordance with the results of *Rizzi and Chiericato (2005)* and *Škrbić et al. (2011)*.

Table 1. External and internal quality of eggs

Factor	Year	Age (weeks)	Weight, g $\bar{x}$	Weight, g SD	Index, % $\bar{x}$	Index, % SD	Albumen height, 0.1 mm $\bar{x}$	Albumen height, 0.1 mm SD	HU $\bar{x}$	HU SD
Year	2019-I		60.26[b]	4.93	78.35	2.39	81.98[b]	14.49	89.12[b]	7.90
	2020-II		64.54[a]	5.09	78.55	2.02	83.26[b]	12.51	89.19[b]	7.41
	2021-III		64.33[a]	5.61	78.57	2.29	92.64[a]	14.30	94.25[a]	7.13
Age		24	58.61[b]	4.59	79.05[a]	1.99	95.21[a]	12.30	96.60[a]	5.65
		35	63.86[ab]	5.30	79.04[a]	1.87	87.17[ab]	12.47	91.62[ab]	6.51
		46	64.08[a]	4.87	78.12[ab]	2.52	79.97[b]	13.81	87.38[b]	7.67
		57	65.29[a]	5.07	77.77[b]	2.25	81.94[b]	14.79	88.10[b]	7.85
Year x Age	2019-I	24	55.37	3.80	79.47	2.06	93.03	13.82	96.03	6.42
		35	60.98	3.96	78.97	1.85	83.63	12.12	90.07	6.29
		46	62.14	3.98	77.77	2.40	71.20	9.87	82.60	6.07
		57	62.56	4.39	77.20	2.58	80.07	13.18	87.77	6.52
	2020-II	24	60.83	4.09	79.04	2.17	91.07	9.22	94.15	4.26
		35	65.15	4.86	78.93	1.76	87.83	13.03	91.70	7.53
		46	65.10	5.34	78.17	2.23	80.10	12.04	87.47	7.06
		57	66.69	4.24	78.1	1.83	74.8	8.47	83.93	6.01
	2021-III	24	59.94	3.93	78.61	1.69	101.54	10.97	99.57	4.68
		35	65.46	5.84	79.23	2.03	90.03	11.76	93.10	5.40
		46	64.99	4.74	78.43	2.91	88.60	13.64	92.07	6.89
		57	66.62	5.49	78.00	2.26	90.97	16.89	92.60	8.47
Two-factor analysis of variance (p value)										
Year			p<0.05		n.s		p<0.05		p<0.05	
Age			p<0.05		p<0.05		p<0.05		p<0.05	
Year x Age			n.s		n.s		n.s		n.s	

n.s - not significant, p>0.05
a, b Mean values in each column without common marks are significantly different at the 5% level

The shell quality of the examined eggs is shown in Table 2. The year and age of the laying hen influenced the eggshell weight. Statistically significantly higher values (p<0.05) of shell weight were found in the third compared to the first year of the study, while with increasing laying age, similar to changes in egg weight, a significant increase in shell weight was also confirmed (significantly higher values (p<0.05) determined in the 46 and 57 weeks compared to the 24 weeks). Both examined factors had no significant effect on shell deformation as an indirect parameter of eggshell quality. The parameter that shows the most negligible cause of eggshell breakage did not differ significantly under the influence of the examined year, but significant (p<0.05) differences occurred between the first three test periods and the fourth, more precisely, the lowest breaking force was found in the oldest layers. Shell color, as an important parameter due to the affinity of end consumers, was under a statistically significant influence ($p<0.05$) of both examined factors. In the third year of the test, a significantly darker color of the shell was recorded compared to the first two years, while at the age of 46 weeks, the color was significantly lighter compared to the other test periods. The interaction of different years of testing and laying age did not cause a statistically significant effect on eggshell quality properties.

Table 2. Eggshell quality

Factor	Year	Age (weeks)	Eggshell properties									
			Weight, g		Thickness, 0.01 mm		Deformation, µm		Breaking force, kg		Colour	
			$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Year	2019-I		8.28^{b}	0.81	34.72^{b}	4.05	19.38	2.80	4.86	1.03	3.48^{b}	0.59
	2020-II		8.64^{ab}	0.99	34.78^{b}	2.31	19.74	2.71	5.03	0.92	3.44^{b}	0.59
	2021-III		8.91^{a}	0.97	36.26^{a}	3.36	19.64	2.73	4.93	0.81	3.71^{a}	0.54
Age		24	7.98^{b}	0.86	33.74^{bc}	3.05	20.07	3.36	4.92^{b}	1.04	3.62^{a}	0.62
		35	8.69^{ab}	0.83	36.16^{a}	2.76	19.29	2.47	5.13^{ab}	0.76	3.60^{a}	0.58
		46	8.85^{a}	1.01	35.36^{ab}	2.37	19.39	2.41	5.24^{a}	0.85	3.37^{b}	0.61
		57	8.88^{a}	0.86	35.67^{ab}	4.54	19.63	2.65	4.48^{c}	0.87	3.59^{a}	0.52
Year x Age	2019-I	24	7.86	0.83	34.50	3.84	19.73	4.01	4.72	1.31	3.40	0.67
		35	8.53	0.68	36.13	2.73	18.63	2.03	5.14	0.81	3.53	0.63
		46	8.27	0.79	35.00	2.39	19.23	2.05	5.18	0.67	3.47	0.51
		57	8.48	0.79	33.23	5.92	19.93	2.59	4.41	1.05	3.53	0.57
	2020-II	24	7.74	0.82	33.67	2.48	20.26	3.06	4.85	0.94	3.59	0.50
		35	8.80	0.98	34.90	1.99	19.13	1.89	5.23	0.80	3.60	0.50
		46	9.01	0.92	35.63	2.20	19.73	2.80	5.48	0.95	3.07	0.69
		57	8.93	0.73	34.80	2.27	19.90	3.00	4.55	0.73	3.50	0.51
	2021-III	24	8.35	0.83	33.00	2.45	20.25	2.95	5.21	0.70	3.89	0.57
		35	8.74	0.80	37.43	2.94	20.10	3.16	5.00	0.66	3.67	0.61
		46	9.29	1.04	35.43	2.53	19.20	2.35	5.05	0.88	3.57	0.50
		57	9.22	0.90	38.97	2.22	19.07	2.33	4.49	0.84	3.73	0.45

Two-factor analysis of variance (p value)	Weight, g	Thickness, 0.01 mm	Deformation, µm	Breaking force, kg	Colour
Year	$p<0.05$	$p<0.05$	n.s	n.s	$p<0.05$
Age	$p<0.05$	$p<0.05$	n.s	$p<0.05$	$p<0.05$
Year x Age	n.s	n.s	n.s	n.s	n.s

n.s - not significant, $p>0.05$

a, b, c Mean values in each column without common marks are significantly different at the 5% level

The quality properties of the shell are particularly influential for economic reasons. A significantly heavier, thicker, and darker shell was found in the third compared to the first year of testing, which confirms the changes under the influence of the year. *Ketta and Tumova (2016)* consider that the main factors determining eggshell quality are oviposition time, age, genotype, and rearing system. In their research, *Suk and Park (2001)* have found a significant increase in shell weight with increasing age of the layer, which agrees with the results obtained in our research. Consumer demands are to obtain fresh eggs with satisfactory shell quality properties (*Pavlovski et al., 2002*). The shell must be clean and without cracks. The colour of the shell is also important due to consumer demands, so it is considered crucial that this property be uniform. Thus, the importance of its examination increases. Examining the effect of laying age on eggshell colour, *Zita et al. (2009)* concluded that the colour of the shell becomes lighter with increasing age, which is in agreement with our results up to 46 weeks of age.

Conclusion

The obtained results showed that during the three annual production cycles of the test, the genetic progress of the egg quality properties of the Isa Brown laying hen was confirmed, which is primarily reflected in a higher egg weight, egg white height value, and Haugh's units in the third compared to the first year of the test. During the investigation of the effect of the mentioned factor, statistically significantly higher shell weight and thickness values were determined. Under the influence of laying age, it was found that egg weight increased significantly with increasing laying age, while the shape index values decreased. The parameters of internal egg quality were worse with increasing laying age. Regarding shell quality properties, it was established that the eggshell weight and thickness increased with the age of the litter, while the lowest value was found for the breaking force in the oldest layers.

Based on the results of the examination of the influence of the year and the age of laying on the properties of egg and shell quality, we can conclude that the examined hybrid gives satisfactory results that are in accordance with its norms and that are improved from year to year. Egg quality properties should also be the focus of selection work in the following period, especially in older laying hens.

Procena kvaliteta jaja i ljuske: Uvidi iz trogodišnjeg istraživanja na ISA Brown nosiljama

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Rezime

Cilj ovog istraživanja bio je da se ispita uticaj godine (proizvodnog ciklusa) i uzrasta nosilja na kvalitet jaja za konzum. Ispitivanje određenih osobina kvaliteta jaja i ljuske vršeno je u periodu od tri godine u kontinuitetu i u četiri ravnomerno raspoređena vremenska intervala (24., 35., 46. i 57. nedelja uzrasta). Utvrđivanje spoljašnjeg kvaliteta jaja obuhvatilo je utvrđivanje mase i indeksa oblika jaja. Ispitivanje unutrašnjeg kvaliteta jaja obuhvatilo je utvrđivanje visine belanca i Haugh-ovih jedinica. Dok je kvalitet ljuske jaja podrazumevao određivanje sledećih osobina: (masa, debljina, deformacija, sile loma i boje ljuske). Dobijeni rezultati su pokazali da je u toku tri proizvodna ciklusa potvrđen genetski progres ovog hibrida koji se pre svega ogleda u statistički značajno većoj ($p<0,05$) vrednosti mase jaja, visine belanca i Haugh-ovih jedinica u trećoj u odnosu na prvu godinu ispitivanja. Takođe su pod uticajem ovog faktora utvrđene statistički značajno veće vrednosti mase i debljine ljuske kao i tamnija boja ljuske u trećoj u odnosu na prvu godnu istraživanja. Za ostale ispitivane parametre pod uticajem ovog faktora nije utvrđeno postojanje statistički značajnih razlika. Pod statistički značajnim uticajem ($p<0,05$) različitog uzrasta nosilja bile su sve ispitivane osobine unutrašnjeg i spoljašnjeg kvaliteta jaja kao i osobine kvaliteta ljuske, izuzev deformacije ljuske. Masa jaja se značajno povećavala sa povećanjem starosti nosilja dok su se vrednosti indeksa oblika, suprotno masi jaja, smanjivale. Parametri unutrašnjeg kvaliteta jaja su bili lošiji sa povećanjem uzrasta nosilja. Kod osobina kvaliteta ljuske ustanovljeno je da su se masa i debljina ljuske, slično masi jaja, povećavale sa povećanjem starosti nosilja dok je za silu loma utvrđena najmanja vrednost kod najstarijih nosilja. Interakcija oba ispitivana faktora nije uslovila statistički signifikantan efekat na sve ispitivane osobine kvaliteta jaja.

Ključne reči: kvalitet jaja, starost nosilja, kvalitet ljuske, Isa Brown

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GENETIC ANALYSIS OF SEMEN FROM DIFFERENT ORIGINS AND THEIR IMPACT ON PRODUCTION TRAITS: A SINGLE AND MULTIPLE TRAIT APPROACH

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Abstract: This study aims to evaluate the genetics of imported semen and assess the genetic trend of production traits in Holstein cows during their first lactation in Iran. The data was collected from 28 different herds in Isfahan province between 2011 and 2020. Variance-covariance components were estimated by the restricted maximum likelihood method and the single and multi-trait animal model. The correlation between breeding values for bulls reported in catalogs and estimated in this was calculated. The mean of the least squares by countries shows that the daughters of Spanish bulls have the highest average for milk production, and the daughters of German, French, Spanish, and American bulls have the highest average percentage of fat and protein and the amount of fat and protein, respectively. Estimated heritability for milk production, fat, and protein percentage, and the amount of fat and protein were 0.34±0.011, 0.48±0.021, 0.41±0.016, 0.40±0.090, and 0.39±0.010 respectively. The mean genetic trend of milk production, fat percentage, protein percentage, fat content, and protein content were 92, 0.010, 0.004, 1.73, and 2.52, respectively. The correlation between the estimated and reported breeding value of bulls for milk production trait, percentage of fat and protein, and the amount of fat and protein was estimated at 0.48, 0.67, 0.69, 0.14, and 0.26, and all of the estimated correlations are statistically significant at the level of 0.05. Based on the results for the most critical production trait in Isfahan herds, milk production, American bulls have the best performance and genetic trend.

Key words: production traits, variance components, genetic evaluation, animal model, genetic trend

Introduction

The aim of implementing a breeding program is to simultaneously enhance multiple traits in a herd. This genetic improvement in productive traits results in a higher herd income *(Matthews et al., 2019).* Breeding companies estimate the amount of genetic progress and the efficiency of the breeding program implemented at the herd level during a certain period *(Kavosi et al., 2016).* The genetic progress of a population is a combination of the rate of improvement in different paths of selection, which are associated with varying intensities and accuracies *(Abdollahi-Arpanahi et al., 2021).* Choosing the bulls based on the breeding goals of a dairy herd is one of the most effective ways to improve its genetic level. To achieve this, it is necessary to estimate the genetic value of female livestock and select appropriate semen accordingly. However, accurate information on female livestock is often lacking, leading semen importing companies to estimate breeding values using the pedigree method. The science of breeding relies on genetic trends to observe changes in average traits over several years, which are based on shifts in breeding values *(Strabel and Jamrozik, 2006).* Therefore, estimating and examining genetic trends is one of the key tools for evaluating the effectiveness of breeding projects. This enables the comparison of different breeding programs, allowing for a better understanding of their performance *(Hanford et al., 2003).* Procurement of semen from foreign countries has led to changes in the genetic makeup of Iran's Holstein cows in recent years. In selecting semen for the herd, one of the crucial criteria is the transferable genetic potential of the bulls to the offspring. Previous studies have investigated the genetic trend of productive traits in herds in Markazi Province, with *Kavosi et al. (2016)* being one of them. A study by *Kavosi et al. (2016)* revealed a significant difference in estimated genetic trend for reproductive traits between daughters born from semen imported from America and those born from semen imported from other countries such as Canada, Dutch, France, New Zealand, and Italy. Another study by *Razavi et al. (2007)* estimated the genetic, phenotypic, and environmental trends of production traits in Holstein cows in Markazi Province. Their report indicated that phenotypic trends for milk production (22.79 kg), milk amount (0.23 kg), and fat percentage (0.05%) increased. The genetic trends for these traits were even higher, with increases of 3.75 kg in milk production, 0.06 kg in milk amount, and -0.02% in fat percentage. For the same traits, the environmental trend was 19.79 kg, 0.21 kg, and 0.07%, respectively. In contrast, *Shirmoradi et al. (2012)* reported a positive genetic trend for production traits such as milk production, fat, and protein percentage, but a negative genetic trend for first calving age and calving interval traits. *Naeemipour Younesi and Shariati (2016)* conducted another study in which they estimated the genetic parameters and genetic and phenotypic trends of some productive and reproductive traits of dairy cows in Yazd province using a multi-trait livestock model.

They found that heritability values for milk production traits, fat amount and percentage, protein amount and percentage, length of the dry season, first calving age, and calving interval were 0.23, 0.27, 0.39, 0.28, 0.41, 0.03, 25.0, and 0.05, respectively. The authors also reported significant phenotypic trends for all production and reproduction traits and significant genetic trends for milk production traits and protein amounts. However, due to the negative genetic correlation between the investigated production and reproduction traits, selecting to increase productive traits may lead to reduced efficiency of reproductive performance. In *Seyedsharifi et al*. *(2018)* study, the estimated heritability for milk production, fat production, and protein production traits was 0.28, 0.49, and 0.42, respectively. For open days and first calving age, the estimated heritability was 69.0, and 0.07, respectively. The estimated heritability for type traits such as body depth, angularity, and udder depth were 0.27, 0.25, and 0.36, respectively. They also reported genetic correlations between milk production and fat production at 0.12 and between milk production and protein production at 0.05. Given that Isfahan province is one of the leaders in the dairy cattle industry and extensively uses imported semen in their herds, it is crucial to perform genetic evaluations and investigate genetic trends and progress obtained from imported semen. This study aims to compare the genetic effects of semen from different origins and estimate the genetic parameters and trends of production traits such as milk production, fat and protein percentage, and the amount of fat and protein belonging to first-parity Holstein cows from the imported semen used in a herd of dairy cows in Isfahan province.

Material and Methods

This research utilized production data from 71,479 first-parity cows in 28 herds located in Isfahan province from 2011 to 2020 to obtain production traits, fat and protein percentages, and amounts. Pedigree data was reviewed and edited using CFC *(Sargolzaei et al., 2006)* software. Table 1 provides descriptive statistics of the examined and compared traits of the daughters of different groups of bulls. Additionally, Figure 1a depicts the frequency of consumed semen by country. The highest frequency of consumed semen was from American bulls, while the lowest was from Spanish bulls, as shown in Figure 1a. Data editing was performed using Visual Fox Pro version 9. Researchers calculated the animals' total production during the first lactation period using test day records. To calculate the total production, a minimum of 210 lactation days and at least seven records of test days with a recording interval between two consecutive records of 25 to 35 days were considered. The researchers then converted the total production records to the standard 305-day record and used these standardized records to evaluate and estimate parameters and genetic trends. Software airemlf90 *(Misztal et al., 2002)* was utilized to estimate variance-covariance components and genetic parameters.

