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Aims and Scope

An international scientific journal **"Veterinarija ir Zootechnika"** since 1924 publishes original research and review papers on all aspects of veterinary medicine, food safety and animal sciences. From 1952 to 1994 journal was published under the title: „Acta of Lithuanian Veterinary Academy". After decision of the Research Council of Lithuania under the auspices of the Government of Republic of Lithuania from year 1995 scientific journal „Veterinarija ir Zootechnika" (Vet Med Zoot) was re-established as the Official Organ of the Veterinary Academy (VA) in collaboration with Veterinary Academy, Veterinary Institute, LVA Animal Science Institute, Lithuanian University of Agriculture and Immunology Institute of Vilnius University.

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CONTENTS

The Effect of Dry Yeast and Folic Acid Treatment on the Reproductive and Physiological Aspects of Quail Stressed with Hydrogen Peroxide

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Keywords: *folic acid, hydrogen peroxide, quail, yeast.*

Abstract. *This study aimed to assess the effect of prebiotics (dry yeast) and folacin (folic acid) on oxidative stress, performance parameters, and blood characteristics in Japanese quails. One-week-old, 180 sexed chicks were randomly distributed into 5 groups with 4 replicates (two replicates/sex). Birds were divided into 5 treatments: 1st – control basal diet (BD); 2nd – H_2O_2 1% in drinking water; 3rd – H_2O_2 + folic acid (F) 5 mg/L in drinking water; 4th – H_2O_2 + yeast (Y) 10% in a basal diet; and 5th – H_2O_2+F+Y. The results showed that hydrogen peroxide treatment caused a notable decrease in the body weight of both sexes, as well as in the relative weights of the female organs, while the weight of some of the male organs increased compared with the control, and all treatments with H_2O_2 caused an increase in the relative weights and the live body weight compared with the peroxide treatment. The peroxide treatment led to a significant decrease in the hematological parameters of both sexes in comparison with the control group, whereas all treatments improved the blood levels compared with the peroxide treatment. The biochemical parameters of both sexes increased with the administration of hydrogen peroxide, particularly at the MDA level, but they improved and returned to the normal condition in the various treatments with H_2O_2, especially in the levels of GSH as opposed to the peroxide treatment. These results indicate that the administration of yeast or folic acid alone or in combination reduces the harmful effects of oxidative stress and improves performance.*

Introduction

Stress increases free radicals by provoking lipid peroxidation in the cell membrane, which then directly releases glucose and lipid metabolism as well as protein catabolism via stress hormone release (Hosseini et al., 2010). Stress may influence negatively the chickens' performance by reducing feed intake, efficiency and weight gain (Odihambe et al., 2006). When oxidative stress occurs, the first line damaged is the intestine, leading to the improper digestion and malabsorption of nutrients, which causes illnesses and occasionally death (Bai et al., 2018).

The antioxidants involve two types: the natural ones such as medicinal grass and processed ones such as vitamins that have a protective role against oxidative free radicals (McDonald et al., 2010). There is worldwide interest in identifying compounds that have antioxidant properties and are pharmacologically effective with low side effects to use in the food industry and preventive medicine (Sati et al., 2010; Aziz et al., 2019).

Since 2006, the European Union Countries (EUC) have planned to use probiotics in broiler chicken rations as a performance enhancer (Castanon, 2007; Kabir, 2009).

Some live yeasts such as S. Cerevisiae and Kluyveromyces Marxianus are probiotics (Gaggia et al., 2010; Kasianenko et al., 2020). Folic acid, also called vitamin B9, is one of B the complex vitamins. The B complex vitamins that help the body convert food into fuel (energy) and folic acid are necessary to transfer donor or acceptor modification to one-carbon units for methylation or protein and DNA synthesis and gene expression. (Dean, 2007; Asaikutti et al., 2016). Folic acid is necessary for the biosynthesis of amino acids and deoxynucleotides required for DNA replication and repair, as well as the methylation of homocysteine to form methionine (Tapiero et al., 2001).

Quails are more resistant to pathogens and have recently attracted interest in the poultry production sector, so reducing stress in poultry remains a topic of concern among scientists and producers. *Coturnix coturnix, Japonica quails* become important livestock especially when it is used in embryological studies; furthermore, a large number of poultry can be kept in a narrow area, where they are easy to handle and have a small body. A limited number of parents can produce a large number of offspring; quails have high egg production; their eggs are also very protein-rich and a good source of riboflavin, iron, phosphorus, and selenium (Devarestti, 2016; Bing, 2022; Sree et

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al., 2016). Poor feed efficiency, feed intake and lower body gain often seen in meat poultry flocks are adverse effects of stress (Fellenberg & Speisky, 2006; Lyasere et al., 2021). The actual study aimed to determine which probiotic and tonic (Vit. B9) can improve the physiological and biochemical parameters in quail stressed with hydrogen peroxide.

Materials and Methods

This work was applied in the animal house of the Veterinary Medicine College at Mosul University. The chicks used were 7 days old, sexed, and then distributed into 5 groups with 4 replicates of 36 birds housed in cages under an artificial lighting program (16 hours / day) at 37° C. The treatments continued for 6 weeks, no vaccination was performed, and birds were fed a commercial ration containing 19.9% crude protein (CP) and 2878 kcal energy (McDonald et al., 2010).

Blood samples were obtained by jugular vein incision at the end of the period, and the collected samples were divided into 2 portions. The first one was kept in containers containing EDTA for blood pictures (RBC, WBC, Hb, PCV, MCV, MCH, and MCHC). The second one was separated directly by centrifuging at 3000 rpm / 10 min to get serum and then was kept at –20° C. After that, it was tested to determine some biochemical parameters, such as TC, TG, HDL, LDL, VLDL, TP and albumen. The GSH and MDA in the serum samples were determined by using commercial kits from the biodiagnostic company (Egypt) in a college research unit, and other parameters of the weight of the organs were obtained directly after bird slaughtering. Using the linear model method, the values were examined for variance (Al-Najjar et al., 2010), and the differences between treatment groups were determined using the Duncan multiple range (Steel et al., 1997).

Treatments were as follows:

1. Birds received a standard diet and were given tap water.
2. Birds received standard food and 1% H_2O_2/L drinking water.
3. Birds received regular food with 1% H_2O_2/L drinking water and 5 mg/L folic acid.
4. Birds received regular food that included dry yeast (10 g/kg ration) and 1% H_2O_2/L drinking water.
5. Birds received standard food of dry yeast (10 g/kg), folic acid (5 mg/L), and 1% H_2O_2/L in drinking water.

Results

Tables 1 and 2 show that administering hydrogen peroxide significantly reduced body weight in both male and female birds. Males also had a significant increase in the relative weight of pancreas and liver tissues, while females had a significant decrease in the relative weights of ovaries, heart, and kidneys compared to the control group ($P < 0.05$). All treatment groups with hydrogen peroxide led to a significant improvement in the weights of the relative organs of the testes, ovaries, kidneys, and heart in both sexes, compared with the hydrogen peroxide group ($P < 0.05$).

Table 1. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on relative organ weights of male Japanese quails

Traits / Treatments	BW g	R. Testis g/100 g BW	L. Testis g/100 g BW	Liver g/100 g BW	Kidney g/100 g BW	Heart g/100 g BW	Pancreas g/100 g BW
Control	207 ± 5.34 a	1.49 ± 0.05 b	1.6 ± 0.04 b	1.42 ± 0.04 c	0.45 ± 0.02 ab	0.83 ± 0.02 c	0.15 ± 0.01 d
H_2O_2	174 ± 1.34 c	1.52 ± 0.02 b	1.77 ± 0.03 b	2.36 ± 0.08 a	0.40 ± 0.01 b	0.85 ± 0.03 c	0.29 ± 0.01 a
H_2O_2+F	186 ± 3.36 b	1.53 ± 0.04 b	1.74 ± 0.04 b	1.65 ± 0.02 b	0.47 ± 0.02 a	1.00 ± 0.03 a	0.23 ± 0.01 b
H_2O_2+Y	191 ± 2.42 b	1.72 ± 0.06 a	2.42 ± 0.11 a	1.6 ± 0.04 b	0.44 ± 0.02 c	0.87 ± 0.02 ab	0.19 ± 0.01 c
H_2O_2+F+Y	206 ± 1.86 a	1.58 ± 0.07 ab	1.77 ± 0.04 B	1.59 ± 0.05 B	0.45 ± 0.01 ab	0.93 ± 0.02 b	0.26 ± 0.02 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 2. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on relative organ weights of female Japanese quails

Traits / Treatments	BW g	Ovary g/100 g BW	Oviduct g/100 g BW	Liver g/100 g BW	Kidney g/100 g BW	Heart g/100 g BW	Pancreas g/100 g BW
Control	233.6 ± 4.05 b	4.13 ± 0.11 b	3.23 ± 0.10 a	2.84 ± 0.08 bc	0.68 ± 0.02 a	0.89 ± 0.01 a	0.25 ± 0.01 ab
H_2O_2	206.5 ± 3.51 d	3.21 ± 0.12 d	3.21 ± 0.07 a	3.03 ± 0.011 ab	0.44 ± 0.03 c	0.81 ± 0.01 b	0.26 ± 0.01 ab
H_2O_2+F	237.6 ± 6.66 c	3.57 ± 0.09 c	3.09 ± 0.04 a	2.65 ± 0.02 cd	0.51 ± 0.01 b	0.81 ± 0.01 b	0.23 ± 0.01 b
H_2O_2+Y	225.0 ± 4.68 a	4.74 ± 0.12 a	3.29 ± 0.08 a	3.13 ± 0.08 a	0.56 ± 0.01 b	0.81 ± 0.02 b	0.28 ± 0.01 a
H_2O_2+F+Y	216.8 ± 5.61 cd	3.31 ± 0.13 cd	3.18 ± 0.01 a	2.47 ± 0.09 d	0.53 ± 0.01 b	0.74 ± 0.01 b	0.28 ± 0.01 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Tables 3 and 4 show the results, which demonstrate the impact on hematological characteristics. When compared with the hydrogen peroxide group, all treatment groups with peroxide improved all blood parameters in both sexes, males and females, whereas the treatment with peroxide significantly decreased all hematological parameters when compared with the control group ($P < 0.05$).

According to Tables 5 and 6, the hydrogen peroxide treatment significantly elevated all the assessed bio-

Table 3. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the blood parameters of male Japanese quails

Traits / Treatments	RBC Cell*10^6	WBC Cell*10^3	Hb g/dL	PCV %	MCH pg	MCHC g/100 mL	MCV fL
Control	4.59 ± 0.05 a	26.86 ± 0.27 c	21.78 ± 0.07 a	42.6 ± 0.48 Ab	47.24 ± 0.24 c	51.25 ± 0.52 a	92.86 ± 1.18 d
H_2O_2	4.00 ± 0.08 b	26.80 ± 0.28 c	17.24 ± 0.35 c	39.9 ± 0.81 c	42.01 ± 0.53 d	42.15 ± 0.67 c	99.76 ± 1.16 c
H_2O_2+F	3.91 ± 0.08 bc	30.08 ± 0.15 b	22.00 ± 0.21 a	43.6 ± 0.54 a	54.91 ± 0.89 b	50.82 ± 0.52 a	110.83 ± 1.45 b
H_2O_2+Y	3.23 ± 0.03 d	33.55 ± 0.21 a	19.71 ± 0.19 b	41.6 ± 0.56 b	60.97 ± 1.2 a	48.02 ± 0.41 b	124.40 ± 2.71 a
H_2O_2+F+Y	3.76 ± 0.6 c	29.39 ± 0.31 b	20.00 ± 0.39 b	41.5 ± 0.22 b	53.11 ± 0.26 b	48.59 ± 0.65 b	110.51 ± 1.84 b

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

chemical parameters in both sexes, especially the MDA levels compared with the control group ($P < 0.05$).

While albumin, total protein, and low-density lipoprotein (LDL) levels were all significantly decreased in all hydrogen peroxide treatment groups and increased significantly in the F+H_2O_2 and F+Y+H_2O_2 groups, respectively, when compared with the control group, all hydrogen peroxide treatment groups significantly increased GSH levels.