Table 1. Descriptive statistics of the studied traits

Traits	Number of Records	Mean ± SD	Minimum	Maximum	CV%
Milk (Kg)	71479	11540.00 ± 1755	5200.00	16545.00	15.00
Fat (%)	69415	3.33 ± 0.45	2.00	5.90	13.60
Fat (Kg)	68573	384.00 ± 58.28	229.00	512.00	15.18
Protein (%)	69124	2.95 ± 0.16	2.25	5.45	5.32
Protein (Kg)	68745	344.00 ± 52.83	208.00	464.00	15.21

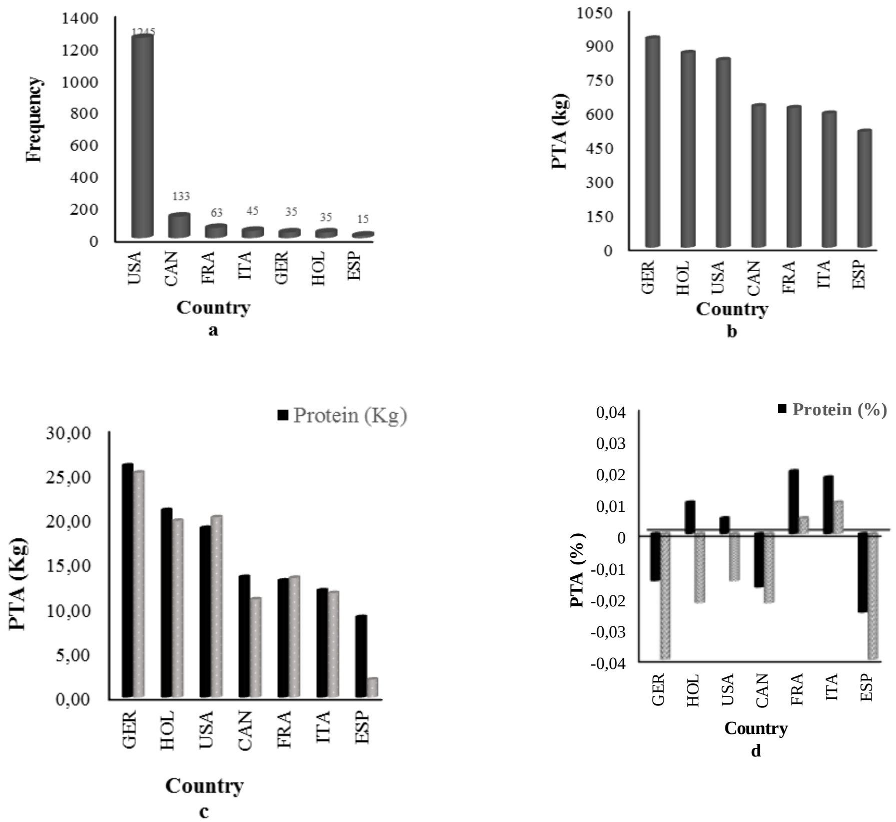

Figure 1. Distribution and frequency of bull semen originated from different countries (a), Graph for mean breeding value for bull semen originated from different countries (b), Graph for mean breeding value for bull semen originated from different countries for protein and fat contents(c) and graph for mean breeding value for bull semen's originated from different countries (d)

The genetic trends of traits were calculated in two ways: expected (using pedigree based breeding values) and observed (using estimated breeding values). To estimate the pedigree breeding value, the basis of calculations is as follows:

$$PTA_O = \left(\frac{1}{2} \times PTA_{Sire}\right) + \left(\frac{1}{4} \times PTA_{\text{MGS}}\right) + \left(\frac{1}{8} \times PTA_{\text{MMGS}}\right)$$

In this regard, PTA Sire, PTA maternal grandsire, and PTA maternal great-grandsire, respectively, include the reported breeding value of the sire, maternal grandsire, and maternal great-grandsire of the cow in question. In the following, single-trait and multi-trait models were used for the production traits to estimate the breeding values.

$$Y_{ij} = \mu + HYS_i + C_k + b(Age - \overline{Age_{ij}}) + a_j + e_{ij}$$

In this model, Y_{ij} = the record of the j^{th} cow in the herd, the i^{th} year and calving season for each trait, μ = the average population, HYS_i = the fixed effect of the herd, the calving year and season, C_k= the fixed effect of the semen's origin country, $b(Age)_j$ = the effect of the animal's calving age as a covariate, a_j = the random additive genetics effect of j^{th} animal, and e_{ij}= the random residual effect. The model mentioned above in the matrix notation is as follows.

$$y = Xb + Za + e$$

Where y = vector ($n \times 1$) of observations, b = vector ($p \times 1$) of fixed effects, a = vector ($q \times 1$) of random animal effects, e = residual vector, and X and Z are the design matrices that relate records to fixed and random effects, respectively. In the end, after estimating the variance and covariance components through the AI-REML Algorithm, predicted breeding values. Finally, genetic trends were fitted as a function of the breeding values in the calving year. In the multi-trait model (5 traits), the structure of the variance-covariance matrix was as follows.

$$\text{var}\begin{bmatrix} a \\ e \end{bmatrix} = \begin{bmatrix} A \otimes G & 0 \\ 0 & I \otimes R \end{bmatrix}$$

Where A = numerator relationship matrix, $\otimes$ Kronecker's multiplication, G is the genetic covariance matrix between traits (5 * 5), R is the residual variance-covariance matrix of the traits, and I is the identity matrix. **a** is the vector of the random additive genetics effect of the animal, and **e** is the residual vector.

Table 2. Comparison of the least square means (LSM) of studied traits between different countries

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
ESP	11610^{a}	3.30^{ab}	386^{a}	2.97^{c}	350^{a}
ITA	11545^{a}	3.32^{ab}	384^{ab}	2.99^{ab}	348^{ab}
FRA	11423^{a}	3.33^{a}	379^{a}	3.02^{a}	344^{ab}
USA	11392^{a}	3.29^{b}	379^{a}	2.98^{c}	343^{a}
CAN	11342^{a}	3.34^{a}	377^{a}	2.99^{b}	342^{a}
GER	11288^{ab}	3.35^{a}	375^{ac}	2.98^{c}	340^{ac}
HOL	11109^{b}	3.31^{ab}	369^{c}	3.01^{a}	335^{c}

In the columns related to traits, the averages that have at least one letter in common statistically do not have significant differences ($P<0.05$).

Results and Discussion

Figure 1 shows the distribution of semen consumed in different countries (a), the average modified value of semen consumed for milk production (b), the amount of fat and protein (p), and the percentage of fat and protein (t) in the studied herds of Isfahan province. According to this graph, it can be seen that America has the largest share in the genetics of the herds of Isfahan province. Regarding the breeding value of milk production, German semen has the highest breeding value. The Netherlands and Germany regarding the amount of fat and protein, and America, Italy, and the Netherlands in terms of the percentage of fat and protein in the semen had the most significant breeding value. Table 1 shows the descriptive statistics of the traits under study. Based on the results of the analysis among the daughters of bulls of different countries, the highest average trait of milk production was related to the daughters of Spanish, Italian, and American bulls, which were equal to 11735, 11690, and 11545, respectively, for the amount of fat. Female American bulls had the highest protein production, 398 and 336 kg, respectively. Daughters of Italian bulls had the highest average for the fat percentage trait (3.98%).

For the protein percentage trait, daughters of French bulls had the highest average (3%), which is consistent with the results of *Kavosi et al. (2016)* and *Nilforooshan and Edriss (2007).* Table 2 compares the least square- mean of various performance traits of bulls' daughters based on country of origin. According to this table, in terms of amount of milk produced, daughters born of Spanish semen had the highest least square-mean. On the other hand, there was a statistically significant difference at the 0.05 level between the averages obtained from Spain, Italy, France, America, Canada, and Dutch bulls. Still, there was no significant difference between daughters born of German and Dutch semen. Table 3 shows the estimation of genetic and phenotypic variance components and heritability of traits based on single-trait and multi-trait models. In all traits and models, the residual variance is greater than the additive genetic variance, which indicates the effect of non-additive genetic and environmental factors on different traits. Several factors, such as health, climate and management conditions, recording accuracy, etc., effectively reduce additive genetic variance and increase residual variance *(Collier et al., 2006).*

Table 3. Estimated genetic and phenotypic variance components and heritability using univariate and multivariate models

Trait	σ_a^2	σ_e^2	σ_P^2	$h^2 \pm SE$
Univariate Model				
Milk (Kg)	3342300.00	6327400.00	9651700.00	0.35 ± 0.007
Fat (Kg)	3871.00	6563.00	10434.00	0.38 ± 0.050
Protein (Kg)	3286.00	6354.00	9740.00	0.35± 0.012
Fat (%)	0.08	0.10	0.18	0.45 ± 0.020
Protein (%)	0.06	0.09	0.15	0.40 ± 0.018
Multivariate Model				
Trait	σ_a^2	σ_e^2	σ_P^2	$h^2 \pm SE$
Milk (Kg)	1047800.00	2021000.00	3068800.00	0.34 ± 0.011
Fat (Kg)	3371.20	5062.60	8433.80	0.40 ± 0.090
Protein (Kg)	3285.30	5153.80	8439.10	0.39 ± 0.010
Fat (%)	0.10	0.11	0.21	0.48 ± 0.021
Protein (%)	0.07	0.10	0.17	0.41 ± 0.016

The heritability of different traits for single-trait and multi-trait models shows that the highest heritability value is for fat and protein percentage. It can be concluded that these two traits are more affected by the additive effects of genes than other traits. The heritability estimated using the single-trait and multi-trait model for milk production was 0.35 and 0.34, respectively, which was consistent with the results of *Moghaddar et al. (2001).* Still, the current estimates are slightly higher than the report of *Shahdadi et al. (2017)*. The heritability rate was 0.45 and 0.48, respectively for the fat percentage trait, which was consistent with the results of *Kavosi et al. (2016)* but was somewhat higher than the rate estimated by *Farhangfar and Naeemipour Younesi (2007*). Table 4 shows genetic and phenotypic correlations between different traits. The genetic correlation between the trait of milk production with fat and protein percentage was -0.65 and -0.54, respectively, consistent with the results of *Toghiani (2012)* and *Pahlavan and Moghimi Esfandabadi (2010).* Still, it was not consistent with the results of *Kavosi et al. (2016).* One of the reasons for the negative correlation was the opposite effect of effective genes on milk production in fat and protein percentage traits. Also, the phenotypic correlation between milk production with fat and protein percentage was -0.11 and -0.30, respectively. Figures 2, 3, and 4 show the studied traits' expected and observed genetic trends (multiple traits). According to the results, for all studied traits, except fat percentage, the expected genetic trends were more than the observed ones. Among the reasons for this difference, we can mention the interaction of genetics and environment (different performance of bulls). Despite this difference, the direction of the expected and observed trends was positive and increasing. Regarding the fat percentage trait, the observed and expected trends had a slight difference, and the direction of the trends increased from 2010 to 2019. The average genetic trends of milk production obtained from the single-trait and multi-trait models were 93 and 92, respectively, very different from the expected value of 289. For other traits, particularly the amount of fat and protein, the average genetic trends were 1.66, 1.73, 2.42, and 2.52, respectively. This result was not consistent with the results of *Kavosi et al. (2016)* and *Razm Kabir et al. (2009).* The average genetic trend based on semen type (country) is presented in Table 5.

Table 4. Estimated genetic (Upper off-diagonal matrix) and phenotypic (Lower off-diagonal matrix) correlation of traits

Traits	Milk (Kg)	Fat (Kg)	Protein (Kg)	Fat (%)	Protein (%)
Milk (Kg)		0.47	0.33	-0.65	-0.54
Fat (Kg)	0.90		0.21	-0.10	0.08
Protein (Kg)	0.91	0.24		-0.09	-0.12
Fat (%)	-0.11	-0.32	-0.28		0.67
Protein (%)	-0.30	-0.30	-0.31	0.25	

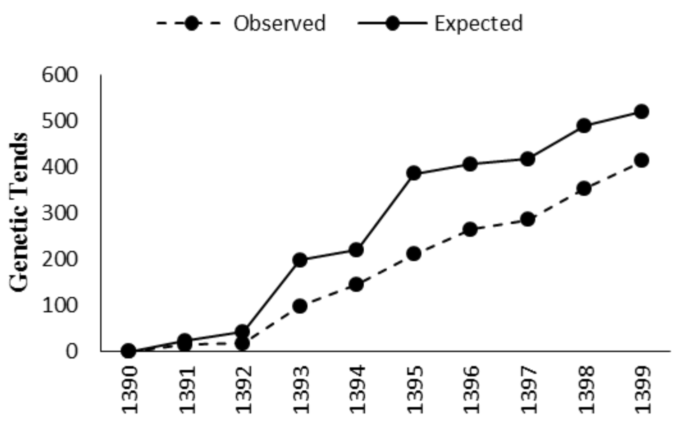

Figure 2. Genetic trends of milk production in different years of birth

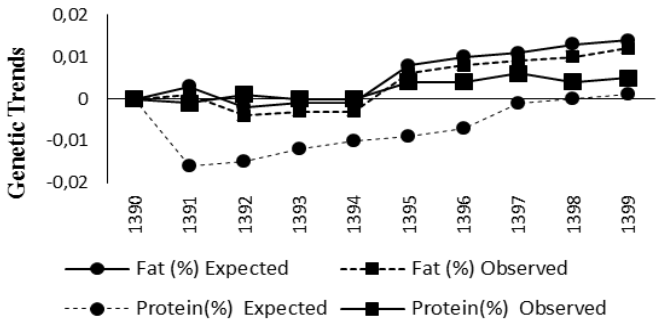

Figure 3. Genetic trends of fat and protein percentage in different years of birth

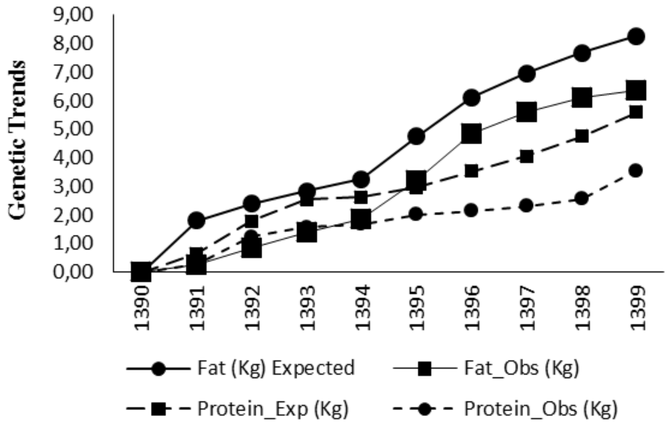

Figure 4. Genetic trends of fat and protein contents in different years of birth

Table 5. The average breeding value of the studied traits of daughter of bulls

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
USA	226	-0.001	2.350	0.007	6.800
CAN	23	0.026	3.490	0.010	2.870
FRA	126	0.001	4.110	0.017	3.770
GER	15	0.023	1.740	0.020	2.570
ITA	9	-0.001	1.380	0.005	-0.310
HOL	17	-0.007	1.600	0.006	-0.560
ESP	158	-0.001	6.200	-0.020	6.300

According to the results of this table, it can be seen that daughters of American bulls had the highest genetic trend in terms of milk production, fat, and protein amounts, and daughters of German bulls had the highest genetic trend for fat and protein percentage traits. These results are consistent with the *Kavosi et al. (2016)* report. Daughters born from American semen have an almost uniform trend with a slight increase from 2011 to 2020, and only a slight decrease can be seen in 2017. The genetic trend of daughters born from French semen fluctuated the most, but since 2017, it has increased with a suitable slope**.**

The correlation between the reported and estimated breeding values (catalog) in this research, milk production, fat and protein percentage, and fat and protein amounts in bulls were equal to 0.48, 0.67, 0.69, 0.14, and 0.26, respectively. The highest correlation was for protein percentage and the lowest for fat amount trait. The correlation of all breeding values of traits was statistically significant at 0.05 level.

The average breeding values estimated for the bulls of different countries using the records of Isfahan's daughters are shown in Table 6. According to the results of this table, the daughters of American bulls for milk production trait are in the first rank, and for fat and protein amounts traits, they are in the second rank after the daughters of Spanish bulls. Daughters of Canadian and Italian bulls had the highest heredity value for fat percentage traits, and daughters of French bulls ranked first for protein percentage traits.

According to the results of the present research, it can be concluded that the semen of bulls originating from different countries has different functions in terms of diverse traits. Generally, the best genetic performance belongs to American bulls in the herds of Isfahan province. The reason can be due to the vast geographical extent of America and different climatic conditions; therefore, bulls are evaluated based on different geographical regions, and their hereditary value is corrected according to those environmental conditions.

Table 6. Average of estimated breeding values for bulls using the records of Isfahan's daughters

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
USA	265	0.009	2.480	0.002	4.210
CAN	35	0.020	-0.680	0.003	-0.650
FRA	26	-0.001	0.850	0.020	0.830
GER	73	0.010	2.470	0.003	2.020
ITA	210	-0.001	6.990	-0.020	6.310
HOL	-9	0.020	-0.390	0.005	-0.310
ESP	23	-0.007	-0.610	0.007	-0.550

Conclusion

The results of this study show that the genetic trend of the studied traits was upward in the Isfahan herds, and the expected and observed genetic trends were parallel. American, Spanish and Italian bulls had the highest average milk production. The least-squares mean by country shows that daughters of Spanish bulls have the highest average milk yield, while German, French, Spanish and American bulls have the highest average fat and protein percentages and fat and protein content. Predicted genetic trends were greater than those for all traits examined except for fat percentage. American bulls had the greatest genetic tendency for milk production, fat and protein content and had the best genetic performance in the herds of Esfahan province. On the basis of our results, it can be concluded that the use of American bulls in Iranian herds gives better results.

Genetska analiza semena različitog porekla i uticaj na proizvodne osobine: model sa jednom i više osobina

Ramin Jafarzadeh Ghadimi, Jalil Shodja, Sadegh Alijani, Abbas Rafat, Mohamadreza Sheikhlo

Rezime

Cilj studije je procena genetike uvezene sperme i procena genetskog trenda proizvodnih osobina kod holštajn krava tokom prve laktacije, u odgajivačkim objektima u Iranu. Podaci su prikupljeni iz 28 različitih zapata u provinciji Isfahan između 2011. i 2020. Komponente varijanse i kovarijanse su procenjene metodom ograničene maksimalne verovatnoće i animal modelom sa jednom i više osobina. Izračunata je korelacija između priplodnih vrednosti bikova navedenih u katalozima i procena dobijenih u ovom istraživanju. Srednja vrednost najmanjih kvadrata po zemljama pokazuje da ćerke španskih bikova imaju najveći prosek proizvodnje mleka, a ćerke nemačkih, francuskih, španskih i američkih bikova imaju najveći prosečni procenat masti i proteina, kao i količinu masti i proteina, respektivno. Procenjene vrednosti heritabiliteta za proizvodnju mleka, procenat masti i proteina, kao i količinu masti i proteina su 0,34±0,011, 0,48±0,021, 0,41±0,016, 0,40±0,090 i 0,39±0,010 respektivno. Srednji genetski trend za proizvodnju mleka, procenat masti, procenat proteina, sadržaj masti i sadržaj proteina bio je 92, 0,010, 0,004, 1,73 i 2,52, respektivno. Korelacija između procenjene i priplodne vrednosti bikova koja je dobijena u istraživanju, za mlečne osobine, procenat masti i proteina, kao i količine masti i proteina, procenjena je na 0,48, 0,67, 0,69, 0,14 i 0,26, respektivno, a sve procenjene korelacije su statistički

značajne na nivou od 0,05. Na osnovu rezultata za najkritičniju proizvodnu osobinu u stadima Isfahana, proizvodnju mleka, američki bikovi imaju najbolje performanse i genetski trend.

Ključne reči: proizvodne osobine, komponente varijanse, genetska evaluacija, animal model, genetski trend

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MORPHOMETRIC AND MORPHOLOGICAL ANALYSIS OF INDIGENOUS MATABELE GOATS OF ZIMBABWE

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Original scientific paper

Abstract: This study aimed to characterize Matabele goats based on their qualitative and quantitative traits, to facilitate their genetic improvement and conservation. Data were collected from 120 goats over 12 months, comprising 20 males and 100 females using a stratified random sampling approach. Body weight and various body measurements were recorded. Five growth curve estimation models were employed to describe the growth pattern of Matabele goats. Descriptive statistics were computed for both qualitative and quantitative traits. T-tests were conducted to assess the impact of sex on morphometric measurements in different age groups. The results revealed an average body weight of 20.93 kg, with significant sex-related differences ($p < 0.05$) observed across all quantitative traits. Most goats displayed horned and bearded characteristics, while black and white coat colours were predominant. Among the growth curve models, the Gompertz model exhibited the highest R^2 value (0.992). Notably, the study found relatively low average morphometric measurements and body weight for Matabele goats, emphasizing the urgent need for genetic improvement efforts and the development of comprehensive breeding standards for this breed.

Key words: body measurements, body weight, growth models, genetic improvement

Introduction

Goats, through millennia, have been integral to human civilization, offering a plethora of valuable products, including meat, milk, and skins (*Mataveia et al., 2021*). Particularly in developing countries like Zimbabwe, goats play an indispensable role, making a substantial contribution, especially to the economically disadvantaged smallholder sector. It is noteworthy that women and youth are prominently engaged in goat rearing. These animals offer distinct advantages such as shorter generation intervals compared to cattle, prolific reproductive rates, ease of marketing, and minimal competition with humans for food resources (*Zvinorova, 2016*).

However, it is disheartening that goats are frequently viewed as subsistence animals with limited profitability (*Assan, 2013*). Consequently, resources and attention often lean toward cattle production, leaving indigenous goat breeds underappreciated and under-supported. Many African nations, including Zimbabwe, suffer from inadequate breeding standards for indigenous goats, a dearth of well-structured small stock improvement programs, and fragmented efforts that yield meagre impacts on goat productivity and the livelihoods of farmers (*Nsoso et al., 2003; Badi et al., 2016*). Compounding these challenges is the concerning trend of uncontrolled breeding and the introduction of exotic animals and crossbreeding practices by breeders and farmers. These practices, while well-intentioned, pose a significant risk through genetic erosion (*Gizaw et al., 2011; Celik, 2019*). This, in turn, threatens to obscure vital traits, such as disease resistance inherent in indigenous livestock breeds and even pushes certain breeds toward extinction (*Khan, 2018*).

Recognizing the need for long-term intervention, morphological characterization of indigenous goats emerges as a crucial avenue for enhancing overall productivity and developing sustainable breeding strategies. This, in turn, can lead to lower-cost initiatives that ultimately elevate the livelihoods of those involved (*Fernandes et al., 2019*). Critical to genetic improvement efforts is a deep understanding of the variation in morphological and morphometric traits (*Nguluma et al., 2016*), an understanding that can be facilitated through the use of body weight and morphometric measurements. While prior phenotypic characterization studies of Matabele and Small East African goats in Zimbabwe were conducted more than a decade ago by *Sikosana (2008)*, information about these goat breeds remains scarce. Recent efforts by the Value Chain Alliance for Livestock Upgrading (*VALUE*) project in 2021 attempted to establish breed standards for Matabele and Mashona goats, albeit with a predominant focus on qualitative traits. Therefore, the primary objective of this study is to furnish comprehensive baseline information regarding the phenotypic characteristics, encompassing both qualitative and quantitative traits, of indigenous Matabele goats in Zimbabwe. In addition, this study seeks to gauge the practical utility of these traits in animal breeding and management. Furthermore, it aspires to identify the most fitting growth curve model for the indigenous Matabele goats.

Materials and Methods

Ethical clearance for this study was obtained from the Lupane State University Animal Welfare and Ethical Committee.

Study site description

The study was conducted at Lupane State University in Lupane district of Zimbabwe. Located in the Matabeleland North Province of Zimbabwe, Lupane District is nestled within the southern African landscape with geographic coordinates ranging approximately between latitude 18.9313° S and longitude 27.7985° E. This semi-arid region experiences a distinct wet and dry season, with a mean annual rainfall of around 600 to 800mm. The climate in Lupane is classified as subtropical, featuring warm to hot temperatures during the day, averaging between 25°C to 32°C in the summer months, and cooler nights, with temperatures ranging from 10°C to 20°C during the dry season. The district's landscape is characterized by rolling plains, valleys, and low hills, adorned with acacia trees, miombo woodlands, and various grass species. These environmental factors, along with Lupane's rich cultural heritage and communal farming practices, make it a significant area for livestock farming, including the rearing of indigenous Matabele goats, which are integral to the local agricultural and economic activities.

Experimental design

The study involved the collection of data from a sample of Matabele goats. A stratified random sampling approach was used for the selection of the target groups based on sex and age. A total of 120 goats were selected, comprising 20 males and 100 females. The age range considered was from zero to 48 months.

Data Collection

To assess the physical characteristics of the animals, body weight (BW) and several morphometric measurements were taken, including wither height (WH), body length (BL), chest girth (CG), rump length (RL), hip width (HW), thurl width (TW), and pin bone width (PW) (*Getahum et al., 2020*). BW was determined using a livestock weighing scale (TAL-TEC, Ptv Ltd Model 1440). WH was measured from the highest point of the withers to the ground while the animal stood on a flat platform, employing a graduated measuring rod. BL was measured as the distance from the anterior points of the shoulder to the posterior extremity of the pin bone, using a tailor's tape measure. The CG represented the circumference of the body immediately behind the shoulder and was measured with a tape measure (*Khan et al., 2018*).

The HH was measured from the highest point of the hip vertically to the ground, utilizing a measuring rod. RL was measured with a tape measure from the hip bone to the pin bone area (*Ndhlovu et al., 2017*). HW, representing the width between the two hip bones, was measured using a digital calliper, while TW, the area between the thurl bones, was also determined with a digital calliper. PW, representing the width between the two pin bones, was measured similarly with a digital calliper. To minimize variations arising from different individuals, all measurements were consistently performed by the same person, with the animals measured in the morning before grazing and watering to mitigate the effects of rumen distention (*Ndhlovu et al., 2017*). To minimize variation due to environmental factors, body weight, and linear measurements were taken in the morning before grazing and watering, aiming to reduce the potential impact of rumen distention effects.

Qualitative traits under scrutiny included coat colour (grey, white, multiple, brown, or black, either singly or in combination), hair pattern (plain or patchy), beard presence or absence, horn presence or absence, ear orientation (erect, pendulous, or horizontal), horn orientation (lateral, backward, or upward), and facial profiles (straight, concave, or convex). The *FAO (2015)* livestock descriptor tool for goats was employed for the morphological trait descriptions, primarily relying on visual assessments (*Hagan et al., 2012; Birteeb et al., 2015*).

Statistical Analysis

Five growth curve estimation models were utilized, including the Gompertz, Bertalanffy, Wood, Logistics, and Brody growth models (*Brody, 1945; Gompertz, 1825; Unal et al., 2018*). To construct the growth curves, data on the age and live body weight of the animals at various stages were collected from farm records.

The equations for the various growth models were as follows:

Gompertz: $Yi = A * \exp(-\alpha * \exp(-\beta t))$

Bertalanffy: $Yi = A * (1 - \exp(\beta t)^3)$

Wood: $Yi = A * t^\alpha * \exp(\beta t)$

Brody: $Yi = A * (1 - \alpha * \exp(\beta t))$

Logistics: $Yi = A / (1 + \alpha * \exp(\beta t))$

Where: Yi: Body weight (kg) at age t (months), A: Body weight at maturity (kg), α: Integration constant, β: Maturation rate, exp: Exponential (natural logarithm) and t: Age (months)

The Shapiro-Wilk test was employed to evaluate the normality of continuous variables using Minitab 19, while Levene's test, also through Minitab 19, assessed homogeneity of variance. Descriptive statistics were employed for the analysis of qualitative and quantitative traits using SPSS version 21 and Minitab 19, respectively. The growth curves were fitted using StaPro version 2.0 (Kyoto University, Japan). To ascertain differences in trait measurements between males and females, a T-test was conducted. The selection of the most appropriate curve was based on the R-square value. All analyses were performed at a 95% confidence interval (CI).

Results

Table 1 shows the descriptive statistics for morphometric traits and body weight of Matabele goats. The traits are body weight, chest girth, wither height, hip height, rump length, chest depth, body length, hip width, thurl width, and pin bone width. The mean, standard deviation, and coefficient of variation are presented for each trait. The results show that the Matabele goat is a relatively small goat breed with moderate to high variation in morphometric traits.

Table 1. Descriptive statistics of grouped morphometric trait data of Matabele goats

	Matabele goats		
Traits	**Mean**	**SD**	**CV%**
Weight (kg)	20.93	7.69	36.74
Chest girth (cm)	62.99	10.18	16.17
Wither height (cm)	56.57	7.47	13.22
Hip height (cm)	57.89	7.53	13.01
Rump length (cm)	17.48	2.70	15.50
Chest depth (cm)	30.55	4.98	16.32
Body length (cm)	55.01	8.44	15.35
Hip width (cm)	10.93	2.07	18.90
Thurl width (cm)	12.69	2.14	16.82
Pin bone width (cm)	7.89	1.82	23.03

Notes: CV- Coefficient of variation, SD-Standard Deviation

Within-sex means of body weight and morphometric measurements are presented in Table 2.

The study found that male Matabele goats are larger and more robust than female Matabele goats, with significant differences in body weight and morphometric measurements at all ages. Males had higher body weight, chest girth, wither height, hip height, rump length, body length, hip width, thurl width, and pin bone width than females. The exception were on hip height, thurl width at 24 months and rump length where the difference was only statistically significant at birth and 12 months of age. No comparisons were made between males and females at the ages of 36 and 48 months because no males had reached these ages in the current study. Where comparisons were made, the males had a higher PW than females across these ages (Table 2).

Table 2. Body weight and morphometric measurement within sexes

Trait	Age (m)	Males	Females	P-value
Body weight (kg)	B	2.49 ± 0.56	1.87 ± 0.41	0.001
	12	18.08 ± 2.57	14.72 ± 2.02	0.001
	24	25.90 ± 4.91	18.64 ± 3.08	0.008
	36	….	27.18 ± 2.65	….
	48	…	31.36 ± 2.49	….
Chest girth (cm)	B	35.26 ± 2.83	32.40 ± 1.92	0.001
	12	48.69 ± 4.09	46.27 ± 2.41	0.044
	24	55.77 ± 3.35	50.42 ± 4.03	0.005
	36	…….	61.59 ± 1.67	...
	48	……	62.42 ± 2.61	….
Withers height (cm)	B	32.23 ± 4.67	28.48 ± 1.74	0.001
	12	53.94 ± 4.91	50.95 ± 7.72	0.041
	24	59.07 ± 3.96	55.34 ± 3.70	0.050
	36	….	59.93 ± 1.19	….
	48	….	64.06 ± 1.35	….
Hip height (cm)	B	32.96 ± 2.12	30.87 ± 1.88	0.001
	12	55.36 ± 5.06	50.59 ± 7.72	0.019
	24	60.46 ± 4.43	56.88 ± 2.73	0.092
	36	….	61.06 ± 1.18	…
	48	….	65.07 ± 1.52	…
Rump length (cm)	B	8.89 ± 0.40	8.18 ± 0.85	0.001
	12	17.19 ± 1.90	15.87 ± 2.84	0.050
	24	19.14 ± 2.12	17.53 ± 1.50	0.103
	36	….	18.93 ± 0.55	….
	48	….	20.77 ± 0.75	….
Body length (cm)	B	30.02 ± 4.83	27.01± 1.30	0.008
	12	53.44 ± 3.77	47.76 ± 8.70	0.005
	24	59.79 ± 4.53	53.28 ± 5.63	0.010
	36	….	58.86 ± 1.01	…
	48	….	62.78 ± 0.33	
Trait	**Age (m)**	**Males**	**Females**	**p-value**
Hip width (cm)	B	5.07 ± 0.804	4.52 ± 0.34	0.004
	12	10.21 ± 1.39	9.32 ± 1.45	0.050
	24	11.73 ± 0.89	10.68 ± 0.73	0.037
	36	….	13.30 ± 1.09	….
	48	….	13.99 ± 2.48	….
Thurl width (cm)	B	6.69 ± 0.50	6.47 ± 0.22	0.005
	12	12.25 ± 1.47	11.05 ± 1.58	0.016
	24	14.89 ± 2.22	12.73 ± 0.67	0.099
	36	….	13.12 ± 0.42	….
	48	….	15.25 ± 0.61	….
Pin bone width (cm)	B	3.93 ± 0.83	3.41 ± 0.35	0.009
	12	7.75 ± 0.94	6.60 ± 0.72	0.001
	24	8.84 ± 0.65	7.86 ± 103	0.012
	36	….	8.20 ± 0.31	….
	48		10.27 ± 0.54	….

Notes: B- at birth; gender values are represented as mean ± SD

Interestingly, morphological results showed that most of the goats were horned (85.4%), and a few percentages (14.6) were polled. Most of the goats under investigation had lateral horns (47.6%), followed by those with upward (26.2%) and backward orientations (11.7%). Another interesting feature in goats is the presence of beards. In the present study, most of the goats had no beards (69.9%), and a few percentages had beards (30.1%). The main hair patterns observed in the studied goats were the patchy type and plain type. The patchy type dominated (70.9) and the plain type (29.1%). There was an unequal representation of the different colours observed in the study. The different colours observed include brown, white, fawn, black, and various combinations of these (multiple). The combination of black and white dominated the herd (27.2%), and white, white, and brown combinations dominated 13.6% of the population. The brown and black colours were equally (8.7%) distributed among the Matabele goats. The fawn and multiple had the least equal distribution of 5.8%. The facial profiles that characterized the Matabele goats were straight and concave types. The convex type was not observed in the study population. The straight facial profiles dominated (97.2%). The concave type was observed in males entirely.

The study found that the Gompertz growth model had the highest coefficient of determination (R2 value), followed by Bertalanffy, Brody, Logistics, and Wood. This indicates that the Gompertz model was the most accurate model for predicting the growth of Matabele goats. The Gompertz model also estimated the highest asymptotic weight (maturity weight; A), followed by Bertalanffy, Brody, Logistics, and Wood. This suggests that Matabele goats reach their maximum weight at around 25.3 kg under this study conditions. The study also found that Matabele goats mature at different rates, with the Gompertz model estimating the slowest maturation rate (0.152) and the Logistics model estimating the fastest maturation rate (0.489). Overall, the study found that the Gompertz growth model is the most accurate model for predicting the growth of Matabele goats. Matabele goats reach their maximum weight at around 25.3 kg and mature at different rates. These results are further presented graphically in Figure 1.

Table 3. Equations of the five growth models and their R-square values

Model	A	A	B	R-square value
Wood	17.550	0.772	0.015	0.655
Von Bertalanffy	27.093	0.512	0.104	0.991
Brody	35.607	0.811	0.035	0.947
Logistics	20.321	13.579	0.489	0.894
Gompertz	25.330	2.342	0.152	0.992

Notes: where A is the mature weight (asymptotic weight); B is the maturation index (growth rate), and α is the integration constant.

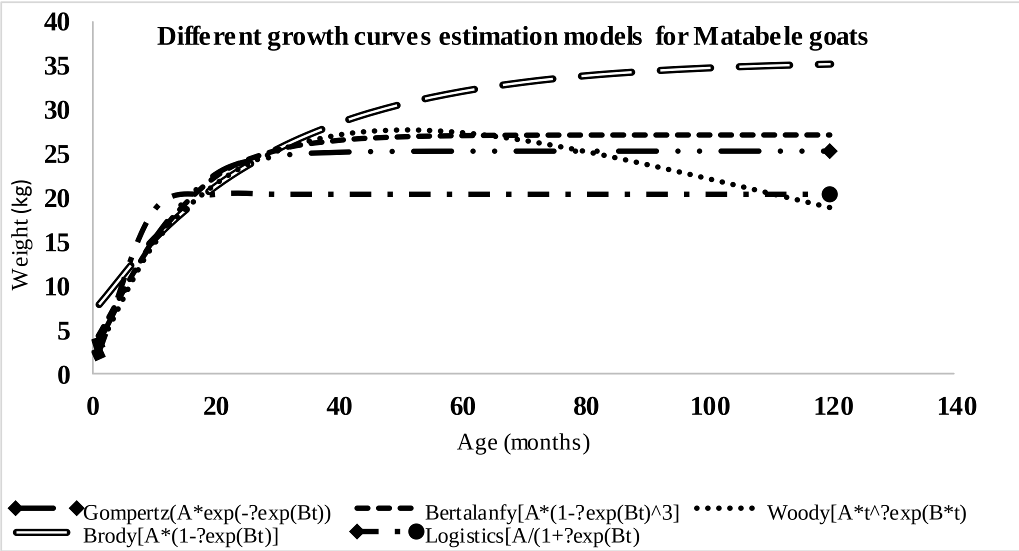

Figure 1. The growth curves of Indigenous goats from different non-linear equations

Discussion

In the current study, the mean BW of the combined sexes was much lower than those reported by *Ndhlovu et al. (2017)* for the same goat type. The study's findings suggest that males are better adapted to growth and development than females and that this may be due to differences in hormonal profiles or other biological factors (*Ojedapo et al., 2007*). A similar scenario was also observed in other body measurements such as CG, CD, RL, WH, HH, HW, and TW. Moreover, the BW and other body measurements were lower than those reported by *Getahum et al. (2020)* in Ethiopian goats and *Diba (2017)* in Nigerian indigenous goats. The difference can be attributed to genetic and environmental variations that can exist within these goat breeds or types (*Toro et al., 2011*). In addition to the source variances discussed above, the average age of a particular population can affect its average body weight and body measurements. In the present study, the average was 19 months. This might be a cause of the difference observed from other studies because younger animals tend to weigh less than mature animals.