Table 4. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the blood parameters of female Japanese quails

Traits / Treatments	RBC Cell*10^6	WBC Cell*10^3	Hb g/dL	PCV %	MCH pg	MCHC g/100 mL	MCV fL
Control	4.41 ± 0.07 a	27.19 ± 0.52 c	20.72 ± 0.30 a	43.2 ± 0.64 a	41.76 ± 1.72 b	48.14 ± 1.39 a	86.46 ± 1.35 b
H_2O_2	3.67 ± 0.12 b	29.86 ± 0.48 b	18.18 ± 0.15 d	39.8 ± 0.41 b	49.57 ± 1.34 a	45.43 ± 0.36 b	109.17 ± 3.10 a
H_2O_2+F	3.89 ± 0.11 b	25.88 ± 0.32 d	19.2 ± 0.17 c	40.2 ± 0.29 b	49.60 ± 1.24 a	49.51 ± 0.64 a	104.24 ± 3.95 a
H_2O_2+Y	3.73 ± 0.11 b	32.30 ± 0.26 a	19.53 ± 0.17 bc	40.89 ± 0.34 b	52.58 ± 1.30 a	47.79 ± 0.59 a	110.35 ± 3.68 a
H_2O_2+F+Y	3.78 ± 0.05 b	28.26 ± 0.23 c	20.08 ± 0.12 b	40.3 ± 0.49 b	53.20 ± 0.67 a	49.88 ± 0.53 a	106.79 ± 1.78 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 5. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the biochemical parameters of male Japanese quails

Traits / Treatments	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	TP g/dL	Albumin g/dL	MDA nmol/mL	GSH nmol/mL
Control	281.7 ± 2.33 a	192.6 ± 1.52 b	63.9 ± 0.88 c	179.29 ± 2.09 a	38.52 ± 0.3 b	3.55 ± 0.06 c	1.24 ± 0.07 c	0.723 ± 0.003 b	1.491 ± 0.004 a
H_2O_2	253.8 ± 1.92 b	286.0 ± 2.3 a	57.4 ± 2.45 d	139.2 ± 3.12 b	57.2 ± 0.46 a	4.26 ± 0.02 a	1.65 ± 0.03 a	0.903 ± 0.002 a	1.027 ± 0.005 e
H_2O_2+F	172.00 ± 3.2 E	122.8 ± 4.0 e	105.6 ± 1.22 A	41.84 ± 2.23 E	24.56 ± 0.8 e	3.40 ± 0.01 d	1.24 ± 0.01 c	0.728 ± 0.002 b	1.397 ± 0.004 c
H_2O_2+Y	188.10 ± 3.3 d	132.6 ± 4.1 d	109.6 ± 1.57 a	51.98 ± 2.31 d	26.52 ± 0.82 d	3.85 ± 0.01 b	1.42 ± 0.03 b	0.713 ± 0.003 c	1.412 ± 0.004 b
H_2O_2+F+Y	238.4 ± 2.6 C	166.6 ± 1.6 c	74.7 ± 0.36 b	130.38 ± 2.53 c	33.32 ± 0.32 c	3.34 ± 0.03 d	1.20 ± 0.01 c	0.732 ± 0.002 b	1.384 ± 0.001 d

Data are shown as mean ± SE. n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 6. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the biochemical parameters of female Japanese quails

Traits / Treatments	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	TP g/dL	Albumin g/dL	MDA nmol/mL	GSH nmol/mL
Control	118.3 ± 0.51 c	247.85 ± 0.83 a	65.60 ± 0.40 c	3.13 ± 0.20 bc	49.57 ± 0.16 a	4.10 ± 0.02 c	1.50 ± 0.02 b	0.757 ± 0.01 bc	1.567 ± 0.015 a
H_2O_2	130.6 ± 0.89 a	248.05 ± 0.85 a	79.00 ± 0.81 a	1.99 ± 0.23 b	49.61 ± 0.17 a	4.24 ± 0.06 c	1.51 ± 0.03 b	0.944 ± 0.006 a	1.011 ± 0.004 c
H_2O_2+F	109.7 ± 0.61 d	247.70 ± 0.80 a	54.80 ± 0.41 d	5.39 ± 0.59 a	49.51 ± 0.15 a	4.85 ± 0.11 a	1.75 ± 0.07 a	0.739 ± 0.002 c	1.500 ± 0.002 b
H_2O_2+Y	110.7 ± 1.08 d	249.4 ± 0.24 a	56.90 ± 0.97 d	3.92 ± 0.28 b	49.88 ± 0.04 a	3.42 ± 0.04 d	1.30 ± 0.02 c	0.755 ± 0.002 bc	1.495 ± 0.003 b
H_2H_2+F+Y	122.00 ± 1.24 b	248.00 ± 1.51 a	70.70 ± 1.15 b	2.39 ± 0.21 cd	49.6 ± 0.30 a	4.54 ± 0.08 b	1.74 ± 0.04 a	0.772 ± 0.003 b	1.493 ± 0.003 b

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Discussion

The results of treatment with hydrogen peroxide H_2O_2 showed a significant decrease in body weights for both sexes (Tables 1, 2), as well as a decrease in the relative weights of vital organs, especially among females (Table 2). This is consistent with what was indicated by Rajkumar et al. (2015) and Jiu et al. (2019) in the parents of broilers and may be attributed to stress providing an opportunity for effective oxygen species to attack the fats that form cell membranes and oxidize them to produce what is known as lipid peroxides, especially MDA as well as other compounds. This series of oxidative reactions negatively encouraged the ability of birds to construct GSH in the tissue, and due to the insufficient availability of the external source (low feed consumption) that promoted the construction of tissue GSH, the matter worsened on various other vital tissues, while the results of yeast and folic acid indicate their ability to provide tissue protection or reduce its action by promoting the antioxidant status and reducing the role of disruptive stress. It is believed that this is due to their role in promoting the construction of tissue glycogen by activating the pentose shunt, which is the main provider of NADPH needed to re-reduce the oxidized glutathione GSSG to a reduced form GSH, and inhibiting the process of gluconeogenesis that destroys body proteins; this is consistent with the results of El-Husseiny et al. (2007) and Li et al. (2021). They claimed that different levels of folic acid (0.5, 0.75, and 1.0 mg/kg) improved performance metrics in broiler chicks fed diets containing methionine (0.45%). Zhang et al. (2021) concluded that folic acid at different levels (0.75, 1.5, and 3 mg/kg) in the diet could significantly improve the average daily gain of broilers. The Association of Official Analysis of Chemists (AOAC, 2000) reported that folic acid is needed for animals with greater production rates or growth because it plays a role in amino acid and DNA metabolism and methylation of the homocysteine to form methionine plays a role in reducing corticosterone levels and then reducing stress (Shareef & Al-Dabbagh, 2009; Whisner & Castillo, 2018). Variations in gut flora and environmental factors may confuse the variable effect of yeast (SAC).

The results for both genders in Tables (Bai et al., 2018; McDonald et al., 2010) about hematological parameters are in line with those obtained by Rasheed and Al-Nuaimmi (2022) and Li and Gatlin (2003). When folic acid was used instead of the oxidative stress group, the adult quails' total WBC count increased significantly. This difference may be explained by the vitamin's increased antioxidant effect, which boosts the activity of phagocytes. However, it was discovered that certain hematological parameters were significantly improved by folic acid therapy (Mohamed et al., 2013). Leukocytosis may be indicative of higher activity of innate immune responses because yeast cell walls contain chitin manner and glucan that have triggered the immune stimulation, these findings are consistent with those

of Reda et al. (2021) and Abdul-Majeed et al. (2021), who observed similar effects when yeast was added to the diet of Japanese quail.

The results provided in the Tables (Sati et al., 2010; Aziz et al., 2019) showing the role of hydrogen peroxide on the biochemical characteristics in both sexes indicate significantly rising levels of cholesterol and triglycerides. This increase in the above criteria may be attributed to the role of stress in encouraging the gluconeogenesis process, which works to liberate glucose from non-carbohydrate sources and raise fat levels through its role in the occurrence of disorders in the metabolic or digestive processes of fat in the stomach and intestines, which is in line with Mousa's (2021) findings, while other treatments with hydrogen peroxide showed an improvement in biochemical parameters, especially in folic acid, which is in line with the results obtained by Poonuru et al. (2011). In chicks, it plays a role in encouraging the methylation process necessary for the production of methionine, which has a role in activating the work of somatic cells, including pancreatic beta cells, which in turn stimulate the secretion of insulin, working indirectly through synthesis of the enzyme lipoprotein lipase (Abdul-Azeem, 2007).

The increase in anaerobic and cellulolytic bacteria that occurred when yeast was added to the experimental diet may have contributed to the positive effects of the dietary supplement on plasma total protein, albumin and total lipid. This in turn contributes to improved lactate utilization and a more stable PH of the gut, which improves nutrient digestibility and growth performance (Gao et al., 2008).

Conclusion

According to the study findings, giving dry yeast and/or folic acid to quails of both sexes that have been exposed to H_2O_2 stress has a positive impact on their hematological efficiency and aspects.

Highlights

1. The folic acid and dry yeast or a combination lessen the stress effects by improving the biochemical aspects in both sexes of quails.
2. Both above materials improved the body metabolism that reflected on body and vital organ weights by decreasing the harmful stress.

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Conflict of interest

There are no conflicts of interest involving the publication of this work, according to the authors.

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Role of Melatonin in Attenuation of Vascular Ang 1–7 Reactivity *via* Oxidative Stress Enzymes and PI_3K/AKT/eNOS Signalling Pathways in Induced Diabetic Rats

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Keywords: *Melatonin, Ang 1–7, vascular tone, oxidative stress enzymes, Mas receptor.*

Abstract. *Diabetes mellitus (DM) is considered as the main complication of the cardiovascular system leading to vascular endothelial dysfunction (VED). Besides, melatonin (MEL) has been known to improve the vascular tone directly or indirectly with MEL receptors (MT_1R and MT_2R) and antioxidant properties, respectively. The rings were extracted from three groups including non-diabetes (non-DM), streptozotocin induced diabetes (STZ-induced DM) and STZ-induced DM treated with MEL (DM+MEL) in male albino rats. The experimental procedure includes thoracic aortic vascular reactivity of angiotensin 1–7 (Ang 1–7) and histological examination. The vascular reactivity was conducted across eight distinct groups, encompassing RO-31-8220 (5 μM), protein kinase C (PKC) inhibitor, Apocyanin [(APO, 10 micromolar (μM)], the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, rotenone (ROT 50 μM), mitochondrial complex I electron transport chain inhibitor, oxypurinol (OXY, 100 μM), xanthine oxidase inhibitor, PI-3065 (1 μM), phosphoinositide 3-kinases (PI_3K) inhibitor, Ipatasertib (1 μM), protein kinase B (AKT) inhibitor, A779 (1 μM), the Mas receptor blocker and N(ω)-nitro-L-arginine methyl ester (L-NAME 200 μM), the nitric oxide (NO) inhibitor pre-incubation. However, it is worth noting that the pre-incubation with OXY resulted in a notably significant rightward shift in this response. Conversely, in the STZ-induced DM group, there was a notable significant rightward shift observed in response to each of APO, ROT, and OXY. MEL appeared to regulate the vascular tone within Ang 1–7 modulation in STZ-induced DM rats. Therefore, MEL could offer many vascular benefits within Ang 1–7 under diabetic condition.*

Introduction

The presence of DM is associated with adverse cardiovascular implications (Aboalgasm et al., 2021). It has been demonstrated that cardiovascular complexities represent a major mortality factor under DM consequences (Beckman and Creager, 2016). Moreover, DM interferes with the protective mechanisms of the cardiovascular system, resulting in a compromised defence system (Chien et al., 2020). Furthermore, the compromised state can lead to dysfunction in the vascular endothelial cells (VECs), marked by a decrease in NO levels and an increase in vasoconstrictors (Sena et al., 2013). Moreover, there is substantial evidence supporting the notion that DM contributes to an elevated production of reactive oxygen species (ROS) in diverse body components, such as VECs, vascular smooth muscle cells (VSMCs), and even within the mitochondria (Tan et al., 2022). Moreover, the elevated glucose levels can serve as a stimulus for the emission of ROS from various origins, including the mitochondrial electron transport chain, NADPH oxidases, xanthine oxidase, and arachidonic acid pathways. Consequently, this may result in the disruption of nitric oxide synthases (eNOS) coupling under such circumstances (Kayama et al., 2015).