The results of the study revealed that sex is an important source of variation in live BW and linear body measurements. A similar observation was made by *Tyasi et al. (2021)*. Most of the linear body measurements were significantly different between the sexes. This indicates that sexual dimorphism was observed in most of the quantitative traits. All quantitative traits were consistently higher in male goats than in female goats. These results are in line with observations made by *Bedada et al. (2019), Monau et al. (2018) and Getahum et al. (2020)* working with different goats. According to *Ndhlovu et al. (2017),* this is a result of intersex differential in hormonal response causing sexual dimorphism. The different hormone profiles in bucks and does invariably translate to differential growth rates. Testosterone is the most important male sex hormone, culpable with most of the male attributes and masculinity.

Peculiar morphological characteristics of Matabele goats include a mixture of coat colour patterns and the presence of horns and beards. A greater percentage of these goats have horns, no beards, and various coat colours. This was similar to findings by several authors on various indigenous breeds (*Ahmed et al., 2015; Alubele et al., 2015; Mukete et al., 2016*). *Castanheira et al. (2010)* asserted that coat colour is also useful in protecting deep tissue against excess exposure to solar shortwave radiation in tropical zones; hence, goats develop this as a form of protection. Moreover, this might be because farmers could unintentionally select these traits as an environmental adaptation mechanism for thermoregulation, cultural purposes, and protection from predators. This is also true with horn presence which can be used as a defense mechanism against predators (*Monau, 2018*).

Most of the goats in this study had pendulous ear orientations. This has been a defining characteristic of Matabele goats throughout the decades (*Mhlanga, 1999*). However, this cannot be the sole indicator that a goat is a Matabele goat type. Other factors such as body posture and horns can be incorporated to describe these goats (*VALUE, 2021*). The predominant mixed coat colour could be advantageous during the country's common seasonal temperature fluctuations. The presence of beards is thought to be associated with superior reproductive characteristics such as high conception, prolificacy, and fertility rates (*Monau et al., 2018*). However, no research has been done to scientifically ascertain this association. Therefore, research is required to determine the true impact of these qualitative traits on Matabele goats' adaptation, performance, and overall productivity. Morphological characteristics are important in defining goat type and breed. Breeding societies rely on these traits to register breeds. Any animal that diverges greatly from the expected morphology might not be considered for registration (*Zimbabwe Herd Book - ZHB, 2019*).

In general, all non-linear models demonstrated good capacities of fitting for describing the growth kinetics of goats. However, in the current study, the Gompertz and von Bertalafny functions had the best fit. *Teleken et al. (2017)* found a similar result in chickens; the Gompertz model fitted the chicken growth curve better than other models. The von Bertalanffy model was found to be a good choice for fitting the gain of body weight for the bulls in cattle (*Brown, 1992*). *Silva et al. (2011)* evaluated five non-linear models for the gain of weight for cows of different biological types and found that Richards' and Brody's models were the most appropriate for Nelore cows. *Beltran et al. (1992)* evaluated the growth patterns of two lines of Angus cows using Brody and Richards' models and concluded that both models provided good results.

Out of the five models evaluated, the Brody model showed a higher asymptotic weight, that is, the maturity weight (A) for the Matabele goats, followed by the von Bertalanffy model, Gompertz, Logistics, and Wood models. However, following the results of this study, it was proven that the Gompertz function had the best fit. In this study, the maturity weight estimation from the Gompertz growth model was estimated to be 25.33kg. However, it should be noted that this figure was influenced by the lower average age of the animals in this study. The average weight can change with the incorporation of older and mature populations. The growth curves represent the growth pattern of that particular population data set; hence the existence of difference is expected Wood showed that after the asymptotic weight, there is a decrease in the weight. This antagonistic behaviour has been reported by other authors (*Coyne et al., 2015*) in pigs and sheep (*Hossein-Zadeh, 2017*). The trend can be explained by the loss of feeding ability and loss of muscles as animals age, hence a decrease in body weight (*Adeyinka and Mohammad, 2016; Bathaei and Leroy, 2016*). The growth rate or maturation index (B) of Matabele, as estimated by the stated models, shows that it ranges from a low value of 0.015kg to 0.489kg per month. The Gompertz growth model, which fits best, estimates this value at 0.152 kg per month. A lower maturation index indicates a slow growth rate (*Ceron et al., 2020; Ali et al., 2020; Sharif et al., 2021*). This translates to the fact that Matabele goats have slow growth, which might indicate a lack of genetic improvement compared to other breeds. A gap remains to estimate the growth curves of other indigenous goats so that a comparative study can be done to assert differences in the growth rate with growth equations so that proper interventions can be taken.

The integration constant (α) shows the animal's birth weight. The Wood estimated the Matabele goat's birth weight at 0.772kg, the von Bertalanffy puts it at 0.512kg, Brody estimates it at 0.811, Logistics at 13.579kg, and the Gompertz at 2.342kg. Wood, Bertalanffy, and Brody underestimated the birth weight of these goats when compared to the actual weights observed. The Logistics function overestimated the birth weight of the Matabele goats. Gompertz gave a closer estimate of the birth weight to the actual observed values. Consequently, the Gompertz model had the most accurate estimation of the initial and mature weights of the goats. Therefore, the Gompertz equation showed the highest reliability and is the most suitable equation for describing the growth of Matabele goats.

It should be noted, however, that these growth models or non-linear equations rely on the data present at a particular time. Therefore, they are specific to the situation and may not present generic information that can be applied to every population (*Kaplan and Gurcan, 2018; Ceron et al., 2020*). This follows that proper use of the curves and estimates generated from these equations needs a proper evaluation of the environment and the population in question.

Conclusion

The findings of this study provide valuable insights into the morphometric measurements and characteristics of Matabele goats, shedding light on the diversity within this indigenous breed. The notable coefficient of variation, particularly when considering both sexes, underscores the substantial variation in morphometric traits among these goats. These variations can be primarily attributed to the animal's genetic makeup and the environmental conditions to which they are exposed. Furthermore, the study highlights the significant influence of sex and age on quantitative traits. Males consistently exhibited larger body measurements compared to females, and these measurements increased with age, emphasizing the importance of considering these factors in assessing and managing goat populations. The morphological characteristics of Matabele goats, including the presence or absence of horns, the presence or absence of beards, various coat colours, facial profiles (concave or straight), and hair patterns (smooth or patchy), were found to be diverse within this breed. These traits contribute to the unique identity of Matabele goats and may have practical implications for their adaptation, performance, and overall productivity. Regarding growth modelling, the Gompertz function emerged as the most suitable model for describing the growth curve of Matabele goats. This model provides valuable insights into the growth patterns of this indigenous breed and can serve as a valuable tool for future research and breeding programs. In summary, this study enhances our understanding of the morphometric diversity and growth characteristics of Matabele goats. These findings contribute to the knowledge base necessary for the sustainable utilization and conservation of indigenous goat breeds like the Matabele, which play a crucial role in the livelihoods of many communities.

Morfometrijska i morfološka analiza autohtonih Matabele koza u Zimbabveu

Sizo Moyo, Fortune N. Jomane, Alban Mugoti, Reagan Mudziwapasi

Rezime

Istraživanje je imalo za cilj da opiše Matabele koze na osnovu njihovih kvalitativnih i kvantitativnih osobina, kako bi se olakšalo njihovo genetsko poboljšanje i očuvanje. Podaci su prikupljeni od 120 koza tokom 12 meseci, uključujući 20 muških i 100 ženskih grla korišćenjem stratifikovanog slučajnog pristupa uzorkovanja. Evidentirane su telesna težina i različite telesne mere. Pet modela za procenu krive rasta korišćeno je da se opiše obrazac rasta Matabele koza. Deskriptivna statistika je izračunata i za kvalitativne i za kvantitativne osobine. Sprovedeni su T-testovi da bi se procenio uticaj pola na morfometrijska merenja u različitim starosnim grupama. Rezultati su otkrili prosečnu telesnu masu od 20,93 kg, sa značajnim razlikama između polova ($p< 0,05$) uočenim u svim kvantitativnim osobinama. Većina koza je imala rogate i bradate karakteristike, dok su preovladavale jedinke crno-bele dlake. Među modelima krive rasta, Gompertz model je pokazao najveću vrednost R2 (0,992). Takođe, studija je otkrila relativno niske prosečne morfometrijske mere i telesnu masu kod koza Matabele rase, naglašavajući hitnu potrebu za naporima za genetsko poboljšanje i razvoj sveobuhvatnih standarda uzgoja za ovu rasu.

Ključne reči: telesne mere, telesna masa, modeli rasta, genetsko poboljšanje

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THE INFLUENCE OF SOME FACTORS ON THE PRODUCTION EFFECTS OF SJENICA SHEEP

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Abstract: Gene expression at the phenotypic level varies due to a number of influences from the environment in which the animals are reared. Ignoring this reality or due to insufficient knowledge, farmers are often disappointed when choosing a population of sheep when they do not get the production results that the breed achieves where they bought it. The investigation of the reproductive and production characteristics of parent herds of Sjenica sheep was conducted on four farms. In the research, it included a total of 921 lambs and 474 sheep. The influence of sheep body weight on the weight of lambs at birth within a farm was analyzed. Based on the research conducted on the sheep population and after the obtained and processed data, we can state the following: certain differences were found in the fertility of sheep depending on the farm and body weight. We also perceived that certain differences in the body weight of the lambs are evident depending on the weight group of the sheep and the farm where the sheep were raised. All of the above leads us to the general conclusion that management is extremely important in sheep farming. If the selection of the breeding population is carried out correctly and adequate measures of keeping, nutrition and reproduction are applied, success is guaranteed.

Key words: sheep, fertility, body weight, farm, production effect

Introduction

As is known in the theory of domestic animal genetics *(Petrović et al., 2015),* gene expression at the phenotypic level varies from a number of genetic factors and the influence of the environment in which the animals are reared. *Gardner et al. (2007)* said that the maternal effect on composition of the body prior to pregnancy and nutrition during gestation also had significant effects on birth weight in the sheep. The efficiency of sheep production is conditioned by fertility. According to some authors, the number of offspring obtained per lambing is more important than the gain of weight *(Petrović et al., 2012).* The frequently used indicators of sheep reproductive performance include fertility *(Vlahek et al., 2023).* Reproductive performance in animal husbandry is a very important trait that affects profitability. Ewe live weights, nutrition, weather, and season have all reportedly influenced reproductive performance *(Gaskins et al., 2005; Akhtar et al., 2012; Aktaş et al., 2015; Behrem et al., 2022).* The evaluation of the genetic potential of sheep in Serbia can be found in the papers *(Petrovic et al., 2009; 2015).* Not having this reality in mind or due to insufficient knowledge, farmers are often disappointed when choosing a population of sheep because they do not get the production results that the breed achieves when they buy it. With this in mind, many authors are studying various factors affecting sheep production *(Askoy et al., 2023*). The Sjenica sheep, as a member of the widespread autochthonous breed that we call Pramenka, plays an important role in Serbian sheep farming, especially in its western and south-western regions. This sheep is also the most numerous population of Pramenka. Because of all this, it is of particular importance to study various factors that contribute in any way to its profitability of breeding. The aim of this paper is to find further contributory factors to this necessity based on prior study (*Lecić et al., 2022*).

Material and Methods

The study of the reproductive and production characteristics of the parent flocks of Sjenica sheep was conducted on four private farms in the area of Kolubar district. It included in the research 474 adult sheep that lambed in the years 2017 and 2018, consisting of 921 total lambs in the four farms.

The determination of the effect of adult sheep body weight on fertility in the years 2017 and 2018 within farms as well as the impact of sheep adult weight on lambs' birth weight, the rest method and the statistical analysis was the same with (*Lecić et al., 2022*).

Throughout the research, the influence of sheep body weight on the weight of lambs at birth within the farm was examined. The statistical analysis of the accessed experimental data was done using the software statistical package Statistics for Window 7 (Stat. Soft. Inc.). The equality of variances of the analysed treatments was also tested using Levene's test.

The impact of body weight of sheep on the fertility and the weight of lambs at birth within the farms was scrutinized using the variance analysis method (one-factor analysis). The differences between the mean values of the investigated treatments were analysed using Fisher's LSD test, T-test, and HSD test. Analyses were performed at a significant level of 0.05 and 0.01, and the obtained results are presented as means ± standard deviation (X ± SD).

Results and Discussion

The influence of sheep's body weight on fertility within farms in 2017 and 2018 is seen in tables 1 and 2.

Table 1. Effect of sheep body weight on fertility in 2017 within observed farms

Farm of sheep	Weigh group of sheep, kg	N	Fertility		
			$\overline{X}$	SD	CV
Farm 1	60-65	39	1.90	0.82	43.16
	66-70	20	1.95	0.94	48.21
Farm 2	60-65	23	1.78	0.85	47.75
	66-70	25	1.84	0.94	51.09
	71-75	3	1.00	0.00	0
Farm 3	70-75	14	2.71	0.99	36.53
	76-80	25	2.68	0.95	35.45
	81-85	12	2.42	0.90	37.19
Farm 4**	60-65	8	1.75^b	0.71	40.57
	66-70	30	2.27^b	1.01	44.49
	71-75	18	2.17^b	1.15	53.00
	76-80	3	4.33^a	2.31	53.35

**P<0.01
a, b - statistically significantly different at the 0.01 level

As can be viewed from the results shown in Table 1, the influence of weight groups of sheep on fertility was highly significant only within farm 4 (P<0.01). Looking in particular at farms 1 and 2, natural mating is applied. It can be noted that the body weight of sheep from farms 1 and 2 has a significantly higher fertility than the one reported in the literature for the Sjenica sheep, which is 1.20. This difference is due to a more intensive breeding method and the application of estrus induction with hormones in some sheep under our research.

On these two farms, two rams are kept constantly with ewes so that some ewes gave birth at the beginning of 2017 (more precisely, in February 2017) and that are well prepared were fertilized in June of this year and lambed again in November of the same year. For this reason, the fertility of the sheep in the table (1) is significantly different from the one mentioned in the literature, because at these two farms, out of the total number of 10 sheep, they gave birth for the second time in the same year, so that we got 4 lambs from some sheep in that year.

Estrus synchronization is applied on farms 3 and 4. A certain number of sheep from farms 3 and 4 also gave birth at the beginning and end of the year.

Table 2. Effect of sheep body weight on fertility in 2018 within observed farms

Farm of sheep	Weigh group of sheep, kg	N	Fertility		
			$\overline{X}$	SD	CV
Farm 1	60-65	18	1.39	0.61	43.88
	66-70	40	1.68	0.76	45.24
	71-75	11	2.00	0.63	31.50
Farm 2	60-65	30	1.90	0.84	44.21
	66-70	30	2.16	0.83	38.43
	71-75	2	2.00	1.41	70.50
Farm 3*	65-70	16	1.63^{A}	0.50	30.67
	71-75	15	1.47^{AB}	0.63	42.86
	76-80	27	1.19^{B}	0.40	33.61
	81-85	14	1.21^{AB}	0.43	35.53
Farm 4	60-65	8	2.25	1.04	46.22
	66-70	27	1.96	0.94	47.96
	71-75	16	2.31	1.20	51.95

*(P<0.05)
A,B, - statistically significantly different at the 0.05 level

As can be spotted from the results shown in table 2, the influence of weight groups of sheep on fertility was highly significant only within farm 3 (P<0.05). The fertility of sheep in 2018 per farm was lower compared to the fertility in 2017. The reason is that in 2018, sheep lambed only once during the year, unlike in 2017, where we had a certain number of sheep on farms that lambed twice. Three weight groups of sheep were recorded on farms 1, 2 and 4, and four weight groups of sheep were recorded on farm 3. Sheep with a body weight of 71-75 kg from farm 4 had the highest fertility out of 2.31. Sheep with a body weight of 76-80 kg from farm 3 had the lowest fertility of 1.19.

Alafar et al. (2022) state that the reproductive performance of sheep varies by genetic factors, but sheep's reproductive performance is significantly affected by non-genetic factors, which should be included in genetic analysis and taken into account for improving sheep herd breeding. *Amnate et al. (2016)* said some factors affecting fertility showed a highly significant difference (P< 0.01) in the weight of the mother from the insemination to the fertility. These authors' notes justify the results we obtained.

The impact of sheep body weight on the birth weight of lambs in 2017 and 2018 are presented in tables 3and 4.

Table 3. The influence of body weight of sheep on lambs' birth weight in 2017

Farm of sheep	Weigh group of sheep, kg	N	Body weight of lambs at birth, kg				
			$\overline{X}$	SD	CV	Min	Max
Farm 1	60-65	73	3.52	0.56	15.91	2.30	4.60
	66-70	39	3.32	0.47	14.16	2.40	4.40
Farm 2*	60-65	42	3.31^{B}	0.56	16.92	2.20	4.70
	66-70	46	3.30^{B}	0.65	19.70	2.10	4.60
	71-75	3	4.20^{A}	0.0	0	4.20	4.20
Farm 3	70-75	38	4.04	0.59	14.60	2.80	5.50
	76-80	65	4.11	0.64	15.57	2.70	5.50
	81-85	30	3.94	0.62	15.74	2.70	4.90
Farm 4**	60-65	14	4.05^{ab}	0.66	16.30	2.90	5.40
	66-70	69	3.80^{b}	0.68	17.89	2.40	5.20
	71-75	39	3.56^{bc}	0.73	20.51	2.70	5.40
	76-80	14	3.04^{c}	0.85	27.96	2.20	5.20

*(P<0.05) **P<0.01
A,B, - statistically significantly different at the 0.05 level
a, b, c - statistically significantly different at the 0.01 level

As noticed in table 3, the influence of sheep body weights on lambs' body weight at birth was significant in farm 2 (P<0.05) and farm 4 (P<0.01). The highest average body weight at birth was 4.20 kg, achieved by sheep lambs from farm 2 with a body weight of 71-75 kg. The least average lambs' weight at birth was 3.04 kg attained by sheep lambs from farm 4 with a body weight of 76-80 kg.

Table 4. The influence of body weight of sheep on lambs' birth weight in 2018

Farm of sheep	Weigh group of sheep, kg	N	Body weight of lambs at birth, kg				
			$\overline{X}$	SD	CV	Min	Max
Farm 1	60-65	26	3.88	0.59	15.21	2.80	5.10
	66-70	70	3.80	0.64	16.84	2.80	5.40
	71-75	22	3.93	0.73	18.58	2.90	5.40
Farm 2	60-65	56	3.42	0.59	17.25	2.50	4.60
	66-70	66	3.36	0.63	18.75	2.50	4.50
	71-75	4	3.63	0.90	24.79	2.70	4.40
Farm 3**	65-70	23	3.77^{b}	0.78	20.69	2.90	4.40
	71-75	26	3.48^{b}	0.43	12.36	2.50	5.10
	76-80	30	4.41^{a}	0.56	12.70	3.10	5.50
	81-85	17	4.30^{a}	0.62	14.42	3.20	4.80
Farm 4	60-65	19	3.74	0.56	14.97	2.90	4.50
	66-70	52	3.55	0.60	16.90	2.30	4.90
	71-75	37	3.77	0.69	18.30	2.80	5.20

**P<0.01
a, b - Means significantly different at the 0.01 level

As recognized in table 4, the influence of maternal body weight on the lambs' body weight at birth was significant only within farm 3 (P<0.01). The highest average body weight at birth was achieved by sheep lambs from farm 3, with a body weight of 76-80 kg from 4.41 kg. The lowest average body weight of lambs at birth was recorded in sheep on farm 2, with a body weight of 66-70 kg from 3.36. Differences in body weight of lambs at birth in relation to weight groups of sheep did not vary statistically significantly within farms 1, 2, and 4.

The study of *Petrović et al. (2015)* observed that lambs in both genotypes, Pirot x Württemberg and Sjenica x Württemberg, were heavier at birth if born from heavier ewes, the later statement partly defend our results. *Aktaş et al. (2015)* investigated the effects of ewe's live weight and age on reproductive performance and lamb growth. Several other authors have tested the effects of environmental factors on the production performance of sheep (*Aliyari et al., 2012; Fraga et al., 2018; Bancheva et al., 2022; Campos et al., 2022).* Besides all of these, *Van Der et al. (2010)* concluded that dam weight had no effect or only minor effect on reproductive performance of the offspring.

It is not directly concerned with our results but possibly correlates with the results of our study, *Fraga et al. (2018)* and *Campos et al. (2022*) that inadequate nutrition of the ewe, especially in the last third of gestation, is associated with a reduction in lamb birth weight and development. The reproductive performance of sheep conceived, born, and reared under different degrees of nutritional adversity provided evidence that inadequate or inappropriate fetal and/or early postnatal nutrition reduces adult reproductive performance *(Gunn et al., 1995; Purushotam Joshi, 2022).* In the study of *Corner-Thomas et al. (2015),* it appears that poor reproductive performance of ewe lambs with a BCS of 2.0 or less was independent of the ewe lamb's live weight, then if ewe lambs have a body condition score of greater than 2.0, differences in fertility and reproductive rate appear to be explained by differences in live weight.

Conclusion

Based on the conducted research on the Sjenica sheep population and after the obtained and processed data, we can state the following.

Certain differences have been found in sheep fertility depending on the farm and body weight. We can notice that some differences are evident in lambs' weight at birth depending on the sheep's weight group and the farm where the sheep were raised. All of the above leads us to the general conclusion that management is highly essential in sheep farming. If we make a proper selection of the breeding population and apply adequate measures of keeping, nutrition, and reproduction, success is guaranteed. The results of our research confirm that this is not always the case on all farms.

Uticaj nekih faktora na proizvodne efekte sjeničke ovce

Nemanja Lečić, Dragana Ružić-Muslić, Nevena Maksimović, Veselin Petričević, Bogdan Cekić, Ivan Ćosić, Violeta Caro Petrović

Rezime

Ekspresija gena na fenotipskom nivou varira zbog brojnih uticaja iz sredine u kojoj se životinje uzgajaju. Ignorišući ovu realnost ili zbog nedovoljnog znanja, farmeri su često razočarani, pri izboru populacije ovaca jer ne dobijaju proizvodne rezultate koje rasa postiže tamo gde su je otkupili. Ispitivanje reproduktivnih i proizvodnih karakteristika matičnih stada sjeničkih ovaca obavljeno je na četiri farme. Istraživanjem je obuhvaćeno ukupno 921 jagnje i 474 ovce. Tokom istraživanja analiziran je uticaj telesne mase ovaca na težinu prirođenih jagnjadi u okviru farme. Na osnovu istraživanja sprovedenog u posmatranoj populaciji ovaca i nakon dobijenih i obrađenih podataka, možemo konstatovati sledeće: utvrđene su određene razlike u plodnosti ovaca u zavisnosti od farme i telesne mase. Takođe vidimo da su evidentne određene razlike u telesnoj masi jagnjadi u zavisnosti od težinske grupe ovaca i farme na kojoj su ovce uzgajane. Sve navedeno nas dovodi do opšteg zaključka da je menadžment izuzetno važan u ovčarstvu. Ako se pravilno izvrši selekcija priplodne populacije i primenjuju adekvatne mere držanja, ishrane i reprodukcije, uspeh je zagarantovan.

Ključne reči: ovce, plodnost, telesna težina, farma, proizvodni efekat

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INNOVATIVE APPLICATION OF INULIN-GEL SUSPENSION IN POULTRY SAUSAGES: TECHNOLOGICAL IMPACT AND NUTRITIONAL ENHANCEMENT

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Abstract: Consumer demand for healthier foods has led the industry to look for alternatives to reduce high-fat levels. Dietary fibers such as inulin have emerged as promising substitutes for fat, increasing nutritional value and reducing fat absorption. The aim of this research was to formulate chicken cooked sausages in which the fat was replaced by inulin suspension at different levels. Four groups of sausages participated in the experiment: group K without fat replacement and groups 50%IN, 75%IN, and 100%IN with 50%, 75%, and 100% fat replacement, respectively. The results show a significantly higher process and cooking loss in the experimental groups compared to group K ($p<0.05$). Groups K and 50%IN had significantly better emulsion stability ($p<0.05$). Increasing inulin content significantly affected color characteristics, including L* (lightness), a* (redness), and b* (yellowness) ($p<0.05$). The experimental groups of sausages had a lighter color. By reducing fat, the caloric value of sausages decreased significantly and differed between groups ($p<0.01$). Inulin can be used to produce cooked sausages with reduced fat content and optimal physical and chemical properties. In addition to the reduced energy value, this meat product also has good nutritional characteristics.

Key words: inulin, fat, chicken sausages, caloric value, functional food

Introduction

Inulin is considered a functional food ingredient widely used in the food industry as a sugar or fat replacer to improve nutritional profile as it reduces the calorie value of the final product, represents the source of dietary fiber, and has the prebiotic potential. From a technological standpoint, inulin is shown to be an excellent gelling agent, viscosity modifier, and texturizing agent in various foods

(*Melilli et al., 2021; Jayarathna et al., 2022*). The fat-substituting capacity of inulin is tightly related to its ability to form highly stable gels. In order to create inulin-gel, inulin powder has to be suspended in an aqueous solution (at 70°C), appropriately mixed, and cooled down at 40°C overnight. Inulin-gel characteristics are temperature-dependent as inulin forms three-dimensional gels by a network of small crystallites. If diluted at a higher temperature, i.e., above 80°C the inulin gelation is inhibited (*Bot et al., 2004; Glibowski, 2010; Jayarathna et al., 2022*). The properties of these gels resemble that of a network of fat crystals in the oil. Because of this similarity, inulin gels have been identified as an exciting new ingredient for creating unique, functional meat products with a mouthfeel and creaminess similar to the one containing solid fats (*Bot et al., 2004; Yousefi et al., 2018*). Therefore, taking into consideration all of the appealing attributes of inulin gels, numerous studies have been reported with successful implementation in different types of meat products, such as cooked (*Selgas et al., 2005; Berizi et al., 2017; Ferjančič et al., 2021*) and fermented sausages (*Menegas et al., 2013; Vasilev et al., 2011; Glišić, 2019*). Since there is limited information regarding the application of inulin-gels in poultry sausages, this paper aims to develop and propose a modification of „full-fat, commercially available products with minimum to zero levels of animal fat, as well as to observe its technological properties.

Material and Methods

Experimental design

Poultry meat and pork fatback were used for the production of sausages. Fresh chicken breast meat, held at 4°C, was obtained from the Piljan Komerc chicken farm (Belgrade, Serbia). Pork backfat was resourced from the Meat Processing Unit of the Institute for Animal Husbandry (Belgrade, Serbia), where chicken f sausages were made.

Four treatments were manufactured: control group (K) - commercially made sausage (100% pork backfat), group 1 (50% IN) – 50% pork backfat replacement, group 2 (75% IN) – 75 % pork backfat replacement, and group 3 (100% IN) – with 100% pork backfat replacement with inulin-gel suspension. Inulin was obtained from Cosucra, Belgium (dry matter 95.9%, ash on dry matter 0.01%, carbohydrates on dry matter 100.00%, free fructose, glucose, and sucrose on dry matter 0.1%, inulin on dry matter 99.9%). Inulin-gel suspension was made by the method described by *Petričević et al. (2022)*. Afterward, the suspension was cooled to 4°C, left overnight, and added to the meat batter mixture.

All groups were manufactured on the same day and in an identical manner. Minced chicken breast meat and pork backfat were mixed with ice, condiments (Fenc company, Serbia), additives (NO_2+NaCl salt commercially available, phosphates, and soy isolate), and in experimental groups, previously preparedinulin suspension was added (groups 50% IN, 75% IN, 100% IN). All components were homogenized in a cutter, and stuffed into collagen casings (⌀ 22 mm). The processes of boiling and smoking were performed in the chamber for boiling/smoking with program settings: drying for 10 min at 50°C, smoking at 60°C for 30 min, and heating to 85° until they reached an internal temperature of 72°C. Sausages were then cooled down by spraying with cold water, left to drain for 15 min, and stored at 4°C. After 24 h, sausages were selected from each group for further analysis. The experiment was repeated three times, and from each batch,three sausages were taken for analysis. For further analysis, the rest of the sausageswere vacuum-sealed and stored at 4°C for 21 days.

Process loss, purge loss, and cooking loss

Sausage weight was measured after stuffing in the casings, heat treatment, and after three weeks of storage in a cooling chamber at 4°C. The process loss was calculated as a weight difference between sausages before and after heat treatment (%). Chicken sausages were vacuumed and left at 4°C in a cooling chamber to determine purge loss at the storage time. After 21 days, sausages were removed from the vacuum packages, bloated carefully with a paper towel to eliminate any liquid on the surface, and weighed in. Purge loss was calculated as a weight difference between sausages before and after storage time (%). Cooking loss was calculated as a weight difference in the sausages samples before and after cooking in distilled water in a closed glass container (at 80°C for 10 min). It was expressed as a percentage of the weight of the sample before cooking.

Emulsion stability, water-holding capacity (WHC), and pH

Emulsion stability was determined in triplicate for each treatment, as described by *Petričević et al. (2022)*. After undergoing two days of refrigerated storage, cuvettes, each holding approximately 25 ml of the meat batter, underwent a thermal treatment in a water bath set at 98ºC for a duration of 45 minutes. Subsequent to this, a rapid cooling phase ensued in ice water, lasting 10 minutes until the cuvettes reached ambient temperature. Following unsealing, the cuvettes were transferred into measured glass containers, where they remained for an hourto allow the drainage of liquids, encompassing both fat and water released during the heat treatment (HT). The quantity of liquid separated in this process, expressed as a percentage (%) of the initial filling weight before the HT, serves as a metric for quantifying the loss incurred throughout the heat treatment.

The method suggested by *Lin and Huang (2003)* was followed to determine WHC: 5 g (± 0.001 g) of homogenized raw meat batter sample was measured into a 50 ml tube, and 10 ml distilled water was added. The tubes were centrifuged at 2000 g for 10 min, and the final weight was obtained after decanting the supernatant. WHC was calculated using equitation (1). The higher WHC value (expressed by grams of H_2O absorbed/grams of meat) indicates that more water was bound, and therefore higher the water-holding ability of the batter.

WHC=[final sample weight-original sample weight]/original sample weight(g) (1) Sausage samples were used for pH analysis for each treatment using a pH meter equipped with a penetration probe (Hanna HI 83141; Hanna Instruments, USA). The pH meter was precalibrated using standard buffer solutions, pH 4.0 and 7.0 (*SRPS ISO 2917:2004*).

Instrumental color

The CIE L*a*b* color coordinates were determined using a Chroma Meter CR-400 (Minolta, Japan), which was previously calibrated using a standard white surface (illumination D65, observer angle 2° and aperture size 8 mm) as described by *Stajić et al. (2014).* Color attributes as are represented in the CIE L* a* b* system (*CIE, 1976*). The lightness is denoted by factor L*, red proportion is encapsulated by a*, and b* corresponds to the yellowness of color of the samples. Triplicate measurements were conducted for each sample, and the ensuing mean value served as the basis for statistical analysis.

Proximate analysis

The proximate analysis of the sausage samples transpired a day following their preparation. Preceding the chemical analyses, the collagen casings were removed, and the sausages underwent homogenization in a blender. Three sausages were randomly taken from each treatment for proximate composition analysis: moisture *(SRPS ISO 1442, 1998)*; fat content *(SRPS ISO 1444, 1998*); protein content (*SRPS ISO 937, 1992*); ash content (*SRPS ISO 936:1999*); carbohydrates were determined by subtracting the obtained values from 100%; NaCl content was determined by *SRPS ISO 1841-1:1999* method. The caloric value was computed by considering the chemical composition of sausages, with protein, carbohydrates, and fat assigned caloric values of 4 kcal, 1.5 kcal, and 9 kcal per gram of the product, respectively (*Garcia-Santos et al., 2019*).

Statistical analysis

The acquired data were processed by analysis of variance in the one-way ANOVA using the program SPSS Statistics 22. The results are displayed as the mean value ± standard deviation. A t-test determined the statistical significance of the difference between mean values.

Results and Discussion

Table 1 shows the chemical composition of cooked chicken sausages, with a noteworthy trend observed concerning the level of inulin replacement. Our analysis revealed a significant increase ($p < 0.01$) in moisture content in the sausage samples as the inulin replacement level escalated. The increased moisture content in the sausages can be attributed primarily to higher levels of inulin and the incorporation of water in the gel-suspension form within the formulation. Furthermore, the increasing moisture content in the sample groups can also be attributed to the intrinsic properties of inulin. The hydrophilic nature of inulin further enhances moisture retention by facilitating solid interactions with water molecules, emphasizing the critical roles of these components in influencing the product's physical properties.

Table 1. Chemical composition of cooked chicken sausages

Parameter	Groups				Statistical significance
	K	**50% IN**	**75% IN**	**100% IN**	
Moisture(%)	$67.80^{d} \pm 0.10$	$72.49^{c} \pm 0.09$	$74.97^{b} \pm 0.28$	$76.60^{a} \pm 0.53$	**
Total fat(%)	$17.83^{a} \pm 0.16$	$9.06^{b} \pm 0.15$	$4.02^{c} \pm 0.27$	$0.36^{d} \pm 0.05$	**
Protein (%)	$11.17^{d} \pm 0.15$	$11.81^{cb} \pm 0.12$	$12.04^{bc} \pm 0.16$	$13.51^{a} \pm 0.15$	**
Ash (%)	2.68 ± 0.16	2.49 ± 0.08	2.42 ± 0.03	2.28 ± 0.07	ns
Carbohydra tes (%)	$0.54^{d} \pm 0.06$	$4.05^{c} \pm 0.12$	$6.53^{b} \pm 0.34$	$7.23^{a} \pm 0.12$	**
NaCl (%)	1.52 ± 0.04	1.47 ± 0.04	1.52 ± 0.02	1.55 ± 0.06	ns
Caloric value (Kcal/100g)	$216.02^{a} \pm 0.90$	$134.83^{b} \pm 0.16$	$94.18^{c} \pm 0.85$	$60.69^{d} \pm 0.93$	**

ns–not significant; ** – $p<0.01$; $^{a, b, c, d}$: values in the same column with different superscript are significantly different ($p<0.01$)

The results confirm significant differences ($p < 0.01$) in the fat content as well as in the caloric value of the product. A product with a significantly reduced ($p < 0.01$) caloric value is obtained by reducing the fat content or increasing the inulin content. The caloric value in the 100% IN group with 100% fat replacement had about 72% lower caloric value compared to the control group. The results of *Šojić et al. (2011)* were consistent with our results. *Petričević et al. (2022)* state that replacing 50% of the fat in sausages reduces the caloric value by 30%, while the results in this study show a reduction of about 37%. The carbohydrate content also increased significantly ($p < 0.01$); the 100%IN group with the maximum amount of inulin had the highest carbohydrate content. Ash and NaCl content did not differ between groups.