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Regarding the build-up of ROS, several studies have indicated that an increased presence of these molecules seems to diminish the availability of NO, potentially leading to intracellular inflammation and apoptosis (Paneni et al., 2013). The renin-angiotensin system (RAS) serves a crucial regulatory function in preserving blood pressure and electrolyte balance within the body (Paz Ocaranza et al., 2020). Specifically, the RAS activity increases with the presence of pathological conditions (Forrester et al., 2018). Initially, renin catalyses the cleavage of angiotensinogen to produce angiotensin 1–10 (Ang 1–10); then, Ang 1–10 is further converted into angiotensin 1–8 (Ang 1–8) through the involvement of angiotensin-converting enzyme (ACE) (Fountain et al., 2023). At the same time, angiotensin-converting enzyme 2 (ACE_2) can cleave both of Ang 1–8 and Ang 1–10 to produce Ang 1–7 and angiotensin 1–9 (Ang 1–9), respectively (Bosso et al., 2020). In recent years, the Ang 1–7 / ACE_2 / MasR axis has been recognised as a critical signalling pathway that neutralizes the vasoconstricting action of the renin / ACE / ANG 1–8 pathway (Tamanna et al., 2021). In addition, the activation of MasR by Ang 1–7 action mostly appears to improve vascular endothelial function *via* reducing ROS production, enhancing endothelial nitric oxide synthase enzyme (eNOS) activity and attenuating NADPH oxidase (Xie et al., 2022). Furthermore, located within cells, PI_3K triggers

signalling cascades that regulate cellular survival, growth, mobility, differentiation, and the modulation of genetic information, thus playing a central role in the progression and development of atherosclerosis (Zhao et al., 2021). Differently, MEL is primarily synthesized by the pineal gland (Karamitri and Jockers, 2019). Many extensive researches have been conducted to explore its significant effects on both the vascular tone and endothelial function (Tobeiha et al., 2022). Particularly, MEL exerts signals directly *via* both of MT_1R and MT_2R in the thoracic aorta (Molcan et al., 2021). The activation of these receptors in VECs is associated with increased NO production as well as vasorelaxation and improved endothelial function (Nikolaev et al., 2021). Correspondingly, MEL could play a role in maintaining proper vascular dilation and regulating blood flow (Ozkalayci et al., 2021). Regarding vasodilation, MT_2R in the VSMCs of the thoracic aorta has been implicated in controlling vascular contractility and inhibiting vasoconstriction, potentially influencing overall vascular tone and blood pressure regulation (Datta et al., 2021). Furthermore, MEL has free radical scavenging, antioxidant, and anti-inflammatory properties, along with a protective effect against cardiovascular disease (Chitimus et al., 2020). Conversely, it has been demonstrated that MEL can increase the presence of the MasR in cardiovascular tissues (Lissoni et al., 2021). Nevertheless, there is an on-going debate regarding the cumulative evidence of MEL's impact on the modulation of Ang 1–7 through oxidative stress enzymes and MasR signalling pathways. The current research seeks to examine how MEL affects the vascular response of the aorta to vasodilation induced by Ang 1–7, both in the presence and absence of RO-31-8220, APO, ROT, OXY, PI-3065, Ipatasertib, A779 and L-NAME pre-incubation in STZ-induced DM rats and non-DM rats.

Materials and Methods

Chemicals

Angiotensin 1–7, RO-31-8220, APO, ROT, OXY, PI-3065, Ipatasertib, A779, L-NAME and STZ were purchased from Sigma Aldrich company (USA).

Animals

The recent research utilized male albino rats (*Rattus norvegicus*) with a body weight (B. wt) ranging from 250 to 300 grams. These rats were bred and housed in the animal house of the Department of Biology at the College of Science, Salahaddin University-Erbil, Iraq. They were allowed to acclimate to standard environmental conditions, which included a temperature of 23 ± 2°C and a 12-hour light / 12-hour dark cycle (with lights on from 06:00 to 18:00). The rats had continuous access to both tap water and food, which was available 24 hours a day. Ethical approval for the study was obtained from the Animal Research Ethics Committee associated with the College of Science at Salahaddin University-Erbil, Erbil, Iraq. This approval, with the reference number 2636, was granted on August 7, 2022.

The present study involved 74 rats randomly and divided into three main groups: non-DM with 34 rats, STZ-induced DM with 18 rats, and STZ-induced DM treated with MEL with 22 rats. The study was commenced on May 6, 2021, and concluded on July 12, 2022. The research was conducted over approximately three distinct phases. The first phase spanned three months and involved the care of rats. Subsequently, diabetes was induced using STZ, and MEL injections were administered daily. The second phase included the assessment of vascular reactivity to Ang 1–7. Finally, the third phase, which lasted about one month, was dedicated to the histological examination.

Diabetes mellitus type 1 induction

To induce diabetes in the rats, about 40 male albino rats received an intraperitoneal (i.p.) injection of 50 mg/kg B.wt of STZ (Mostafavinia et al., 2016). The STZ was dissolved in sodium citrate buffer with a pH of 4.5. After the STZ injection, the rats were given access to a drinking solution containing 5% dextrose for a period of 24 hours. The presence of diabetes was confirmed after 72 hours by assessing the rats' blood glucose levels through the tail blood sample. Diabetes was considered established when the blood glucose levels exceeded 250 mg/dL, and this measurement was made 72 hours after the STZ injection.

Melatonin dose preparation

Melatonin tablets, specifically Melaplan 10 mg (PLANTE PHARMA, Poland), were dissolved in sterilized distilled water containing 1% ethanol. This dissolution resulted in a MEL solution with a concentration of approximately 150 mg/mL. Following 14 days after inducing DM in the rats using STZ, the rats were subjected to treatment. In this treatment, the administration of the MEL solution was carried out orally through gavage at a daily dosage of 30 mg/kg B.wt for a continuous 14 executive days.

Preparation of rat aortic rings

Following anaesthesia with an i.p. injection of combined 90 mg/kg B.wt ketamine and 10 mg/kg B.wt xylazine, the chests of the animals were opened using a midline incision. This procedure aimed to isolate the thoracic aorta from the aortic arches. A total of 294 aortic rings were prepared from 40 rats of non-DM, SZT-induced DM and STZ-induced DM treated with MEL groups. These rings were carefully removed and immediately placed in a Petri dish filled with cold Krebs-Henseleit buffer solution (KHBS) containing 122 mM NaCl, 4.7 mM KCl, 15.5 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.0 mM $CaCl_2$, and 11.5 mM D-glucose., with a pH of 7.4. The excess surrounding tissues were removed, and then, four aortic rings, each approximately 3 mm in length, were obtained for further experimentation.

Vascular reactivity measurements

To evaluate vascular reactivity, the previously

prepared aortic rings were horizontally suspended using L-shaped stainless-steel hooks within a 5 mL organ bath vessel (Automatic organ bath, Panlab Harvard apparatus, USA) filled with KHBS. The bath solution was kept at a constant temperature of 37°C and continuously oxygenated with a gas mixture of approximately 95% oxygen and 5% carbon dioxide. The aortic rings were initially subjected to a basal tension of 2 gm for duration of 60 minutes. Subsequently, the rings were gradually stretched using KHBS and allowed to equilibrate for approximately 60 minutes. During this equilibration period, the rings were periodically washed and adjusted every 15 minutes to maintain a maximum stable constriction. To assess the functional integrity of the prepared aortic segments, a solution containing 60 mM KCl was employed. Following this, the endothelial integrity was evaluated by exposing the aortic rings to a solution of 1 µM acetylcholine in rings that had been pre-contracted with 1 µM phenylephrine (PE). Once these assessments were completed, such prepared rings were ready for the evaluation of the changes in the dose response curve (DRC) of Ang 1–7 induced aortic dilation, allowing for the measurement of how Ang 1–7 affected the dilation of the aortic rings.

Experimental design

The current study included nine experiments (experiments I to VIII), collectively addressing vascular reactivity, involving the assessment of DRC triggered by Ang 1–7 across a concentration range from $5x10^{\wedge-12}$ to $10^{\wedge-6}$ µM. These experiments were conducted in non-DM rats, STZ-induced DM rats, and rats with STZ-induced DM treated with 300 mg/kg body weight of MEL. Each vascular reactivity experiment (I to VIII) included pre-incubation for 20 minutes with specific blockers or inhibitors or without blockers or inhibitors (control group) as following: Experiment I (vascular reactivity): RO-31-8220 (5 µM), a PKC inhibitor; Experiment II (vascular reactivity): APO (10 µM), a NADPH oxidase inhibitor; Experiment III (vascular reactivity): ROT (50 µM), a mitochondrial complex I electron transport chain inhibitor; Experiment IV (vascular reactivity): OXY (100 µM), a xanthine oxidase inhibitor; Experiment V (vascular reactivity): PI-3065 (1 µM), a PI3K inhibitor; Experiment VI (vascular reactivity): Ipatasertib (1 µM), an AKT inhibitor; Experiment VII (vascular reactivity): A779 (1 µM), a MasR blocker; and Experiment VIII (vascular reactivity): L-NAME (200 µM), an NO inhibitor. Meanwhile, experiment IX focused on histological examination of thoracic aortae of non-DM (n = 17), STZ-induced DM (n = 13), and STZ-induced DM treated with 300 mg/kg B. wt of MEL (n = 19). The isolated tissues were fixed in a 10% buffered formo-saline solution and subsequently embedded in paraffin for quantitative histological analysis. Hematoxylin-eosin (H and E) staining was employed for microscopic analysis, which included the counting of smooth muscle cells (SMCs) nuclei and measurement of tunica media thickness (TM). The analysis was performed in a double-blinded manner using ImageJ software version 1.8.0.

Statistical analysis

The study reported various parameters, including maximal effect (Emax), drug potency (pD_2, expressed as $-logIC_{50}$), dAUC% (difference area under the curve percentage), TM thickness, and SMCs nuclei count. These values are present as means and standard errors of the mean (SEM) to distinguish the impact of inhibitors and blockers on vascular responses to Ang 1–7 in aortic segments from non-DM, STZ-induced DM, and STZ-induced DM treated with MEL. The data were analysed using one-way analysis of variance (ANOVA) to compare TM thickness, SMCs nuclei count, Emax, and pD_2 among the studied groups, and the results were displayed as figures and tables. Additionally, two-way ANOVA was employed to compare the groups using DRC, followed by the Dunnett post hoc test. Furthermore, the Student *t* test was employed to compare pD_2 values of the groups against the control group, and this comparison was illustrated in figures. Finally, the level of $P < 0.05$ was employed as the criterion for establishing the statistical significance level in the study.

Results

Effect of melatonin on the vasodilatory response to Ang 1–7 via PKC activity

The isolated aortic rings were pre-incubated by RO-31-8220 (PKC inhibitor) revealing a non-significant difference of a vasodilatory response triggered by Ang 1–7 in the non-DM group as compared with the control group (Figure 1A). On the other hand, the aortic rings of STZ-induced DM rats showed a slight alteration (Figure 1B). Conversely, in diabetic rats treated with MEL, the response was slightly changed, while a significant declination in potency ($P = 0.0067$) occurred as compared with the control rats (Figure 1C). Likewise, the level of dAUC% in STZ-induced DM rats treated with MEL exerted a significant decrease ($P < 0.05$) as compared to the STZ-induced DM rats (Fig. 1D).