Alaei et al. (2018) stated that the group of chicken sausages with 100% fat replacement with inulin had the best physicochemical, textural, and colorimetric characteristics. The results of the proximal analysis were in line with our results for the same group (100% fat replacement), following 72.63%, 29.90%, and 51.34% for moisture, carbohydrates, and protein content, respectively. They state that fat content was reduced with increased inulin content. The same authors suggest that inulin can be recommended as a fat substitute in making chicken sausages. Contrary to our results, research conducted by *Huang et al. (2011)* showed that the moisture content of sausage samples decreased with an increased level of inulin. This disparity may arise from variations in sausage sample formulations, inulin types, and the specific manner of incorporation into the formulation.

In the present study, substituting fat with inulin suspension is the primary strategy for creating a final product with reduced fat content, leading to decreased energy intake upon consumption. *Menegas et al. (2013)* verified this approach, demonstrating that sausages with 50% fat replacement exhibited significantly lower fat content (28.2%) compared to the control group (45.4%), aligning with findings by *Alaei et al. (2018)*. These results conform with our study, affirming the consistent impact of inulin replacement on fat reduction, as also reported by *Méndez et al. (2015)* and *Huang et al. (2011)*. Notably, *Vasilev et al. (2011)* showcased that cooked sausages containing an 8% inulin suspension had more moisture (63%) and less total fat (22-23%) than conventional counterparts, emphasizing the potential of inulin in altering sausage composition. Additionally, *Selgas et al. (2005)* introduced inulin as dietary fiber in cooked sausages, resulting in a 33-37% reduction in fat content and a 25% decrease in calories.

In our research, increasing the level of inulin suspension in formulationsled to a significant ($p < 0.01$) increase in protein content in chicken sausages, likely due to its calculation in dry matter. While the protein content between the 50% IN and 75% IN groups did not differ, both significantly ($p<0.01$) differed from the other groups. These results were consistent with observations made by *Menegas et al. (2013)* and *Méndez et al. (2015)*, who reported analogous conclusions. *Mendoza et al. (2001)* further supported these findings, demonstrating that protein content increased with higher inulin concentrations up to 12%, beyond which a decline was observed. *Vasilev et al. (2011)* reported protein content ranging from 10.2 to 10.4% in cooked sausages produced with an inulin suspension of about 3.0%, highlighting the intricate relationship between inulin concentration and protein content. In contrast to the conclusions drawn by *Šojić et al. (2011)* and *Mendoza et al. (2001)*, our study produced differing results regarding the ash content of sausages. This variance may be attributed to discrepancies in the methods used for calculating ash content.

The capacity to bind and retain water stands as a crucial determinant in assessing the technological quality of sausages (*Lu et al., 2021)*. In this study, sausages prepared with an inulin-gel exhibited a markedly higher mass loss after heat treatment compared to the control group, as evidenced by the data presented in Table 2 ($p<0.05$). Specifically, the control group exhibited significant variation ($p<0.05$) from the other experimental groups. However, intriguingly, the 75% IN group demonstrated no significant difference from the 50% IN and 100% IN groups, indicating a consistent trend in mass loss across these inulin concentrations.

Additionally, our findings revealed a substantial increase in weight loss after cooking sausages across all experimental groups, with significant differences observed ($p<0.05$). This occurrence can be ascribed to the higher water content in the filling, resulting from the replacement of the fat portion with an aqueous suspension of inulin, as outlined in Table 2. These outcomes emphasize the importance of understanding the impact of inulin-gel incorporation on mass loss during heat treatment and cooking processes, shedding light on potential applications in the food industry.

Table 2. Technological characteristics of cooked chicken sausages

Parameter	**Groups**				**Statistical significance**
	K	**50% IN**	**75% IN**	**100% IN**	
Process loss (%)	$19.90^{c} \pm 0.19$	$26.76^{ab} \pm 0.19$	$27.47^{ab} \pm 0.19$	$28.47^{a} \pm 0.19$	*
Purge loss (%)	1.58 ± 0.63	1.50 ± 0.50	1.45 ± 0.28	1.4 ± 0.63	ns
Emulsion stability (%)	$16.20^{b} \pm 0.35$	$18.61^{b} \pm 0.86$	$24.50^{a} \pm 0.99$	$25.80^{a} \pm 0.78$	*
Cooking loss (%)	$12.97^{d} \pm 0.19$	$16.69^{c} \pm 0.19$	$17.54^{b} \pm 0.19$	$24.75^{a} \pm 0.19$	*
pH	6.21 ± 0.12	6.22 ± 0.17	6.21 ± 0.08	6.20 ± 0.09	ns
WHC (%)	1.0927 ± 0.01	1.0998 ± 0.02	1.1069 ±0.01	1.1260 ± 0.01	ns
Instrumental color					
L	$73.62^{b} \pm 0.82$	$75.56^{ab} \pm 2.01$	$76.01^{ab} \pm 1.62$	$77.96^{a} \pm 0.83$	*
a*	$5.96^{a} \pm 0.19$	$5.06^{b} \pm 0.13$	$4.25^{c} \pm 0.04$	$1.17^{d} \pm 0.06$	*
b*	$10.76^{b} \pm 0.22$	$11.31^{a} \pm 0.11$	$11.80^{a} \pm 0.24$	$11.51^{a} \pm 0.20$	*

WHC – water-holding capacity; L – lightness, a* – redness; b* – yellowness; ns – not significant; * significant at the level of $p<0.05$; [a, b, c, d]: values in the same column with different superscript are significantly different ($p<0.05$).

Color represents a pivotal characteristic of this product type. Introducing fiber into the sausages had a noticeable impact on their coloration. Notably, reducing fat content led to sausages exhibiting a lighter coloration. The color nuances were quantified using the L*, a*, and b* values, as detailed in Table 2, with significant differences observed among the groups ($p<0.05$).

Our analysis revealed a considerable trend in the L* values, which increased proportionally with higher inulin content, reaching its peak in the 100% IN group. This aligns with the findings of *Alaei et al. (2018)*, who observed a similar increase in L* values upon the addition of inulin. In contrast, the study by *Ferjančič et al. (2021)* reported a decrease in L* values when dietary fiber was incorporated. The observed alterations in color could be attributed to the introduction of dietary fiber, especially considering that the inulin-gel mixture itself is predominantly white.

Interestingly, the indicator of redness (a*) exhibited a decrease in our study, contrary to the findings of *Alaei et al. (2018)* and *Ferjančič et al. (2021)*, both of whom reported that the addition of inulin enhanced the natural redness of chicken sausages. This discrepancy underscores the complexity of color modulation in food products and highlights the need for comprehensive investigations into the interplay of various ingredients.

Furthermore, in the present study, the indicator of yellowness (b*) demonstrated an increase with reduced fat content. This contrasts with the research conducted by *Ferjančič et al. (2021)*, where b* values exhibited a decrease, likely influenced by the more pronounced redness of chicken sausages in their study. These variations underscore the intricate relationships between fat content, inulin addition, and color attributes, emphasizing the multifaceted nature of color development in sausages. The research conducted by *Choe et al. (2013)* and *Petričević et al. (2022)* has demonstrated the significant enhancement of emulsion stability in cooked sausages when fibers are introduced, particularly in formulations with reduced fat content. In addition, *Furlan et al. (2014)* conducted a study exploring the impact of replacing fat with inulin in cooked sausages. Based on inulin incorporation at two distinct levels (1% and 2%), their findings revealed a marked improvement in emulsion stability. This enhancement can be attributed tothe water-binding properties of inulin, coupled with its emulsifying capabilities, making it a valuable additive in sausage formulations.

Furthermore, the outcome of *Petričević et al. (2022)* scientidic article sheds light on the nuanced dynamics of inulin content in sausages. Their research elucidates that higher levels of inulin in sausage formulations lead to elevated process loss and cooking loss. This intriguing observation suggests a delicate balance between emulsion stability and overall product characteristics, highlighting the complex interplay of ingredients in sausage production. These findings and current research findings underscore the importance of careful formulation to achieve desired product attributes.

Conclusion

In conclusion, our study demonstrated the potential of inulin-gel suspension as a promising ingredient in poultry sausages, showcasing its impact on mass loss during heat treatment and cooking processes. The inulin-gel addition significantly influenced the sausages' color attributes, leading to nuanced changes in lightness (L*), redness (a*), and yellowness (b*), highlighting the complexity of color development in inulin-modified sausages. Furthermore, the incorporation of inulin suspension resulted in alterations in the sausages' chemical composition, including reduced fat content and caloric value, and increased protein and carbohydrate content, emphasizing the potential of inulin as a fat replacer and a nutritional enhancer. These findings contribute valuable insights into the utilizationof inulin-gel suspension in poultry sausages, offering opportunities for the development of healthier and more nutritionally balanced meat products. However, further research is warranted to explore the sensory aspects and consumer acceptance of these modified sausages, providing a holistic understanding of their viability in the market.

Inovativna primena inulin-gel suspenzije u pilećim kobasicama: Tehnološki uticaj i poboljšanje nutritivne vrednosti

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Rezime

Potražnja potrošača za zdravijom hranom navela je prehrambenu industriju da traži alternative za smanjenje visokog nivoa masti. Dijetalna vlakna kao što je inulin su se pojavila kao obećavajuća zamena za masti, povećavajući nutritivnu vrednost i smanjujući apsorpciju masti. Cilj ovog istraživanja je bio formulisanje pilećih kuvanih kobasica u kojoj je mast zamenjena suspenzijom inulina u različitimnivoima. U ogledu su učestvovale četiri grupe kobasica: grupa K bez zamene masti i grupe 50% IN, 75% IN i 100% IN sa 50%, 75% i 100% zamene masti respektivno. Rezultati pokazuju značajno veći gubitak process and cooking loss oglednih grupau odnosu na grupu K ($p<0,05$). Grupe K i 50%IN su imale značajno bolju stabilnost emulzije ($p<0,05$). Povećanje sadržaja inulina značajno je uticalo na karakteristike boje uključujući L* (svetloća), a* (udeo crvene boje) i b* (udeo žute boje) ($p<0,05$). Ogledne grupe kobasica su imale svetliju boju. Smanjenjem masti, kalorijska vrednost kobasica se značajno smanjila i razlikovala među grupama ($p<0,01$). Inulin se može koristiti u proizvodnju kuvanih kobasica sa smanjenim sadržajem masti i optimalnih fizičko-hemijskim svojstvima. Ovaj mesni proizvod pored smanjene energetske vrednosti ima i dobre nutritivne karakteristike.

Ključne reči: inulin, mast, pileće viršle, energetska vrednost, funkcionalna hrana

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EFFECT OF pH ON GERMINATION AND SEEDLING GROWTH OF MAIZE

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Abstract: Soil pH is one of the most important limiting factors for crop cultivation, including maize. About 30-40% of the world's arable land is acidic, and over a billion ha is alkaline. Today, there are 205 million ha of arable land under maize in the world, so it ranks third in area after rice and wheat. Maize hybrids have wide genetic variability and high pH tolerance. We tested the tolerance of maize hybrids seedlings of ZP 4708 and ZP 5797 to pH 5, 6, 7, and 8. Results showed that hybrid ZP 4708 has a higher adaptability to low and high pH, due to the higher value of germination energy (GE), shoot length (ShL), shoot fresh weight (ShFW), shoot dry weight (ShDW), germination rate index (GRI), and seedling vigor index (SVI) than hybrid ZP 5797. Results further showed, on average for hybrids, that pH 5 and pH 8 significantly decreased the GE, root length (RL), ShL, root fresh weight (RFW), ShFW, root dry weight (RDW), shoot fresh weight (ShDW), and SVI. These parameters did not differ significantly between pH 6 and pH 7. Seed germination was not affected by the pH, indicating that the seed vigor of both hybrids supports full germination potential. However, it could be expected that exposure to the lowest and highest pH values of the medium could affect seedlings growth in the field conditions, even though germination is high. SVI showed a positive and significant correlation with all investigated parameters, except germination (G) and relative seedling water content (RSWC).

Key words: correlation, germination, maize, pH, seedling

Introduction

Maize is one of the top-produced crops in the world. Its chemical composition makes it suitable for human consumption, animal feed, and industrial uses. The total world production of maize in 2021 was 1210 billion tons on about 205 million ha *(FAO, 2023)*. Serbian production of maize in 2022 was 4.283.293

tons on 952.216 ha *(Statistical Yearbook of the Republic of Serbia, 2023)*. However, there are many limiting factors for cultivation, including soil pH. Soil with a pH of 4.5-5.5 is strongly acidic, pH 5.6-6.5 slightly acidic, pH 6.6-7.3 neutral, and pH 7.4-8.4 slightly alkaline *(Ahmed et al., 2018)*. Maize thrives best on soil between 6.5 and 7.0 pH. The tolerance degree to soil acidity depends on the maize genotype, the concentration of hydrogen and aluminum ions, and the physicochemical soil properties. On soils with a reaction pH<5, due to aluminum and manganese toxicity, the root system and plant growth are reduced *(Rahman et al., 2018)*, with occurrence of phosphorus deficiency. The result is a yield reduction in over 69% *(Tandzi et al., 2015)*. On such soil, it is necessary to carry out amelioration measures such as calcification, humification, and application of phosphorus and microelements fertilizers. By growing tolerant hybrids, the harmful effects of acidic soil reactions on maize production can be mitigated. On the other hand, alkaline soils reduce the maize yield by 20–46% *(Fu et al., 2017)*, due to root poisoning by ions and osmotic imbalance, which reduces its growth, inhibits biomass and plant growth, and causes wilting and eventual death of plants *(Guo et al., 2014)*. Germination and early seedling growth are the most important factors to achieve the appropriate number of plants per unit area and yield *(Amini et al., 2015)*.

According to *Kolasinska et al. (2000)*, germination test results in laboratory conditions correlate well with emergence in the field. This provides a good basis for predicting seed and seedling performance under field conditions. In this regard, laboratory experiments that are faster and cheaper were settled to test maize hybrids regarding the various soil pH. This research aimed to determine to what extent the pH value of the medium influences the differences and possible advantages of germination and initial seedling development (vigor) of two maize genotypes.

Materials and Methods

Laboratory experiments were conducted at the Institute for Animal Husbandry in Belgrade, Republic of Serbia in 2023. New hybrids ZP 4708 and ZP 5797 selected in Maize Research Institute "Zemun Polje" were used as material. The seeds were first sterilized with 1% sodium hypochlorite for 5 minutes, washed 3 times in sterile distilled water (pH 7), and dried. Each treatment contained 100 seeds per application placed on filter paper in a sterilized plastic box with a cover to prevent water loss (15cm x 21cm x 4cm). The pH 5 and pH 6 solutions were made by adding 0.1 M hydrochloric acid solution to distilled water. The pH 8 solution was made by adding 0.1 M sodium hydroxide to distilled water. The filter papers were soaked with 10 ml of media. Experiments were carried out at 20 °C for 10 days.

In the study, a randomized complete block design with four replicates was used, i.e. a 2 × 4 design (factor hybrid with 2 levels and factor pH with 4 levels).

Germination e Germination energy (GE) and germination (G) were determined according to ISTA Rules on the fourth and seventh days, respectively *(ISTA 2019)*. Other quantitative parameters (root length - RL, shoot length - ShL, root fresh weight - RFW, shoot fresh weight - ShFW, root dry weight - RDW, and shoot dry weight - ShDW) were determined ten days after sowing. Rate germination index (RGI), seedling vigor index (SVI), and relative seedling water content (RSWC) were calculated by the formulas described in research by *Mandić et al. (2014)*:

$$RGI = \frac{\text{No. of seeds germinated at 3 day}}{\text{No of seeds germinated at 7 day}} \times 100$$

SVI = (Root length + Shoot length) x Germination percentage

$$RSWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

The data analysis was processed in Statica version 13 (StatSoft, Tulsa, Oklahoma, USA) while comparing the means using Tukey's test at a 5% level. Pearson's correlation coefficient was run to measure the relationship between the parameters.

Results and Discussion

Results of ANOVA showed that hybrid had a highly significant effect on GE, ShL, ShFW, ShDW, RGI, and SVI (Table 1). These parameters were higher in the hybrid ZP 4708 than in ZP 5797. Thus, GE was higher for 42.5%, ShL for 1.15 cm, ShFW for 29.1 mg, ShDW for 2.3 mg, RGI for 61.1%, and SVI for 210.6 than in ZP 5797 (54.38, 3.44 cm, 99.4 mg, 11.2, 49,4%, and 1374,4, respectively). *Tandzi et al. (2018)* also reported that maize hybrids have different levels of pH tolerance. *Torres et al. (2019)* stated that genetic diversity can be established already on seed and seedling traits. According to *Egli and Rucker (2012)*, maize hybrid seeds with a high vitality index germinate more uniformly and in less favorable environmental conditions, which we observed in our research. It should be noted that although we only focused on the effect of pH on these hybrids, the results are useful for future field experiments related to the expansion of the area of their cultivation.