Effect of melatonin on the vasodilatory response to Ang 1–7 via NADPH oxidase activity

The aortic rings pre-incubated with APO (NADPH oxidase inhibitor) lead to non-significant changes of the Ang 1–7 effect in non-DM rats. However, the potency was decreased significantly ($P = 0.0003$) as compared with the control (Figure 2A). Conversely, in the STZ-induced DM, the rings showed a significant rightward shift in the vasodilatory response induced by Ang 1–7 at mentioned doses, but the potency remained unchanged as compared with the control group (Figure 2B). On the other hand, in STZ-induced DM treated with MEL, the vasodilatory response induced by Ang 1–7 showed a significant decrease ($P < 0.05$) at the 10^{-6} dose,

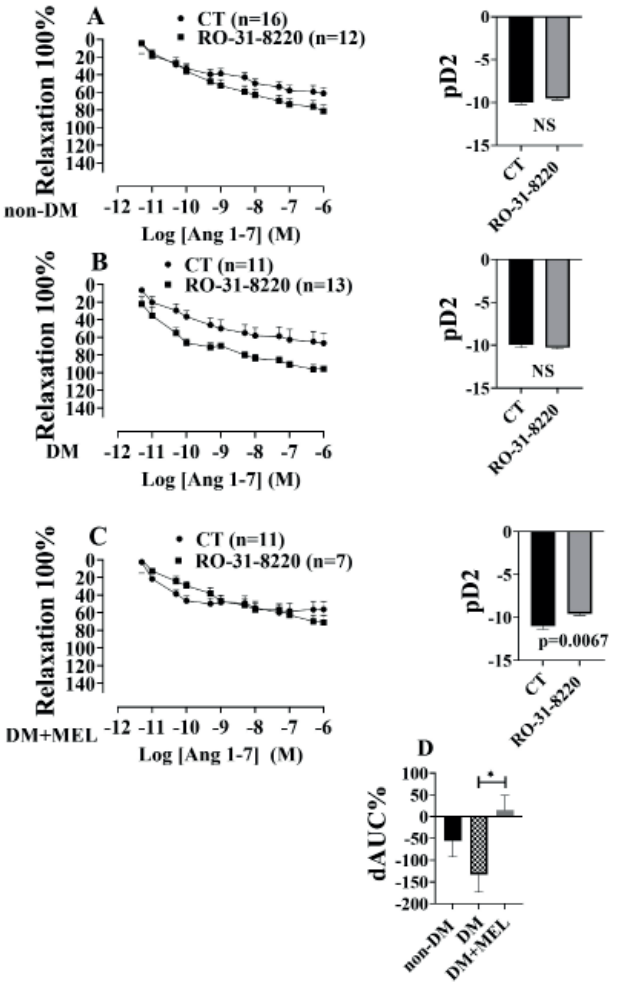

*Fig.1 A*ng 1–7 DRC of aortic rings pre-contracted with PE (1 µM) in the presence of RO-31-8220 (5 µM). The data points in the study are represented as the mean values ± SEM. (CT, control; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD_2, the potency of Ang 1–7; dAUC %, the percentage of difference area under curve; n, sample size). [* $P < 0.05$].

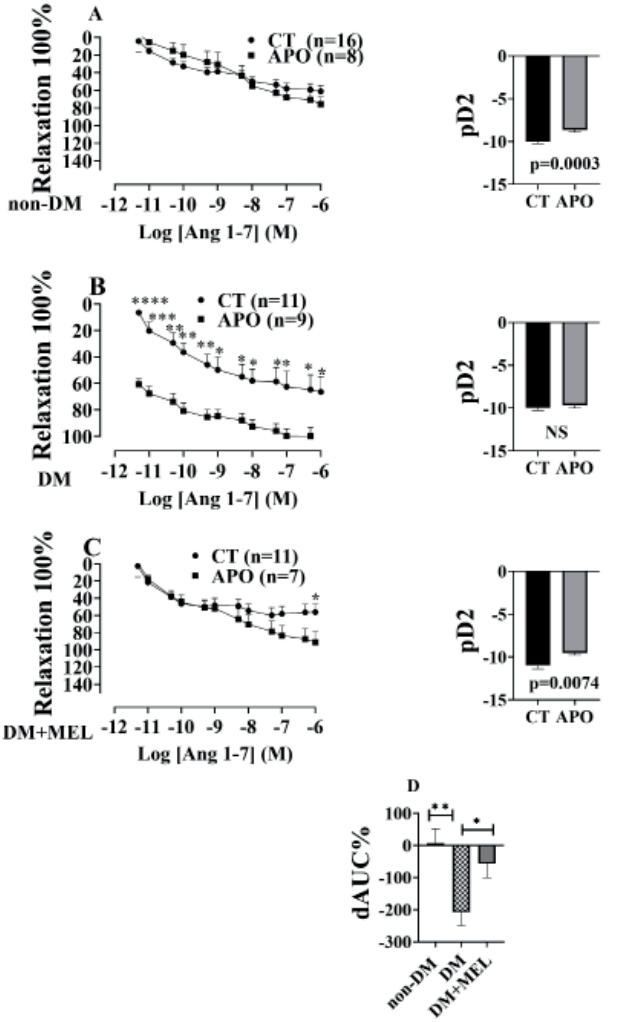

Fig. 2. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 µM) in the presence of APO (10 µM). The data points in the study are represented as the mean values ± SEM. (CT, control; APO, apocyanine; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of difference area under curve). [*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$].

followed by a significant ($P = 0.0067$) decrease in the potency of Ang1–7 as compared with the control (Figure 2C). Additionally, the dAUC% of such an inhibitor was increased significantly ($P < 0.01$) in the STZ-induced DM group as compared with the non-DM group. However, the dAUC% was declined significantly ($P < 0.05$) in the diabetic group that was administered with MEL as compared with the STZ-induced DM group (Figure 2D).

Effect of melatonin on the vasodilatory response to Ang 1–7 via mitochondrial activity

To investigate whether the complex I electron transport chain of mitochondria has roles in the vascular response to Ang 1–7, ROT was pre-incubated with the aortic rings. In the non-DM group, there were no significant differences observed in the vasodilatory response induced by Ang 1–7, and Ang 1–7 potency remained unchanged (Figure 3A). While diabetes rings exhibited a notable reduction to Ang 1–7 activity, there were no statistically significant alterations in Ang 1–7 potency (Figure 3B). Nonetheless, when isolated rings were subjected to MEL treatment, the Ang 1–7 impact was alleviated through significant ($P = 0.0137$) enhancement in the effectiveness of Ang 1–7 as compared with the control group (Figure 3C). Furthermore, the pre-incubation with ROT caused a significant ($P < 0.05$) increase in dAUC% in the STZ-induced DM group as compared with the control rats. Conversely, under MEL influence, the dAUC% was restored in a significant ($P < 0.01$) fashion as compared with the STZ-induced DM (Figure 3D).

Effect of melatonin on the vasodilatory response to Ang 1–7 via the xanthine oxidase activity

To assess the impact of xanthine oxidase on the vascular response to Ang 1–7, OXY (xanthine oxidase inhibitor) was pre-incubated. In the non-DM group, the vasodilatory response induced by Ang 1–7 exhibited a significant decrease at doses ranging between $5x10^{-7}$ to 10^{-6}, followed by a highly significant ($P = 0.001$) elevation in potency as compared with the control group (Figure 4A). In contrast, the vascular response of STZ-induced DM rings was declined significantly at doses ranging from 10^{-11} to 10^{-6} as compared with the control group along with non-significant changes in potency (Figure 4B). Conversely, in the diabetic condition and MEL administration, the previous effect was restored slightly as compared with the control group, while the potency remained unchanged as well (Figure 4C). Ultimately, the dAUC% value of OXY pre-incubation showed a dramatic ($P < 0.01$) elevation in the STZ-induced DM rats as compared with the non-DM rats. However, the diabetic rings treated with MEL restored diabetic changes significantly ($P < 0.01$) as compared with the diabetic group.

Effect of melatonin on Ang 1–7 Emax via PKC and oxidative stress enzymes activity

Table 1 shows the multiple comparison of the maximal response to the Ang 1–7 effect on isolated aortic rings with or without MEL administration and inhibitors. The control maximal responses to Ang 1–7 was increased significantly ($P = 0.0048$) in STZ-DM groups compared with the control of non-DM aortic rings. On the other hand, the presence of RO-31-8220 produced a significant ($P = 0.0313$) declination in STZ-induced DM treated with MEL against STZ-

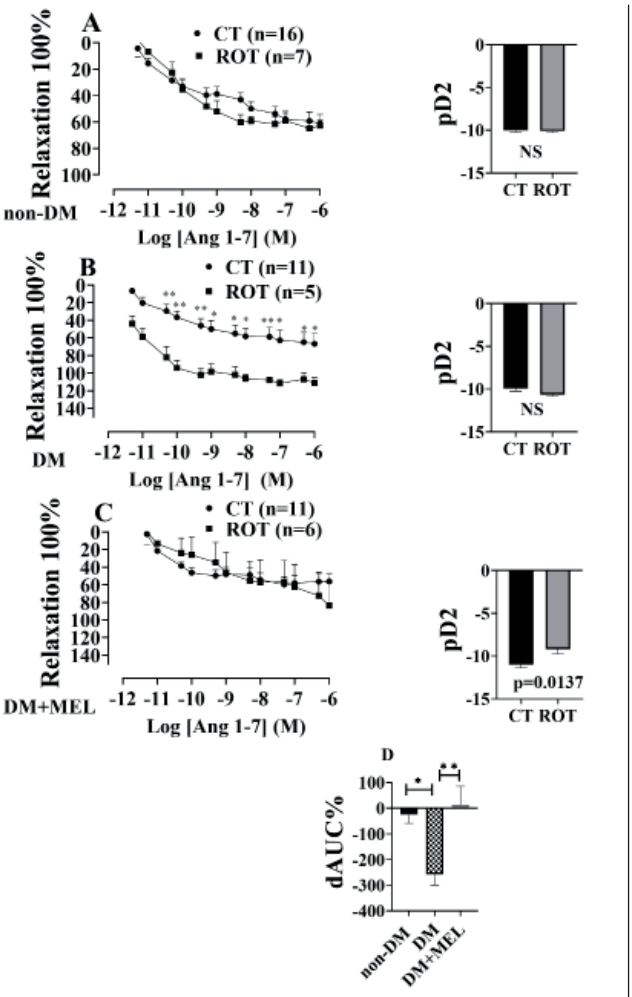

Fig. 3. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 μM) in the presence of ROT (50 μM). The data points in the study are represented as the mean values ± SEM. (CT, control; ROT, rotenone; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of difference area under curve; n, sample size). [*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$].

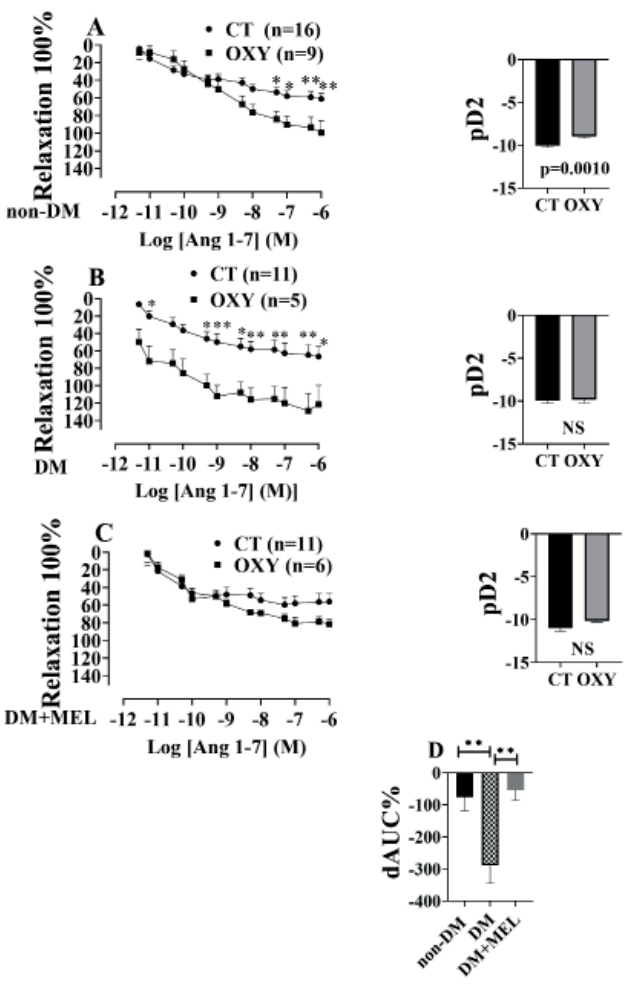

Fig. 4. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 μM) in the presence of OXY (100 μM). The data points in the study are represented as the mean values ± SEM. (CT, control; OXY, oxypurinol; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of difference area under curve; n, sample size). [*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$].

induced DM rats. Meanwhile, APO mediated slight changes between all compared groups. In contrast, the presence of ROT in that response led to a significant ($P = 0.0437$) increase in diabetic rats treated with MEL as compared with the STZ-induced DM group. Furthermore, the slight difference occurred in the presence of OXY in the studied groups.

Effect of melatonin on Ang 1–7 potency via PKC and oxidative stress enzymes activity

Table 2 shows the multiple comparisons of Ang 1–7 potency with or without MEL administration and applied inhibitors. The Ang 1–7 potency produced slight changes of control groups in all comparison groups. In contrast, the Ang 1–7 potency in the presence of RO-31-8220 was increased dramatically ($P = 0.0133$) in STZ-induced DM as compared with the non-DM group, while the selected parameter was decreased significantly ($P = 0.0300$) in STZ-induced DM treated with MEL as compared with the STZ-induced DM group. Similarly, the pre-incubation aortic ring with APO exhibited a significant ($P = 0.0211$) declination of potency in STZ-induced DM as compared with the DM group. On the other hand, the multiple comparison of ROT pre-incubation revealed a significant ($P = 0.0220$) decrease of Ang 1–7 potency in diabetic rats treated with MEL from the diabetic group. Additionally, in presence of OXY, the Ang 1–7 potency of isolated rings was also increased significantly ($P = 0.0082$) in STZ-induced DM treated with MEL as compared with the non-DM group.