Table 1. The effects of hybrid and pH on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW), shoot dry weight (ShDW), rate germination index (RGI), seedling vigor index (SVI), and relative seedling water content (RSWC) of maize

Parameters	Hybrid (H)	pH				Means
		5	6	7	8	
GE, %	ZP 4708	96.25^{a}	96.25^{a}	97.50^{a}	97.50^{a}	96.88^{a}
	ZP 5797	37.50^{c}	61.25^{b}	68.75^{b}	50.00^{bc}	54.38^{b}
	Means	66.88^{b}	78.75^{ab}	83.12^{a}	73.75^{ab}	75.63
G, %	ZP 4708	100.00	100.00	100.00	98.75	99.69
	ZP 5797	100.00	96.25	98.75	98.75	98.44
	Means	100.00	98.12	99.38	98.75	99.07
RL, cm	ZP 4708	9.22	11.69	13.27	11.08	11.31
	ZP 5797	8.45	11.11	12.10	10.46	10.53
	Means	8.84^{b}	11.40^{a}	12.68^{a}	10.77^{ab}	10.92
ShL, cm	ZP 4708	4.19	5.00	5.62	3.56	4.59^{a}
	ZP 5797	1.99	4.04	4.17	3.58	3.44^{b}
	Means	3.09^{c}	4.52^{ab}	4.90^{a}	3.57^{bc}	4.02
RFW, mg	ZP 4708	136.9	246.1	277.9	184.9	211.4
	ZP 5797	135.3	242.8	232.8	183.5	198.6
	Means	136.1^{c}	244.4^{ab}	255.4^{a}	184.2^{bc}	205.0
ShFW, mg	ZP 4708	115.8^{abcd}	146.3^{ab}	183.1^{a}	68.8^{cd}	128.5^{a}
	ZP 5797	52.7^{cd}	122.5^{abcd}	117.0^{abcd}	105.3^{bcd}	99.4^{b}
	Means	84.2^{b}	134.4^{a}	150.0^{a}	87.1^{b}	113.95
RDW, mg	ZP 4708	20.4	28.3	31.8	23.2	25.9
	ZP 5797	15.3	27.9	29.0	23.5	23.9
	Means	17.8^{c}	28.1^{ab}	30.4^{a}	23.3^{bc}	24.9
ShDW, mg	ZP 4708	11.8	14.6	16.8	11.0	13.5^{a}
	ZP 5797	7.3	13.2	12.7	11.8	11.2^{b}
	Means	9.6^{c}	13.9^{ab}	14.7^{a}	11.4^{bc}	12.4
RGI, %	ZP 4708	77.5	85.0	82.5	74.7	79.9^{a}
	ZP 5797	16.2	20.9	26.6	11.5	18.8^{b}
	Means	46.9	53.0	54.6	43.1	49.4
SVI	ZP 4708	1341.0	1668.0	1889.2	1441.8	1585.0^{a}
	ZP 5797	1044.2	1457.1	1606.2	1390.0	1374.4^{b}
	Means	1192.6^{c}	1562.5^{ab}	1747.8^{a}	1415.9^{bc}	1479.7
RSWC, %	ZP 4708	87.5	89.2	88.8	85.2	87.7
	ZP 5797	88.2	88.7	88.0	85.8	87.7
	Means	87.9	89.0	88.4	85.5	87.7

Factor	GE	G	RL	ShL	RFW	ShFW	RDW	ShDW	RGI	SVI	RSWC
H	**	ns	ns	**	ns	*	ns	**	**	**	ns
pH	*	ns	**	**	**	**	**	**	*	**	ns
H × pH	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

The testing the effects of pH on maize seedling parameters in laboratory conditions, we concluded that pH greatly affects the germination energy, growth, and development of seedlings. This study demonstrated that the germination energy, seedling performances, RGI, and SVI were significantly dependent on pH media. The G, RGI, and RSWC were non-significant. Data also showed that maximum GE (83.12 %), RL (12.68 cm), ShL (4.90 cm), RFW (255.4 mg), ShFW (150.0 mg), RDW (30.4 mg), ShDW (14.7 mg), SVI (1747.8) were attained at optimal, pH 7. The values of these parameters were not significantly different from those at pH 6 (GE – 78.75 %, RL – 11.40 cm, ShL – 4.52 cm, RFW – 244.4 mg, ShFW – 134.4 mg, RDW – 28.1 mg, ShDW – 13.9 mg, and SVI – 1562.5). The values of investigated parameters were reduced when the pH reaction was at pH 5, and pH 8. The minimum values of (GE – 66.88 %, RL – 8.84 cm, ShL – 3.09 cm, RFW – 136.1 mg, ShFW – 84.2 mg, RDW – 17.8 mg, ShDW – 9.6 mg, and SVI – 1192.6) were recorded at pH 5.

Results showed that the seeds of the tested hybrids can germinate in a wide range of pH from 5 to 8, but the best performances of the seedlings are in the pH 6 and pH 7 treatments. Similarly, *Sikalengo (2016)* reports that the maize germination and dry matter content are reduced in soil with pH 5.0 and pH 8.5. The research by *Neina (2019)* and *Msimbira et al. (2023)* has shown that a slightly acidic pH value of the medium slightly increases the germination of maize seeds and has a significant role in interactions with the environment.

The effect of hybrid × pH interaction was the most important in the expression of GE and ShFW. Hybrid ZP 4708 had the highest GE value in all pH media. The highest average value of GE was in hybrid ZP 4708 at pH 7 and pH 8 medium (97.5 %), and the lowest in hybrid ZP 5797 at pH 5 medium (37.5 %). In both hybrids, ShFW varied significantly depending on the pH of the medium. Hybrid ZP 4708 had the highest ShFW value in the pH 7 treatment (183.1 mg), and hybrid ZP 5797 had the lowest value in the pH 5 treatment (52.7 mg).

On the other hand, seed germination was not affected by the pH, indicating that the germination potential of both hybrids was high. However, seedling parameters of the tested hybrids depended to a large extent on the medium pH. Exposure to the lowest and highest pH values of the medium resulted in similar performance of the hybrids suggesting that seedling development is highly affected by extreme pH, even though germination was high.

GE shows a strong, positive, and highly significant correlation with RGI (0.89**), RL with SVI (0.95**), RDW (0.85**), and RFW (0.83**), ShL with ShDW (0.94**), SVI (0.89**), and ShFW (0.84**), RFW with RDW (0.88**), SVI (0.84**), and ShDW (0.80**), ShFW with ShDW (0.88**), RDW and ShDW (0.88**), and SVI (0.86**), and ShDW with SVI (0.89**), Table 2. Also, *Vengilat et al. (2019)* have found that SVI positively correlated with RL and ShL. However, these authors also have found a positive correlation between G and RL, G and ShL, and G and SVI, which is contrary to our results. *Arshad et al (2020)* have found a significant positive correlation between ShFW and ShL (0.68**) and RFW and RDW (0.50*), while *Naseem et al (2020)* between RL and ShL (0.81*). According to our results, seedling root and shoot parameters and SVI are important for high crop population density under various pH conditions.

Table 2. Relationship of germination and seedling growth parameters under pH media

Parameters	GE	G	RL	ShL	RFW	ShFW	RDW	ShDW	RGI	SVI
G	0.18									
RL	0.36*	-0.06								
ShL	0.54**	-0.015	0.71**							
RFW	0.21*	-0.11	0.83**	0.72**						
ShFW	0.38*	-0.08	0.64**	0.84**	0.71**					
RDW	0.34	-0.18	0.85**	0.76**	0.88**	0.75**				
ShDW	0.46**	-0.09	0.76**	0.94**	0.80**	0.88**	0.88**			
RGI	0.89**	0.23	0.30	0.49**	0.19*	0.36*	0.25	0.39*		
SVI	0.48**	0.06	0.95**	0.89**	0.84**	0.76**	0.86**	0.89**	0.43*	
RSWC	-0.05	0.08	0.12	0.27	0.34	0.12	0.08	0.15	0.06	0.20

Germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW), shoot dry weight (ShDW), rate germination index (RGI), seedling vigor index (SVI), and relative seedling water content (RSWC) of maize

Conclusion

Both hybrids ZP 4708 and ZP 5797 can successfully grow on media with a pH value in the range of 5.0 to 8.0. However, maize hybrids do not have the same ability to adapt to such conditions. Results showed that maize hybrid ZP 4708 has better seedlings performance (GE, ShL, ShFW, ShDW, GRI, and SVI) compared to hybrid ZP 5797. Results further showed that the GE, RL, ShL, RFW, ShFW, RDW, ShDW, and SVI did not differ between treatments pH 6 and pH 7. Accordingly, a slightly acidic pH value of the medium could increase the growth of seedlings. The decrease (pH 5) and increase in pH level (pH 8) decreased the seedling growth. The practical importance of the achieved results indicated that the new hybrid ZP 4708 showed better performances of seedlings on slightly acidic to neutral levels media (pH 6 and pH 7) and could be expected to achieve better emergence, crop growth, and suitable plant density at different pH values.

UTICAJ pH VREDNOSTI NA KLIJANJE I RAST KLIJANACA KUKURUZA

Violeta Mandić, Vesna Krnjaja, Aleksandar Simić, Maja Petričević, Marija Gogić, Milan Brankov, Aleksandar Stanojković

Rezime

pH zemljišta je jedan od važnih ograničavajućih faktora za gajenje useva, a samim tim i kukuruza. Oko 30-40% svetskih obradivih površina je kisele reakcije, a preko milijardu ha je alkalno. Danas je u svetu 205 miliona ha obradivih površina pod kukuruzom, tako da on zauzima treće mesto po površini posle pirinča i pšenice. Hibridi kukuruza imaju široku genetsku varijabilnost i toleranciju za pH. Ispitivali smo toleranciju klijanaca novih hibrida kukuruza ZP 4708 i ZP 5797 na pH 5, 6, 7 i 8. Rezultati su pokazali da hibrid ZP 4708 ima bolju sposobnost adaptacije na nisku i visoku pH vrednost jer je imao veću energiju klijanja (EK), dužinu stabla (DS), svežu masu stabla (SvMS), suvu masu stabla (SuMS), indeks klijavosti (IK) i vigor indeks (VI) od hibrida ZP 5797. Rezultati su dalje pokazali, u proseku za hibride, da su niski (pH 5) i visoki pH nivo medijuma (pH8) značajno smanjili EK, dužinu korena (DK), DS, svežu masu korena (SvMK), SvMS, suvu masu korena (SuMK), SuMS i VI. Ovi parametri se nisu razlikovali između tretmana pH 6 i pH 7. pH medijuma nije uticala na klijanje semena što ukazuje da snaga semena oba hibrida podržava pun potencijal klijanja. Međutim, izlaganje najnižim i najvišim pH vrednostima medijuma je dovelo do toga da nije moguće očekivati jake klijance, iako je klijavost visoka. VI je pokazao pozitivnu i značajnu korelaciju sa svim ispitivanim parametrima, osim sa klijanjem semena i relativnim sadržajem vode u klijancima.

Ključne reči: korelacija, klijavost, kukuruz, pH, klijanac

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OCCURRENCE OF AFLATOXINS AND FUMONISINS IN MAIZE GRAINS HARVESTED IN THE TERRITORY OF BELGRADE (R. SERBIA) FROM 2018 TO 2022

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Original scientific paper

Abstract: The aim of this study was to evaluate the occurrence of total aflatoxins (AFs) and type-B fumonisins (FBs) in 65 maize grain samples collected during harvest in 2018 (13 samples), 2019 (11 samples), 2020 (9 samples), 2021 (14 samples) and 2022 (18 samples) from different locations in suburb of Belgrade (Republic of Serbia). The average levels of AFs and FBs in mycotoxin-positive samples were 5.43 and 2910 μg kg^{-1} (2018), 5.28 and 2710 μg kg^{-1} (2019), 2.35 and 10980 μg kg^{-1} (2020), 6.81 and 4950 μg kg^{-1} (2021) and 5.32 and 20310 μg kg^{-1} (2022), respectively. In 23.08% (2018), 18.18% (2019), 22.22% (2020), 64.29% (2021) and 27.78% (2022) of maize samples, the co-occurrence of AFs and FBs was established. The maximum limits of 10 μg kg^{-1} for AFs in maize and 4000 μg kg^{-1} for FBs in unprocessed maize prescribed by regulations of Serbia and the European Union were exceeded for AFs in 14.29% (2021) and 5.56% (2022) of maize samples and for FBs in 7.69% (2018), 66.67% (2020), 28.57% (2021) and 41.67% (2022) of maize samples. Multiple linear regression analyses showed a statistically significant influence of climate factors (air temperature, relative humidity and total rainfall) in July-September (2018-2022) on FBs levels. These results indicate the need for continuous monitoring of the health status of harvested maize grains and risk assessment of the potential presence of mycotoxins in the food chain to avoid adverse effects on human and animal health.

Key words: maize, aflatoxins, fumonisins

Introduction

Maize is one of the important cereal crops for animal and human diets. However, it is susceptible to mycotoxin contamination in the field and storage.

Mycotoxins are toxic secondary metabolites produced by different fungal species. *Aspergillus* and *Fusarium* species are the most common mycotoxin producers in maize grains. *A. flavus* Link and *A. parasiticus* Speare are the primary aflatoxin producers. There are four main aflatoxins, B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), with AFB_1 as the most toxic. Total aflatoxins (AFs) are carcinogenic to humans and classified as Group 1 carcinogenic compounds by the International Agency for Research on Cancer (IARC). Aflatoxin M_1 (AFM_1) is a metabolite of AFB_1 and can occur in milk and its products through contaminated feed with AFB_1. It is potentially carcinogenic to humans and classified in Group 2B *(IARC, 2002)*. In livestock, the risk of chronic exposure to AFs manifested in low productivity with reduced daily weight and growth, especially in poultry and pigs. In ruminants, the major constraint of AFs is the production of metabolite AFM_1 in milk and dairy products *(Ferrari et al., 2022)*. In farm animals, aflatoxicosis is manifested as acute and chronic liver disease. AFB_1 may cause dysfunction of the liver and reduced egg and milk production and immunity *(Pleadin, 2015)*. In general, aflatoxins are carcinogenic, teratogenic, mutagenic and immunosuppressive and are the main causative agents of liver cancer in humans and animals *(Barošević et al., 2022)*.

Fusarium species, *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg are the most common fumonisin producers. *Aspergillus niger* van Tieghem also produces fumonisins *(Frisvad et al., 2007)*. There are A, B, C and P types of fumonisins, from which B-type fumonisins (FBs) are the most frequent in maize grains and products. Fumonisin B_1 (FB_1) is potentially carcinogenic to humans and classified in Group 2B to IARC. Fumonisins induce neurotoxicity, immunotoxicity, hepatotoxicity and carcinogenicity in organisms. Mycotoxicoses caused by fumonisins are equine leukoencephalomalacia in horses, porcine pulmonary edema and cardiovascular changes in swine, renal injuries in sheep, rabbits and rats, than esophageal cancer and neural tube defects in humans. After aflatoxins, fumonisins represent a significant threat to animal and human health *(Haschek et al., 2001; Palacious et al., 2015; Qu et al., 2022)*.

In many countries, permissible maximum limits of mycotoxins in food and feed are prescribed to avoid their harmful effects on human and animal health. According to Serbian *(Official gazette RS 22/2018)* and the European Union (EU) regulations, the maximum limits of AFs and FBs in unprocessed maize are 10 $\mu g\ kg^{-1}$ (2010/165/EC) and 4000 $\mu g\ kg^{-1}$ (2007/1126/EC), respectively.

Climatic conditions and agro-technical practices (crop rotation, tillage, sowing time, maize genotypes, fertilisation, applying insecticides) influence the occurrence and incidence of *Aspergillus* and *Fusarium* species on maize grains. Aspergillus ear rot (AER) and Fusarium ear rot (FER) are the most common fungal diseases of maize, which are affected by drought years *(Stumpf et al., 2013)*. *Fusarium* species overwinter on crop residues of the previous crops which are the main source of inoculum for subsequent infections *(Pfordt et al., 2020)*. Drought and high temperatures also contribute to increased insects as a vector of fungal diseases in maize (Miller, 2001). In Serbia, the most sensitive period for infestation of maize by *Fusarium* species is during the flowering and silking stages in July *(Krnjaja et al., 2022)*. High temperatures during the reproductive period of maize and humid and wet weather at the physiological maturity stage favour the growth of FER causative pathogens and fumonisin synthesis in maize grains *(Berardo et al., 2011; Akello et al., 2021)*. On the other hand, AFs production is affected by dry and hot maize growing seasons with prolonged drought periods in spring and summer *(Kos et al., 2017)*. The synthesis of aflatoxins is affected by environmental conditions and fungal strains. There are aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* species. Suitable environmental factors for aflatoxin and fumonisin production in maize grains are high temperatures of 25-35°C and 20-25°C and water activity (a_w) of 0.99-0.95 and 0.98, respectively *(Giorni et al., 2019)*. Additionally, FBs production increases with increasing water activity (up to 1), and optimal temperatures for FBs production can range from 10 to 37°C *(Santiago et al., 2015)*.

Ongoing efforts to control fungal contamination in maize grains are part of integral pest management in maize production worldwide. However, mycotoxins occur as unavoidable maize contaminants. Therefore, the main aim of this research was to evaluate levels of total aflatoxins (AFs) and the sum of fumonisins B_1, B_2 and B_3 (FBs) in harvested maize grain samples in the five-year study (2018-2022) with a focus on the discussion about the influence of climatic conditions in Serbia on aflatoxin and fumonisin contamination of maize.

Materials and Methods

A five-year (2018-2022) survey was conducted in order to determine the presence of AFs and FBs in grain samples originating from maize fields in the suburb of Belgrade (area of Zemun and Surčin). Maize crops were grown under dry farming conditions applying standard agro-technical practices. Maize genotypes belong to different maturity groups, mostly from the middle-late maturity group. A total of 65 maize grain samples were collected in the harvest period of 2018 (13 samples), 2019 (11 samples), 2020 (9 samples), 2021 (14 samples) and 2022 (18 samples). The sample size was about 1 kg. The samples were packed in paper bags and stored in a refrigerator at less than 4°C. Before mycotoxin analyses, a sub-sample of about 200 g was dried for 72 h at 60°C and ground into a fine powder using an analytical mill (IKA A11, Germany).

A competitive ELISA (enzyme-linked immunosorbent assay) method was used to quantify the total AFs and FBs using Celer AFLA and FUMO kits (Eurofins Technologies, Budapest, Hungary). The test is performed in plastic micro-wells that are coated with anti-mycotoxin antibodies. Competition is conducted between enzyme conjugates and standard solutions or samples for binding sites of anti-mycotoxin antibodies. Then, unbound molecules are removed in the washing process. The activity of the bound enzyme is determined by adding chromogenic substrates. The enzyme converts the colourless chromogen into a blue product. Colour intensity is inversely proportional to the mycotoxin level in the sample or standard. Finally, a reagent is added that stops the enzymatic reaction and changes the colour from blue to yellow. Absorbance was measured with a microplate reader (Biotek EL × 800TM, USA) at 450 nm. The detection limits for AFs and FBs in cereals were 2 and 750 µg kg^{-1}, respectively.

Statistical analyses of results in mycotoxin-positive samples were performed using IBM SPSS Statistics 20 with a descriptive data display (average, minimum, maximum and median). The significance between medians of mycotoxin levels in investigated years was determined by non-parametric the Kruskal–Wallis statistical test. The correlation between AFs and FBs levels was determined using Pearson's correlation test for all samples, considering statistical significance at $p \leq 0.05$ and $p \leq 0.01$. Multiple linear regression was used to determine whether mean air temperature and relative humidity and the total rainfall from the silking (in July) to maturity (in September) maize stages influenced AFs and FBs levels in grains. In correlation and regression analyses, values of detection limits for mycotoxin-non-detected maize samples were used. The co-occurrences AFs and FBs were also determined.