Effect of melatonin on the vasodilatory response to Ang 1–7 via the PI_3K signalling pathway

In the presence of PI-3065 (PI_3K inhibitor), the maximal vasodilatory response induced by Ang 1–7 and Ang 1–7 potency showed non-significant changes in the non-DM group as compared with the control group (Figure 5A). On the other hand, the presence of PI-3065 produced a slight increase in the response with unchanged potency as compared with the control group (Figure 5B). Correspondingly, the STZ-induced DM treated with MEL also produced slight changes of the vasodilatory response induced by Ang 1–7 with unchanged potency as compared with the control groups (Figure 5C). Besides, the dAUC% exhibited no changes among the studied groups (Figure 5D).

Table 1. The Emax value for Ang 1–7 reactivity *via* PKC enzyme and NADPH oxidase activity

Groups	Emax (PE)			Multiple Comparison
	non-DM (A)	DM (B)	DM+MEL ©	
CT	50.55 ± 8.922	66.46 ± 11.53	96.00 ± 8.000	(A vs. C) $P < 0.0048$
RO-31-8220	81.20 ± 6.697	95.54 ± 2.909	70.79 ± 7.761	(B vs. C) $P < 0.0313$
APO	75.60 ± 8.018	102.9 ± 6.283	91.02 ± 11.93	NS
ROT	62.77 ± 4.304	111.2 ± 6.174	83.49 ± 24.84	(A vs. B) $P < 0.0437$
OXY	99.45 ± 13.59	121.4 ± 21.78	81.6 ± 5.472	NS

Data are presented as mean ± SEM. One-way ANOVA was employed to analyse the studied groups followed by the Tukey test as post hoc. [Emax, maximum response; n, sample size, CT; control; MEL, melatonin; APO, apocyanin; OXY, oxypurinol; ROT, rotenone; non-DM, non-diabetes mellitus; DM, STZ-induced diabetes mellitus; *P*, probability value; PE, phenylephrine; NS, non-significant difference; vs, versus].

Table 2. The potency value for Angiotensin 1–7 reactivity *via* PKC enzyme and NADPH oxidase activity

Groups	pD_2			Multiple Comparison
	Non-DM (A)	DM (B)	DM+MEL (C)	
CT	–10.03 ± 0.207	–9.947 ± 0.305	–11.00 ± 0.399	NS
RO-31-8220	–9.516 ±0.180	–10.27 ± 0.159	–9.561 ± 0.190	$P < 0.0133$ (A vs. B)
				$P < 0.0300$ (B vs. C)
APO	–8.648 ± 0.249	–9.688 ± 0.292	–9.520 ± 0.249	$P < 0.0211$ (A vs. B)
ROT	–10.12 ±0.172	–10.64 ± 0.250	–9.202 ± 0.566	$P < 0.0220$ (B vs. C)
OXY	–8.965 ± 0.179	–9.831 ± 0.416	–10.14 ± 0.145	$P < 0.0082$ (A vs. C)

Data are presented as mean ± SEM. One-way ANOVA was employed to analyse the studied groups followed by the Tukey test as post hoc. [pD_2, Ang1–7 potency; n, sample size, CT; control; MEL, melatonin; APO, apocyanin; OXY, oxypurinol; ROT, rotenone; non-DM, non-diabetes mellitus; DM, STZ-induced diabetes mellitus; *P*, probability value; NS, non-significant difference; vs, versus].

Effect of melatonin on the vasodilatory response to Ang 1–7 via the AKT signalling pathway

The aortic rings were pre-incubated by Ipatasertib (AKT inhibitor), showed slight changes in the maximal vasodilatory response induced by Ang 1–7 and potency in the non-DM group as compared with the control group (Figure 6A). Likewise, that response was changed slightly in the STZ-induced DM group with no changes in potency as compared with the control group (Figure 6B). Similarly, the STZ-induced DM treated with MEL also produced slight changes in the vasodilatory response induced by Ang 1–7 with no changes in its potency against the control group (Figure 6C). Additionally, the dAUC% showed slight changes in all studied groups (Figure 6 D).

Effect of melatonin on the vasodilatory response to Ang 1-7 via Mas receptor

The maximum vasodilatory response caused by Ang 1–7 revealed slight changes of isolated aortic rings pre-incubated with A779 in the non-DM group as compared with the control. However, the Ang 1–7 effectiveness produced a significant decrease ($P = 0.0001$) in the diabetic group as compared with the control group (Figure 7A). Similarly, under the STZ effect, the vasodilatory response triggered by Ang 1–7 showed a significant decrease ($P = 0.002$) in the potency as compared with the control groups (Figure 7B). In contrast, under the diabetic condition with MEL treatment, the significant decrease ($P < 0.05$) of the vasodilatory response occurred at 10^{-7}, $5x10^{-7}$, and 10^{-6} concentration, but the potency of Ang 1–7 remained unchanged as compared with the control group (Figure 7C). Conversely, the dAUC% showed slight changes in all the studied groups (Figure 7D) as well.

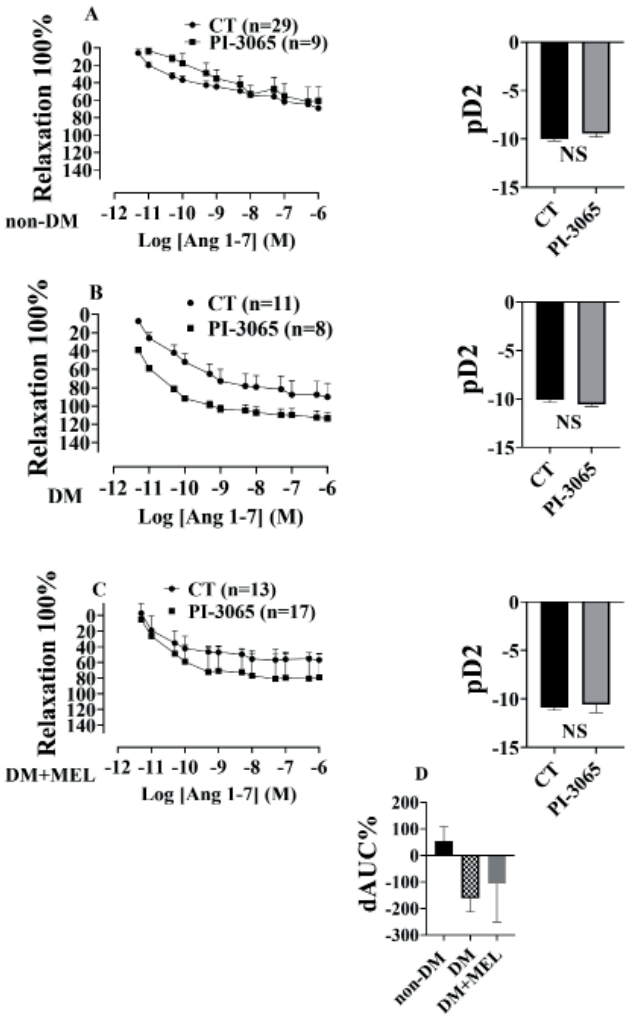

Fig. 5. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 µM) in the presence of PI-3065 (1 µM). The data points in the study are represented as the mean values ± SEM. (CT, control; PI-3065, PI_3K inhibitor; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL).

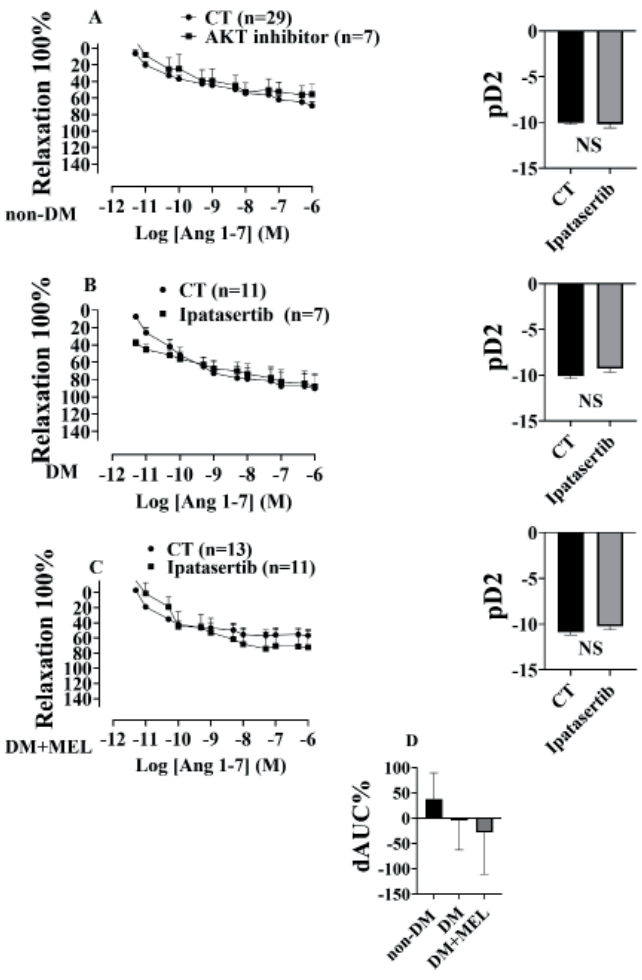

Fig. 6. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 µM) in the presence of Ipatasertib (1 µM). The data points in the study are represented as the mean values ± SEM. (CT, control; Ipatasertib, AKT inhibitor; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of difference area under curve).

Effect of melatonin on the vasodilatory response to Ang 1–7 via the NO activity

The Ang 1–7 effect of aortic rings pre-incubated with L-NAME showed non-significant changes in the non-DM group as compared with the control. However, in diabetic rats, the potency was elevated dramatically ($P = 0.0001$) from to the control rats (Figure 8A). Similarly, in STZ-induced DM, the vasodilatory response triggered by Ang 1–7 showed non-significant changes, but the dramatic increase ($P = 0.001$) in the potency occurred (Figure 8B). In contrast, in diabetic rats treated with MEL, a significant decrease ($P \leqslant 0.05$) in the vasodilatory response of Ang 1–7 occurred at concentrations of $5x10^{-7}$ and 10^{-6}, while the potency of Ang 1–7 remained unchanged as against control rats (Figure 8C). Additionally, the dAUC% showed slight differences in all the studied groups (Figure 8D).

Effect of melatonin on the maximal response to Ang 1–7 reactivity via $PI_3K/AKT/eNOS$ signalling pathway

Table 3 shows the multiple comparison of the maximal response to Ang 1–7 with or without MEL and applied inhibitors as well as blockers. The control maximal response in diabetic rats administered with MEL was decreased significantly ($P = 0.0492$) as compared with the control in STZ-induced DM rats. In contrast, the maximal response to Ang 1–7 with each of PI-3065, Ipatasertib and L-NAME pre-incubation was declined slightly in diabetic rats treated with MEL as compared with the diabetic rats, except in the presence of A779 that increased non-significantly.

Effect of melatonin on to Ang 1–7 potency via the $PI_3K/AKT/eNOS$ signalling pathway

Table 4 shows multiple comparisons of Ang

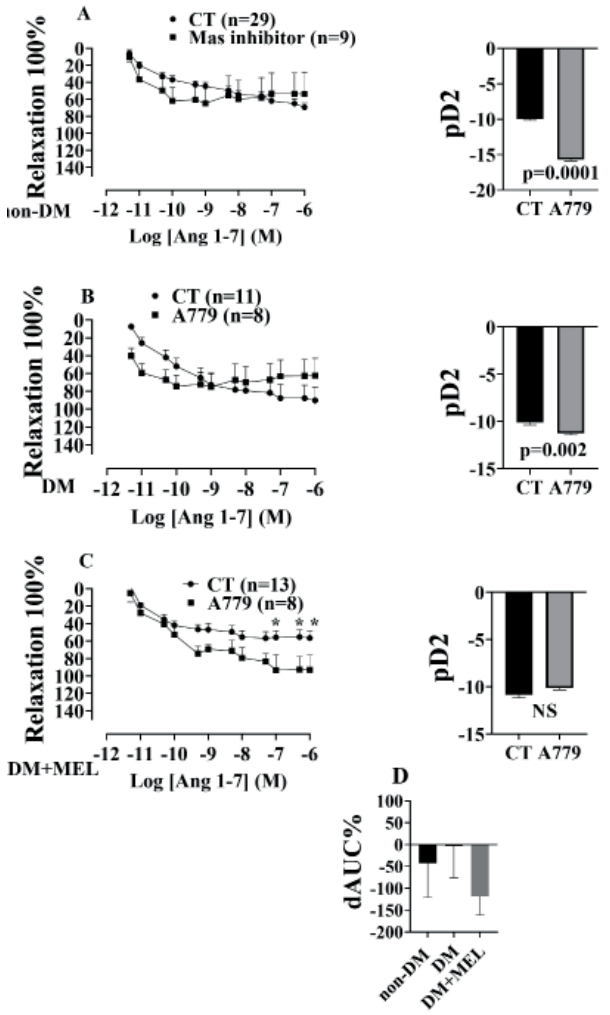

Fig. 7. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 μM) in the presence of A779 (1 μM). The data points in the study are represented as the mean values ± SEM. (CT, control; A779, Mas receptor blocker; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of the difference area under curve). [*, $P < 0.05$; **, $P < 0.01$].

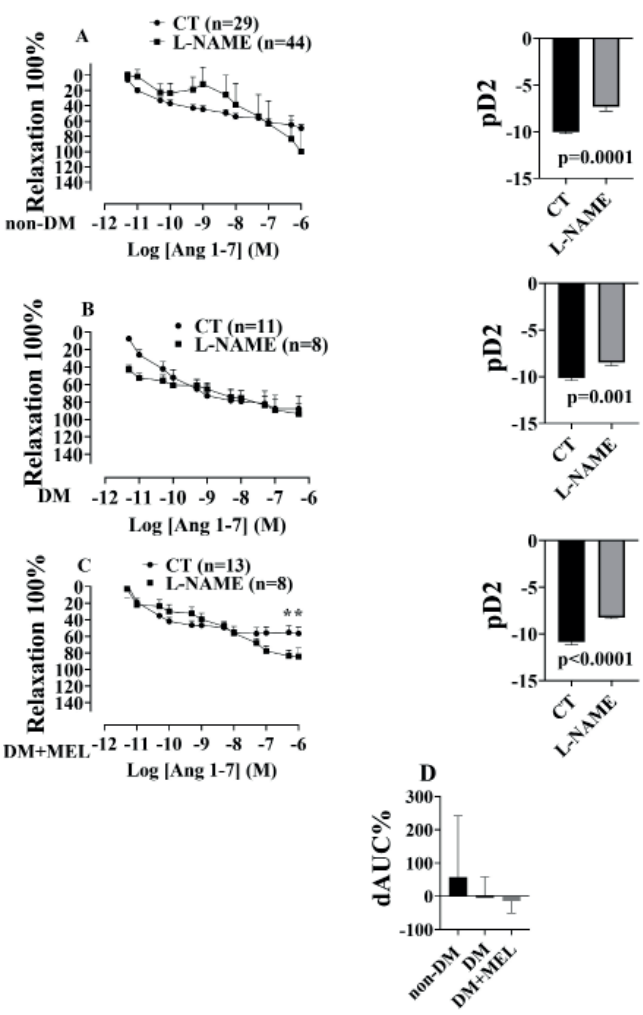

Fig. 8. Ang 1–7 DRC in aortic rings pre-contracted with PE (1 μM) in the presence of L-NAME (200 μM). The data points in the study are represented as the mean values ± SEM. (CT, control; L-NAME, NO inhibitor; non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; pD2, the potency of Ang 1–7; dAUC%, the percentage of difference area under curve). [**, $P < 0.01$].

Table 3. The Emax value to Ang1–7 reactivity *via* the PI3K/AKT/eNOS signalling pathway

Groups	Emax (PE)			Multiple Comparison
	Non-DM (A)	DM (B)	DM+MEL ©	
CT	69.29 ± 5.109	90.08 ± 14.78	56.53 ± 7.957	(B vs. C) $P < 0.0492$
PI-3065	60.76 ± 16.60	113.5 ± 6.611	79.11 ± 29.45	NS
Ipatasertib	55.41 ± 11.85	88.08 ± 14.41	72.00 ± 21.63	NS
A779	53.74 ± 25.71	62.35 ± 19.22	93.01 ± 16.89	NS
L-NAME	100.0 ± 31.11	93.32 ± 11.41	84.21 ± 10.11	NS

Data are presented as mean ± SEM. One-way ANOVA was employed to analyse the studied groups followed by the Tukey test as post hoc. [Emax, maximum response; CT, control; MEL, melatonin; Ipatasertib, AKT inhibitor; L-NAME, NO inhibitor; A779, Mas receptor blocker; PI-3065, PI_3K inhibtor; non-DM, non-diabetes mellitus; DM, STZ-induced diabetes mellitus; *P*, probability value; PE, phenylephrine; NS, non-significant difference; vs, versus].

Table 4. The potency value to Angiotensin 1–7 reactivity *via* the PI_3K/AKT/eNOS signalling pathway

Groups	pD_2			Multiple Comparison
	Non-DM (A)	DM (B)	DM+MEL (C)	
CT	–10.02 ± 0.1689	–10.09 ± 0.274	–10.87 ± 0.2957	$P = 0.0296$ (A vs. C)
PI-3065	–9.446 ± 0.3513	–10.6 ± 0.1584	–10.6 ± 0.8261	NS
Ipatasertib	–10.18 ± 0.4124	–9.276 ± 0.4164	–10.23 ± 0.3993	NS
A779	–15.69 ± 0.2132	–11.27 ± 0.1593	–10.14 ± 0.1907	$P < 0.0001$ (A vs. B)
				$P < 0.0001$ (A vs. C)
				$P = 0.001$ (B vs. C)
L-NAME	–7.313 ± 0.4674	–8.482 ± 0.3555	–8.239 ± 0.1455	NS

Data are presented as mean ± SEM. One-way ANOVA was employed to analyse the studied groups followed by the Tukey test as post hoc. [pD_2, Ang1–7 potency; CT, control; MEL, melatonin; Ipatasertib, AKT inhibitor; L-NAME, NO inhibitor; A779, Mas receptor blocker; PI-3065, PI_3K inhibitor; non-DM, non-diabetes mellitus; DM, STZ-induced diabetes mellitus; *P*, probability value; PE, phenylephrine; NS, non-significant difference; vs, versus].

1–7 potency with or without MEL and applied inhibitors as well as blockers. The potency of Ang 1–7 of diabetic rats treated with MEL was decreased dramatically ($P = 0.0296$) as compared with the control diabetic rats. On the other hand, the Ang 1–7 potency with A779 pre-incubation showed a significant ($P < 0.0001$) decrease in the diabetic group as compared with the non-DM group. Meanwhile, the potency of Ang 1–7 in diabetic rats treated with MEL under A779 produced a significant ($P < 0.0001$) decrease as compared with both STZ-induced DM and non-DM groups, respectively. In contrast, this potency showed slight changes in the presence of PI-3065, Ipatasertib and L-NAME between the studied groups.

Effect MEL tunica medial layer thickness and smooth muscles nuclei count

To evaluate whether the histological examination of MEL on diabetic rats' aortae, the TM layer thickness (μm) and SMCs nuclei count were measured. Tunica media layer thickness was decreased significantly ($P < 0.05$) in diabetic rats from the non-DM group (Figure 9A). In contrast, MEL administration ameliorated the diabetic effect through a compensation of such degeneration significantly ($P < 0.05$) as compared with the STZ-induced DM group. On the other hand, the SMCs nuclei count was decreased dramatically ($P < 0.01$) in STZ-induced DM groups as compared to the non-DM group (Figure 9B), whereas the value in SZT-induced DM treated with MEL was increased non-significantly as compared with STZ-induced DM, and it was decreased significantly ($P < 0.05$) compared with non-DM rats.

Discussion

The obtained results revealed a minor rightward shift of the Ang 1–7 response when a PKC inhibitor was used in aortic rings of non-DM subjects. It has been suggested that PKC could offer a regulatory role of the vascular tone, which influences the diameter of blood vessels as well (Khalil, 2013). It is important to note that various subtypes of PKC can have different effects, with some promoting vasoconstriction and others promoting vasodilation (Thengchaisri et al., 2021a). Regarding PKC roles, the complexity of subtypes could highlight their significance roles

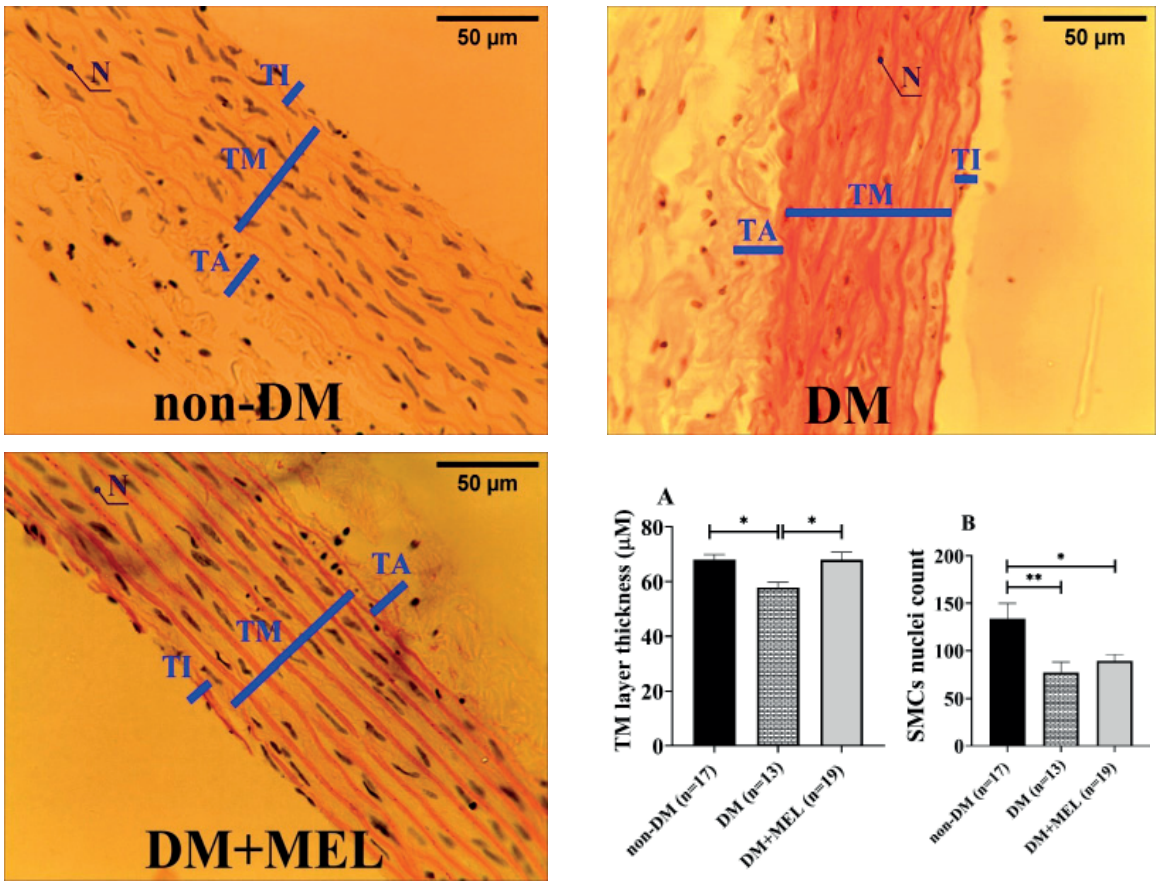

Fig. 9. Quantitative effect of MEL on aortic histology, A; tunica media thickness (µm), B; smooth muscle nuclei count. The bar graph represents the mean ± SEM from the total sample size. (non-DM, non-diabetes mellitus rats; DM, Streptozotocin induced-diabetes mellitus rats; DM+MEL, Streptozotocin induced-diabetes mellitus in rats treated with melatonin; SMCs, smooth muscle cells; n, sample size; N, smooth muscle cell nucleus; TI, tunica intima; TM, tunica media; TA, tunica adventitia). [*, $P < 0.05$; **, $P < 0.01$].

in modulating the blood vessel responsiveness and ultimately affecting the vascular function (Ringvold and Khalil, 2017). In contrast, in the STZ-induced DM group, the vasodilatory response induced by Ang 1–7 was more pronounced. This suggests that in the presence of diabetes and VED, PKC provokes a significant role in the functioning of VECs (Knapp et al., 2019). This heightened response of Ang 1–7 in the presence of diabetes and elevated PKC activity underscores the complex regulatory mechanisms involved in the vascular function under diabetic conditions (Wang et al., 2015). Additionally, one of the main outcomes of diabetes on the vascular system is the emergence of VED, which is subsequently followed by a decline in the vascular tone (Nie et al., 2019). Furthermore, under the diabetes condition, PKC mediates some vasoconstrictors to induce vasoconstriction (Jackson et al., 2016). Based on our findings, the isolated aorta exhibited strong vascular responses toward Ang 1–7 under diabetic conditions; hence, the dAUC% provided such an effect. Existing data suggest that the inhibition of PKC may be involved in vascular tone regulation through MEL antioxidant activity (Huang et al., 2022). Regarding PKC, MEL could exert its modulatory effects *via* specific receptor binding including MT_1R and MT_2R through various signalling pathways (Liu et al., 2016).