Results and Discussion

This study investigated the natural occurrence and co-occurrence of AFs and FBs in maize grain samples collected during harvest in five growing seasons (2018-2022) in Serbia (a suburb of Belgrade). Climatic conditions in Serbia are convenient for maize infections by toxigenic fungal species, producers of AFs (*Aspergillus* spp.) and especially primarily producers of FBs (*Fusarium* spp.).

The percentage of aflatoxin-positive maize samples was the highest in 2021 (64.29%), followed by 2022 with 27.78%, 2018 with 23.08%, 2020 with 22.22% and 2019 with 18.18% aflatoxin-positive maize samples. The highest average AFs level was in 2021 (6.81 µg kg^{-1}), while it was the lowest in 2020 (2.35 µg kg^{-1}). Significant differences between the investigated years for medians according to the Kruskal-Wallis test were observed (Table 1). In the previous study in Serbia, the epidemic occurrence of aflatoxins in maize grains, with an average AFs level of 36.3 µg kg^{-1} in 68.5% of positive samples, was noticed in 2012 *(Kos et al., 2013)*. According to the results of *Krnjaja et al. (2013)*, in 2012, there were 36.69% of maize grain samples contaminated by *A. flavus*. Based on the four-year survey (2018-2021) of maize grains from Serbia, *Pleadin et al. (2023)* have also established the highest percentage of aflatoxin-positive samples in 2021 (84%), followed by 2019 (11%), 2018 (8%) and 2020 (5%), with 5.7 times higher the average level of AFs in 2021 (38.8 μg kg^{-1}) than in this study (6.81 μg kg^{-1}). The explanation for that is the differences in the number and origin (location) of maize samples and the methods for AFs detection. Likewise, the distribution of AFs in maize grains was highly heterogeneous and depended on the agro-ecological region *(Weaver et al., 2021)* and farming practices *(Cheng et al., 2022)*.

The levels of AFs above the maximum limit (10 μg kg^{-1}) were found in 14.29 and 5.56% of maize samples in 2021 and 2022, respectively (Table 2). In a similar four-year (2009–2012) study in Serbia, *Kos et al. (2013)* determined 24 and 29.5% of maize grain samples with 10–50 μg AFs kg^{-1} and 50–90 μg AFs kg^{-1}, respectively in 2012, while in other years AFs were not detected. In a recent study, *Pleadin et al. (2023)* established even 61% of examined Serbian maize grain samples in 2021 with AFs above the maximum limit (10 μg kg^{-1}).

Table 1. Level of total aflatoxins in maize grains harvested in 2018, 2019, 2020, 2021 and 2022

Year	No. of samples	Positive samples (%)	Level in positive samples (μg kg^{-1}) Average	Minimum	Maximum	Median
2018	13	23.08	5.43	3.02	7.00	6.27
2019	11	18.18	5.28	2.38	8.18	5.28
2020	9	22.22	2.35	2.30	2.40	2.35
2021	14	64.29	6.81	2.87	13.30	7.29
2022	18	27.78	5.32	3.55	10.05	4.65

Table 2. Percentage of maize samples with a level of AFs above the maximum limit (10 μg kg^{-1}) prescribed by Serbian and EU regulations

Level of AFs (μg kg^{-1})	Year 2018	2019	2020	2021	2022
>10	0	0	0	14.29	5.56

The percentage of fumonisin-positive maize samples was the highest in 2020 and 2021 (100%), followed by 2022 (88.89%), 2018 (69.23%) and 2019 (45.45%). The highest and lowest average FBs level was in 2022 (20310 μg kg^{-1}) and 2019 (2710 μg kg^{-1}), respectively. According to the Kruskal-Wallis test, significant differences between the investigated years for medians were determined (Table 3). In a similar multi-year study in Serbia, *Jakšić et al. (2019)* reported the percentage of fumonisin-positive maize grain samples collected before storage ranged from 51% in 2005 to 100% in 2012 production years. *Stanković et al. (2011)* have also determined high percentages of fumonisin-positive maize samples collected in Serbia during 2006-2009, ranging from 60.7% in 2006 to 80.1% in 2007. Likewise, *Obradović et al. (2018)* have established a higher percentage of FBs-positive maize samples in 2012 (78.4%) than in 2013 (33.3%), with average FBs levels of 1300 μg kg^{-1} (2012) and 2800 (2013 μg kg^{-1}).

The levels of FBs were above the maximum limit for unprocessed maize (4000 μg kg^{-1}) in 7.69% (2018), 66.67% (2020), 28.57% (2021) and 41.67% (2022) of maize samples (Table 4). Similarly, in an earlier study by *Jakšić et al. (2019)*, the percentage of inappropriate maize grain samples collected before storage during the long-term period in Serbia with FBs level above 4000 μg kg^{-1} ranged from 8% in 2013 to 44% in the 2012 production year.

Table 3. Level of fumonisins in maize grains harvested in 2018, 2019, 2020, 2021, and 2022

Year	No. of samples	Positive samples (%)	Level in positive samples (μg kg^{-1})			
			Average	Minimum	Maximum	Median
2018	13	69.23	2910	830	13230	1210
2019	11	45.45	2710	800	3650	2090
2020	9	100	10980	1160	20250	11790
2021	14	100	4950	810	19300	2420
2022	18	88.89	20310	860	51650	16940

Table 4. Percentage of maize samples with a level of FBs above the maximum limit (4000 μg kg^{-1}) prescribed by Serbian and EU regulations

Level of FBs (μg kg^{-1})	Year				
	2018	2019	2020	2021	2022
>4000	7.69	0	66.67	28.57	41.67

The highest percentage of maize samples with co-occurrence AFs and FBs was established in 2021 (64.29%), followed by 2022 (27.78%), 2018 (23.08%), 2020 (22.22%) and 2019 (18.18%) (Table 5). The occurrence of more mycotoxins in cereals is affected by high fluctuations in temperatures and rainfall. So, in tropical regions of Southeast Asia with hot and humid weather most of the year, the co-occurrence of AFs and FB_1 was in 96.8% of maize samples *(Siri-anusornsak et al., 2022)*. On the contrary, in Brazil, the co-contamination AFs with FBs in only 7% and 8% of maize grain samples were reported by *Rocha et al. (2009)* and *Moreno et al. (2009)*, respectively.

Table 5. Co-occurrence of aflatoxins (AFs) and fumonisins (FBs) in maize samples

Year	No. of samples with AFs and FBs/ No. of total samples	% co-occurrence
2018	3/13	23.08
2019	2/11	18.18
2020	2/9	22.22
2021	9/14	64.29
2022	5/18	27.78

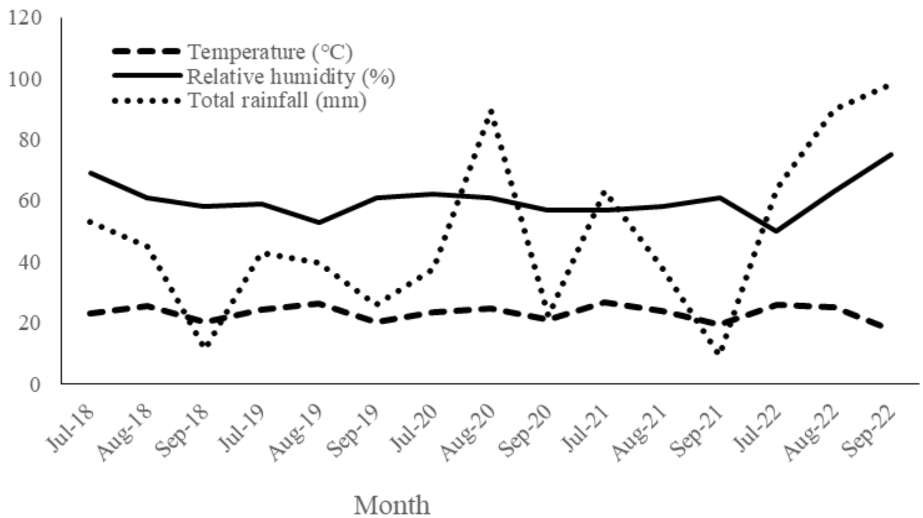
Considering maize grain samples in all investigated years, a statistically insignificant positive correlation between AFs and FBs levels (r = 0.086) was observed (Table 6). As with investigating the contamination of maize hybrids with aflatoxins and fumonisins, *Abbas et al. (2006)* have determined a statistically significant positive correlation between these mycotoxins, indicating that natural *Fusarium* infection did not reduce AFs levels.

Table 6. Correlation between levels of aflatoxins and fumonisins for all maize samples tested

		Aflatoxins	Fumonisins
Aflatoxins	Pearson Correlation	1	0.086
	P value		0.498^{ns}
	Number of samples	65	65
Fumonisins			
	Pearson Correlation	0.086	1
	P value	0.498^{ns}	
	Number of samples	65	65

ns, not significant at p≥0.05

Meteorological data (mean monthly air temperature and relative humidity (RH), and total monthly rainfalls) from July to September in the five years 2018-2022 for the Belgrade area were obtained by the Republic Hydrometeorological Service of Serbia and are shown in Graphic 1.



Graphic 1. Meteorological data from July to September in the five years 2018-2022 (Belgrade area)

In this study, a high percentage of AFs-positive maize samples in 2021 (64.29%) could be explained by the high mean air temperature in July (26.6°C) and fewer total rainfalls in September 2021 (9.4 mm) (Graphic 1). However, the average AFs level of 6.81 μg kg^{-1} in 2021 was below the maximum limit in maize (10 μg kg^{-1}) set by Serbian and EU regulations. Based on previously reported results in Serbia *(Stanković et al., 2011; Krnjaja et al., 2013; Kos et al., 2014; Obradović et al., 2018; Jakšić et al., 2019)*, the natural FBs contamination was an expected occurrence in maize crops due to constant favourable weather conditions for *Fusarium* development. Conversely, higher AFs production in maize was rare in Serbia and depends on specific weather conditions, such as prolonged periods of drought with high night temperatures *(Krnjaja et al., 2013)* and a higher number of days with temperatures above 30°C and 35°C and lower rainfall *(Kos et al., 2013)* which have noticed in 2012. The contamination of maize grains was 50.5% and 18.9% by *A. flavus* and 11.7% and 33.4% by *F. verticillioides* in 2012 and 2013, respectively, according to the reports of *Obradović et al. (2018)*. In addition, *Fusarium* and *Aspergillus* infection of maize grains can be facilitated by injuries of insect European corn borer (ECB) (*Ostrinia nubilalis* Hbn.) *(Tančić Živanov et al., 2019)*. In temperate regions, *F. verticillioides* infection and fumonisin contamination were promoted by ECB *(Blandino et al., 2015)*. Air temperatures and precipitation affected ECB occurrence. There was a significantly positive correlation between mean air temperatures and ECB damages to maize hybrids in Croatia *(Bažok et al., 2020)*. Climate changes contribute to ECB proliferation worldwide *(Eitzinger et al., 2013)*.

Considering average FBs levels in maize samples during five years (2018–2022), the highest FBs level was in 2022 (20310 μg kg^{-1}), followed by 2020 (10980 μg kg^{-1}), 2021 (4950 μg kg^{-1}), 2018 (2910 μg kg^{-1}) and 2019 (2710 μg kg^{-1}). The weather conditions were convenient for *Fusarium* infection during reproductive stages in July 2022, with a mean temperature of 25.9°C and total rainfalls of 63.9 mm. Likewise, ideal weather conditions (optimal temperatures and precipitation) for FBs production were during the grain filling and the physiological maturity maize stages in August and September 2022, with mean air temperatures and total rainfalls of 25°C and 89.7 mm and 18°C and 98 mm, respectively (Graphic 1). Kruskal-Wallis test indicated that there were significant differences in AFs and FBs levels between the investigated years. Similarly, by studying the presence of FBs in harvested maize in five years (2012-2016), *Kos et al. (2013, 2017)* have established the significant effect of climatic factors per investigated years and regions on FBs contamination of maize. *Stanković et al. (2011)* and *Jakšić et al. (2019)* have also stated the significance of the agroecological conditions in Serbia for the natural FBs occurrence in maize. In contrast, by studying the effect of sowing time on FBs levels in two maize genotypes and in two growing seasons (2016-2017) in Serbia, *Krnjaja et al. (2022)* have determined a significant effect of genotype but not of the season on FBs occurrence and high FBs levels. It is noticed that there is a lack of consistency in the expression of resistance of maize genotypes to mycotoxin contamination, depending on environmental conditions. Therefore, identifying and breeding genotypes with stable resistance to both AFs and FBs under different climates and geographic regions should be one of the main preventive measures to mitigate fungal contaminants in maize *(Guo et al., 2017; Barošević et al., 2022)*.

Since weather conditions during the silking, reproductive and maturity maize stages are important prerequisites for AFs and FBs production, in regression analyses, we considered as predictor variables mean of total rainfalls, air temperatures and RH in the July–September period for five years (2018-2022). Positive coefficients (R) between response and predictor variables were determined by multiple enter regression analyses with no significant dependence of predictor variables on AFs level ($p = 0.054$) and highly significant ($p = 0.000$) on FBs level in maize samples. Based on the results of adjusted R squares, levels of AFs and FBs were 11.7% and 28.4%, respectively, determined by predictor variables (Tables 7-8).

Table 7. Results of multiple enter regression model during the five-year season (2018-2022)

Dependent variable	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
AFs level	1	0.342[a]	0.117	0.074	2.39158	1.856
FBs level	1	0.533[a]	0.284	0.249	10.90650	1.831

a. Predictors: (Constant), mean of total rainfall (July-September), mean air temperature (July-September), mean RH (July-September)

Table 8. Results of analyses variance (ANOVA) for predictors (mean of total rainfall (July-September), mean air temperature (July-September) and mean RH (July-September)) and AFs and FBs levels as response (dependent) variables

Dependent variable	Model		Sum of Squares	df	Mean Square	F value	Sig.
AFs level	1	Regression	46.257	3	15.419	2.696	0.054[ns]
		Residual	348.898	61	5.720		
		Total	395.154	64			
FBs level	1	Regression	2882.938	3	960.979	8.079	0.000**
		Residual	7256.059	61	118.9522		
		Total	10138.998	64			

ns – not significant, **significance $p \leq 0.01$

Results of regression analyses indicate that FBs levels in maize grain samples were more affected by climatic conditions in five-year seasons from July to September (2018-2022) than AFs levels. Generally considering, this study confirmed a higher percentage of FBs-positive than AFs-positive maize grain samples and a more common occurrence of FBs than AFs in maize in Serbia, as reported by *Moreno et al. (2009)* in Brazil, *Griessler et al. (2010)* in Southern

Europe, *Akello et al. (2021)* in Zimbabwe, *Cabrera-Meraz (2021)* in Honduras, *Mesterhazy et al. (2022)* in Hungary and *Odjo et al. (2022)* in Mexico and Central America. The temperate climate in Serbia favours the natural occurrence of fumonisin-producing *Fusarium* species in maize crops more than aflatoxin-producing *Aspergillus* species, which require extreme weather conditions such as high temperatures and dry seasons.

Conclusion

Based on the obtained results, it can be concluded that the effect of year was statistically significant on AFs and FBs contamination of maize grain samples. The highest average AFs and FBs levels were in 2021 and 2022, respectively. By regression analyses, weather conditions during July-September of the investigated years had a significant influence on FBs contamination. The percentage of mycotoxin-positive and inappropriate maize samples was not negligible. Hence, the necessity of constant maize monitoring in the agro-ecological conditions of Serbia to find measures to reduce these contaminants in the food chain will always be an actual strategy in the concept of integrated pest management in maize production.

Pojava aflatoksina i fumonizina u uzorcima zrna kukuruza na teritoriji Beograda (R. Srbija) u periodu od 2018. do 2022. godine

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Rezime

Cilj ovih istraživanja bio je da se utvrdi pojava ukupnih aflatoksina (AFs) i fumonizina B grupe (FBs) u 65 uzoraka zrna kukuruza sakupljenih u vreme žetve u 2018. (13 uzoraka), 2019. (11 uzoraka), 2020. (9 uzoraka), 2021. (14 uzoraka) i 2022. godini (18 uzoraka) iz različitih lokaliteta u okolini Beograda. Prosečne koncentracije AFs i FBs u mikotoksin-pozitivnim uzorcima bile su 5,43 i 2910 µg kg^{-1} (2018), 5,28 i 2710 µg kg^{-1} (2019), 2,35 i 10980 µg kg^{-1} (2020), 6,81 i 4950 µg kg^{-1} (2021) i 5,32 i 20310 µg kg^{-1} (2022), respektivno. Združena pojava AFs i FBs ustanovljena je u 23,08% (2018), 18,18% (2019), 22,22% (2020), 64,29% (2021) i 27,78% (2022) uzoraka kukuruza. Maksimalni limiti od 10 µg kg^{-1} za AFs u kukuruzu i 4000 µg kg^{-1} za FBs u neprerađenom kukuruzu, propisani pravilnicima Srbije i Evropske Unije, bili su premašeni za AFs u 14,29% (2021) i 5,56% (2022) ispitivanih uzoraka kukuruza i za FBs u 7,69% (2018), 66,67% (2020), 28,57% (2021) i 41,67% (2022) ispitivanih uzoraka kukuruza. Primenom višestruke linearne regresije ustanovljen je statistički značajan uticaj klimatskih faktora (temperatura i vlažnost vazduha i ukupne padavine) za period jul-septembar u ispitivanim godinama na koncentracije FBs u uzorcima kukuruza. Rezultati ovih istraživanja ukazuju na potrebu stalnog ispitivanja zdravstvenog stanja požnjevenog zrna kukuruza i ocene rizika od potencijalnog prisustva mikotoksina u lancu ishrane kako bi se izbegli štetni efekti na zdravlje ljudi i životinja.

Ključne reči: kukuruz, aflatoksini, fumonizini

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Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[1]

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

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EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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