The obtained results of NADPH oxidase inhibitor demonstrated a dramatic increase in the potency of Ang 1–7 in both non-DM and STZ-induced DM rats treated with MEL. Recent studies have suggested that the elevated NADPH oxidase activity can lead to pathological changes and a reliance on hydrogen peroxide (H_2O_2) to regulate the vascular tone due to excessive production of superoxide (O^{2-}) and H_2O_2 (Sylvester et al., 2022). Additionally, the excessive production of O^{2-} is associated with various pathological obstacles, including vascular complications of diabetes (Thompson et al., 2017). However, in diabetic rats, the Ang 1–7 by itself improved the vascular tone by inhibiting oxidative enzymes (Raffai et al., 2011a). Furthermore, the significant effects were observed with dAUC% in the diabetic group treated with MEL. Our findings align with previous research and reinforce the evidence from other studies that MEL has beneficial effects in mitigating diabetic complications through various mechanisms (Pourhanifeh et al., 2020).

In our study, the inhibited mitochondrial electron transport chain showed a reduction in the vascular response triggered by Ang 1–7 in diabetic subjects. However, such a response was reversed significantly

in the STZ-induced DM treated with MEL group. It is broadly recognized that mitochondria provide a central role in cellular ROS generation through their catabolic processes (Supinski et al., 2020). Regarding ROS activity, it may be attributed to the positive outcomes of MEL which could be linked to its capacity in mitochondrial ROS reduction.

The observations of xanthine oxidase inhibition were also involved in our results; the Ang 1–7 vasodilatory response induced in diabetic rats increased dramatically. However, when diabetic rats were treated with MEL, this effect was reduced. Interestingly, in the group of diabetic rats treated with MEL, such responsiveness may be attributed to MEL's counteraction against VED and the declined vasodilatory response to Ang 1–7. Our explanation is supported by a previous attempt which proposed that the inhibition of xanthine oxidase-mediated O^{2-} generation with a substance like OXY can improve endothelium-dependent vasodilator responses (Thengchaisri et al., 2021b). In particular, xanthine oxidase is well-known to be a potent source of oxidative stress in the vascular system through the production of uric acid (Liu et al., 2021). Furthermore, studies have depicted that the protective effects of MEL are in part mediated through its binding to MT_2R (González-Flores et al., 2023). Additionally, MEL has been shown to decrease certain pro-inflammatory factors in STZ-induced diabetic rats (Maher et al., 2020).

Data obtained from PI_3K inhibitor reveal the dysregulation of vasodilation by Ang 1–7. Numerous attempts have shown that the PI_3K regulatory roles through different physiological mechanisms were linked to a variety of intracellular growth factors (Zhao et al., 2021). However, diabetes could generate both the advanced glycation end products (AGEs) and the receptor of advanced glycation end product (RAGE) contributing to the improvement of downstream PI_3K/AKT signals (Figure 5) (Yuan et al., 2020). Surprisingly, in diabetic rats treated with MEL, the Ang 1–7 vasodilation under PI3K inhibitor effect was increased. Experimental research has supplied findings that endorse the notion that MEL has the potential to enhance insulin signalling, potentially improving insulin sensitivity. This is achieved through the production of insulin-like growth factor and the augmentation of phosphorylation on the tyrosine residues of insulin receptors (Picinato et al., 2008). Hence, the increased vasodilation under MEL action could be involved through insulin improvement.

In our results, the Akt molecule inhibition by Ipatasertib revealed non-significant changes of vasodilation triggered by Ang 1–7 in all studied groups. It confirmed that hyperglycaemia could cause glycosylation to occur at the AKT phosphorylation site in eNOS, ultimately leading to the suppression of the eNOS function (Figure 6A, B, C) (Alghanem et al., 2021). On the other hand, dysregulation of AKT signalling pathways can lead to abnormal vascular remodelling (Jia and Sowers, 2021). In contrast, MEL could offer antioxidant properties of VECs activity through direct free radical scavenging, declined NADPH oxidase, and elevating SOD activity, hence, promoting NO and/or vasodilation *via* MT_2R as well (Song et al., 2022).

Our findings under MasR blocker indicate that Ang 1–7 effectively promotes the dilation of the thoracic aorta, and its potency is notably enhanced in the group with experimentally induced diabetes using STZ. Furthermore, the administration of MEL also significantly induced vasodilation. These results demonstrate that the external Ang 1–7 successfully restored the dilatory capacity of diabetic rats' aortae (Zhang et al., 2015). Additionally, it has been suggested that the signalling pathway of Ang 1–7 primarily operates through the MasR (Chen et al., 2023). Indeed, Ang1–7 *via* its interaction through MasR triggers specific intracellular signalling pathways, resulting in the generation of NO and the stimulation of cAMP release (Tetzner et al., 2016). Meanwhile, several studies have reported that Ang 1–7 can also communicate through the AT_2R (Raffai et al., 2011b). Thus, our finding has uncovered a novel aspect suggesting that both MEL and Ang 1–7 could synergistically potentiate the vascular tone through eNOS activity.

The results obtained from using the NO inhibitor by L-NAME revealed a reduction in the potency of Ang 1–7 without a change in vasodilation, as well as in dAUC% in both diabetic rats and diabetic rats treated with MEL. This suggests that there may be impaired endothelial eNOS signalling pathways, leading to a subsequent decrease in NO production (Xie et al., 2021). Meanwhile, in the STZ-induced DM treated with MEL group, the elevated maximum response was observed (Figure 8C). Studies have indicated that MEL has the potential activity to restore or compensate the decreased NO levels (Simko et al., 2018).

The quantitative histopathological findings demonstrate dysfunction in the development of aortic layers, characterized by a reduction in the number of VSMCs nuclei and a decrease in TM thickness, as well as the disrupted structural organization. These changes are attributed to the significant impact of diabetes on VSMCs, primarily resulting from prolonged exposure to hyperglycaemia followed by vascular complications (Ullah Wazir et al., 2023). On the other hand, the precise cellular-level mechanism responsible for these changes remains unclear. Furthermore, the contraction or shrinking of VSMCs and an increase in collagen bundles within the tissue can lead to alterations in the size, shape, and number of cell nuclei (Silva-Velasco et al., 2023). These combined factors are attributed to be responsible for the observed changes in the aortic diameter. However, the results of STZ-induced DM treated with MEL

showed a notable reduction in the adverse effects of diabetes, and significant improvement was observed, indicating a substantial amelioration of diabetic-related consequences. Indeed, accumulated evidence supports the idea that MEL can improve metabolic irregularities and dysfunction in the adipose tissue due to its antioxidant properties (Cui et al., 2023). Additionally, MEL's effects may be mediated through their membrane receptors (Xia et al., 2020). Moreover, MEL has been found to enhance the activity of the mitochondrial activity, especially those mechanisms linked to oxidative enzymes (Reiter et al., 2016).

Conclusions

The study findings indicate that STZ negatively affects the vascular response to Ang 1–7 in isolated thoracic aortae. However, the administration of MEL effectively reversed these diabetes-induced alterations, leading to improved vasodilation in response to Ang 1–7. The involvement of PKC and NADPH oxidase had minimal effects on Ang 1–7-induced vasodilation in non-diabetic aortic rings. Conversely, MEL induced slight changes in the diabetic group induced by STZ, suggesting that MEL plays a regulatory role in vascular responsiveness to Ang 1–7 in diabetic aortic rings. Particularly, MEL mitigates the adverse effects of mitochondrial complex I inhibition on the vascular function in diabetes and partially counteracts the effects of xanthine oxidase by modulating oxidative stress pathways.

The study also suggests that PI3K may not be a primary regulator of Ang 1–7-induced vasodilation in these experimental conditions. The impact of AKT inhibition on the vascular response to Ang 1–7, with or without MEL treatment, was relatively modest across the experimental groups. The study underscores the crucial roles of MasR in maintaining the efficacy of Ang 1–7-induced vasodilation in both non-diabetic and diabetic conditions. However, under MEL treatment in diabetic rats, there was a reduction in the maximal vasodilatory response to Ang 1–7 when MasR was blocked, although these interventions on vascular responses were subtle.

Furthermore, the study concludes that NO has a counterregulatory role in the efficacy of Ang 1–7-induced vasodilation, as evidenced by a significant increase in potency when NO is inhibited. The complex interactions among NO, MEL, and Ang 1–7 in regulating vascular responses provide valuable insights into potential therapeutic strategies for addressing vascular dysfunction in diabetes. Finally, MEL may have potential protective and restorative effects on the structural integrity of the tunica media layer thickness and vascular smooth muscle cell (VSMC) density in diabetic conditions.

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Conflict of Interest

There were no conflicts of interest as declared by the authors.

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Effects of a Non-Fasting Moulting Treatment and Extended Cold Storage on Some Egg Quality Traits of a Commercial White Laying Hen

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Abstract. *This study was aimed at investigating the effects of non-fasting moulting treatment and extended cold storage on table egg quality traits of a commercial white layer hen. For this research, 360 eggs were collected from moulted and non-moulted flock four weeks after the end of the moulting program. The hens in each group were allowed ad libitum access to water and their respective diets as grain barley for the moulting group and standard layer feed for the control group during the moulting period of 10 days and then a complete layer ration after the moulting program through the laying period. Sample eggs of each of these two groups were numbered, weighed and further assigned to a different storage period as daily fresh eggs: 15, 30, 45, 60 and 75 days. All eggs were stored between 4°C and 5°C and 55% and 60% relative humidity throughout the experiment. Egg weight, egg length, egg width, shell strength, shell weight, shell thickness, yolk height, yolk diameter, yolk colour, albumen height, albumen width, and albumen length were measured at each consecutive analysis period for egg quality comparison. Non-fast moulting treatment significantly affected egg weight, egg-width, egg length and breaking strength ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.020$). There were no significant effects of the length of a cold storage period on the external quality traits of egg weight, egg width, egg length and egg-shape index. Shell thickness and breaking strength of eggs were affected significantly ($P < 0.001$ and $P < 0.02$) by the length of a storage period. The yolk height and yolk colour scores were found to be significantly greater in eggs from the moulting group while all internal quality characteristics were significantly affected by the length of a storage period ($P < 0.001$). In conclusion, even if it was cold storage, egg quality tended to decline in the extended storage period and non-fasting moulting treatment improved the external and internal egg quality.*

Introduction

Induced moulting is a management application to extend the productive life of laying hen (Zhang et al., 2022). Moulting treatment generally improves post-moult productive performance, egg weight, egg quality and results in increases in percentage of A or AA grade eggs (Kakhki et al., 2018; Mishra et al., 2022; Lei et al., 2023; Wang et al., 2023). In practice, there are lots of moulting methods influencing body weight loss, mortality rate, post-moult egg production and egg mass of laying hens (Kakhki et al., 2018; Ga et al., 2022). An ideal moulting program should be providing good layer welfare and bird health without causing much stress and without feed-removal (Mishra et al., 2022). Currently, there is a big concern against conventional feed and water removal moulting methods due to greater body weight losses and higher mortality rates (Mishra et al., 2022). The feed removal also causes much more stress on hen body and declines immunity (Fard et al., 2020). As a result of increasing consumer awareness about animal welfare, egg producers from The United States, The United Kingdom and some European countries have decided to sell eggs produced only through non-feed withdrawal moulting programs (Mishra et al., 2022) and have adopted non-fasting moulting methods in commercial egg production. Non-fasting moulting is less stressful to hens, causes less liver damage, and improves second period production performance of hens by ensuring good animal welfare of animals (Fard et al., 2020; Lei et al., 2023). Zinc-based poultry diet has been one of the most popular non-fast moulting treatments in egg production. But the use of zinc in poultry feed should be strictly regulated due to the environmental effects of zinc (Ga et al., 2022). Therefore, non-fasting moulting treatments as alfalfa (Petek & Alpay, 2008) and whole wheat diets (Mishra et al., 2022) are getting popular in both table or breeder egg production. These diets can be economically acceptable and allow hens to meet their maintenance requirement. Non-feed removal moulting programs based particularly on whole grain barley have a positive effect on egg quality traits especially in yolk colour and Haugh unit (Petek et al., 2008; Onbaşılar et al., 2020).

With an extended laying period, egg production and eggshell quality tend to decline while egg weight increases (Alfonso Carrillo et al., 2021; Benavides-

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Reyes et al., 2021). Egg quality of end of layer hens can rapidly undergo quality deterioration during storage than that of younger birds, causing a major economic loss to the poultry industry (Nasri et al., 2019). The rate at which these changes occur during storage depends on several factors as temperature, humidity, length, etc. (Kopacz & Drazpo, 2018; Mishra et al., 2022). In general, longer period storage of eggs results in a decrease of albumen height, influences yolk and blastoderm quality, gas exchange and embryonic metabolism (Feddern et al., 2017; Quan et al., 2021). Eggs from older hens are more susceptible to longer period storage (Poletti & Vieira, 2021) as it reduces their shelf life and freshness of eggs. Freshness and shelf life of eggs, which are the most important quality characteristics, are a major concern of consumers. There are many factors contributing to the extended of shelf life of table eggs (Addo et al., 2018). Packing eggs under modified atmosphere and covering with shrink film may increase their shelf life (Petek et al., 2014; Giampietro-Ganeco et al., 2015). The main criteria of freshness are Haugh unit, egg weight loss and egg air cell size (Quan et al., 2021). Studies have shown that storage of eggs in a refrigerator or cold conditions will retain their nutritional value longer compared to room storage conditions (Faris et al., 2011; Nadia et al., 2012). The storage method had a significant effect on most egg quality traits, and eggs stored at 4°C were of good quality and were even classified as extra class eggs even after 28 days (Kopacz & Drazpo, 2018). If eggs storage in room temperature it's quality rapidly declines compare to cold storage (Jones et al., 2018). The main factors influencing internal egg quality are length and temperature of a storage period, and there is a significant interaction between both these factors (Grashorn et al., 2016; Feddern et al., 2017; Malfatti et al., 2021). There are a lot of research evidence about the effects of moulting and a storage period on post-moult laying performance and egg quality of both commercial layer and breeder hens. However, there is almost no research about the effects of an extended storage period for more than one month on post-moult egg quality. Therefore, this study was planned to compare the effects of an extended cold storage period on internal and external quality of table eggs collected from non-fast moulted and non-moulted commercial laying hens.

Material and Methods

The eggs for this research were collected from a moulted and a non-moulted flock housed in Research and Experimental Farm of the Faculty of Veterinary Medicine in Bursa Uludag University in Türkiye. This study did not require ethical permission according to Animal Experiments Ethics Committees Regulation on Working Procedures and Principles, Article 8 19-k (Official Gazette, 2014).

Eggs and management of the flocks

A total of freshly laid 360 eggs from moulted (180 eggs) and non-moulted flocks (180 eggs) were collected randomly for this research. The hens (Nick Brown) in both flocks were 70-week-old and they were the optimum age for moulting (Hy-Line International Technical Update, 2019) with similar body weights and laying rates. They were provided ad libitum access to a complete layer ration and water for a period of two weeks to assure that all hens were healthy and in active production before the moulting process. After the acclimation process, the hens were randomly divided into two treatment groups as non-fast moulting groups with 100% whole grain barley and non-moult control with a commercial layer ration. All birds in each group were allowed ad libitum access to water and their respective diets (whole grain barley for the moulting group and standard layer feed for the control group) during the moulting period for 10 days and then a complete layer ration after the moulting program. A commercial layer diet including 16.45% CP and 2800 ME kcal/kg for the hens was used from the acclimation period to the end of the laying period in the experiment. Light was provided 16 L:8 D in the pre- and post-moulting periods and was reduced to 11 hours (only natural light) during the moulting period. Both flocks were kept in the same house throughout the study period, under the same feeding and management conditions, except for the moulting period.

The eggs were collected by 4 weeks after the end of the moulting and transferred to the Egg Quality Laboratory. At arrival in the Egg Quality Laboratory of the University of Bursa Uludag, the eggs were assigned to each of six storage lengths (0, 15, 30, 45, 60 and 75 days) according to the moulting process as non-moulting and non-fast moulting (2 x 6 : 12 interactive groups). Standard home type refrigerators were used for the storage of eggs. All eggs in the control and moulting groups were kept in between 4°C and 5°C and 55% and 60% humidity conditions for all treatments. The storage temperature was slightly under the advice by the EU regulation EG 589/2008, which defines 5°C as the minimum storage temperature for eggs (Grashor et al., 2016). All tests were completed immediately after egg removal from the refrigerator on each target day.

Data

The first analysis was carried out on the first day after oviposition for fresh eggs. At this age, the eggs should qualify as "extra fresh" according to Turkish Food Codex on Egg and Egg Products (2008). Measurements on each egg included egg weight, egg width, egg length, shell thickness, breaking strength, yolk diameter, yolk height, albumen height, albumen width, albumen length and yolk colour. Eggs were weighed with a precision digital scale (0.01 precision). After weighing, the width (*along the equatorial axis*) and length (*along the longitudinal axis*) of the eggs were measured with a caliper to 0.1 mm. The egg shape index was determined from these measurements according to Anderson et al. (2004) as given with the

formula: *width/length x 100*. The breaking strength of eggshell was measured with an eggshell force gauge and breaking point (Balnave & Muheereza, 1997) recorded as Newton (N) force required to crack the shell surface. After all eggs were broken on to a glass flat surface, the height of the albumen was measured with a tripod micrometer. A caliper was used to measure the length and width of albumen of eggs. The color of the yolk was determined using a color fan (DSM, 2022). Shell thickness (without inner and outer shell membranes which were removed manually) was measured at three areas (broad end, middle portion and narrow end of the shell), by using a micrometer according to Chowdhury (1990). The Haugh unit was calculated from the records of albumen height and egg weight using the following equation (Haugh 1937):

$$HU = 100.Log\ (H - 1.7W^{0.37} + 7.6)$$

Where HU is the Haugh unit, H is albumen height (mm), and W is egg weight (g).

Statistical analysis

The collected data were statistically analysed using SPSS 18.0 statistical package (SPSS Inc. 2018). All data were subjected to using two-way analysis of variance (ANOVA) with moulting process (*moulted and non-moulted*) and storage duration (*0, 15, 30, 45, 60 and 75 days*) as the main effects and all interactions between the two effects (Snedecor & Cochran, 1994). In all used statistical tests, differences at $P < 0.05$ were considered as significant.

Results

Some exterior egg quality traits in the experimental groups are presented in Table 1. Moulting treatment

Table 1. Some exterior egg quality traits in the experimental groups (mean ± SEM)

Groups	Egg weight (g)	Egg width, mm	Egg length, mm	Shell thickness, (mm/100)	Egg shape index, (%)	Breaking strength, (N)
Moulting (M)						
Control (C)	67.9 ± 0.58	45.0 ± 0.15	61.8 ± 0.26	36.4 ± 0.33	72.8 ± 0.35	28.9 ± 0.07
Moulting (M)	74.1 ± 0.59	46.1 ± 0.14	64.0 ± 0.25	34.9 ± 0.32	72.3 ± 0.34	30.9 ± 0.08
Storage Length (SL)						
Fresh	73.1 ± 0.90^{a}	45.5 ±0.25	63.3 ± 0.44	38.2 ± 0.004^{a}	72.0 ± 0.56	31.6 ± 1.34^{a}
15 d	71.9 ± 1.00^{a}	45.5 ±0.27	62.5 ± 0.47	37.5 ± 0.003^{a}	72.9 ± 0.59	30.8 ± 1.42^{a}
30 d	71.3 ± 1.09^{ab}	45.5 ±0.26	62.8 ± 0.48	36.6 ± 0.004^{a}	72.5 ± 0.62	30.9 ± 1.48^{a}
45 d	70.6 ± 1.08^{bc}	45.9 ±0.25	63.0 ± 0.44	35.5 ± 0.005^{a}	72.9 ± 0.56	30.0 ± 1.34^{a}
60 d	70.0 ± 0.90^{bc}	45.4 ±0.25	62.7 ± 0.44	32.5 ± 0.005^{b}	72.4 ± 0.57	28.4 ± 1.37^{b}
75 d	69.0 ± 1.00^{c}	45.5 ±0.24	63.2 ± 0.48	33.3 ± 0.006^{b}	72.4 ±0.59	25.5 ± 1.43^{b}
Moulting x Storage Length						
Cx0 d	69.8 ±1.33	44.9 ±0.35	62.3 ± 0.60	39.6 ± 0.008	72.0 ± 0.77	31.1 ± 1.82
Cx15 d	68.6 ±1.54	44.9 ±0.40	60.9 ± 0.69	38.8 ± 0.009	73.8 ± 0.89	30.0 ± 1.97
Cx30 d	67.9 ±1.42	44.6 ±0.37	61.2 ± 0.64	38.0 ± 0.008	73.0 ± 0.82	30.6 ± 1.97
Cx45 d	67.6 ± 1.42	45.6 ± 0.37	62.4 ± 0.64	36.0 ± 0.009	73.2 ± 0.82	29.2 ± 1.96
Cx60 d	66.9 ± 1.42	44.4 ± 0.37	60.9 ± 0.64	33.0 ± 0.009	72.9 ± 0.82	27.6 ± 1.97
Cx75 d	66.6 ± 1.38	45.5 ± 0.36	62.8 ± 0.62	32.8 ± 0.008	72.4 ± 0.80	24.7 ± 1.90
Mx0 d	76.4 ± 1.43	46.3 ± 0.36	63.8 ± 0.64	36.8 ± 0.008	72.6 ± 0.82	32.1 ± 1.97
Mx15 d	75.1 ± 1.38	46.1 ± 0.37	64.1 ± 0.62	36.1 ± 0.008	72.1 ± 0.80	31.6 ± 1.90
Mx30 d	74.7 ± 1.61	46.3 ± 0.42	64.4 ± 0.69	35.2 ± 0.007	72.1 ± 0.93	31.3 ± 2.22
Mx45 d	73.5 ± 1.34	46.2 ± 0.35	63.7 ± 0.64	35.1 ± 0.008	72.6 ± 0.77	30.8 ± 1.84
Mx60 d	73.1 ± 1.38	46.3 ± 0.36	64.5 ± 0.64	32.1 ± 0.008	72.0 ± 0.80	29.3 ± 1.90
Mx75 d	71.4 ± 1.54	45.5 ± 0.40	63.6 ± 0.72	33.9 ± 0.008	72.4 ± 0.89	26.2 ± 2.13
ANOVA						
M	0.001	n.s	n.s	n.s	n.s	0.02
SL	0.05	n.s	n.s	0.001	n.s	0.05
M x SL	n.s	n.s	n.s	n.s	n.s	n.s

n.s. non-significant

a-c; a-b; within the same columns, values with different superscripts were found significantly different

significantly affected the egg weight and breaking strength of egg ($P < 0.001$ and $P < 0.020$) whereas egg weight, shell thickness and breaking strength were affected significantly ($P < 0.05$, $P < 0.001$ and $P < 0.020$) by the length of storage. There were no significant moulting x storage length interactions for all exterior egg quality traits investigated.

Mean and standard errors of some internal egg quality traits in the groups are shown in Table 2. There were significant differences for yolk height and yolk colour score between the control and moulting groups ($P < 0.001$ and $P < 0.001$). All internal quality traits investigated were found to be significantly different due to the effects of the length of the storage period ($P < 0.001$, $P < 0.002$, $P < 0.029$). No moulting x storage length interaction on all interior egg quality traits was observed.

Discussion

Induced moulting extends the productive life of a layer flock by improving egg production, shell quality, and albumen height of eggs (Hy-Line International Technical Update, 2019; Lei et al., 2023). Improved egg shell contributes to saving the internal egg quality, especially in the extended storage condition. Storage conditions used to store the eggs can have a major impact on both table egg or breeder egg quality and the subsequent viability of chicken embryos (Adriansen et al., 2022). Similarly, to some previous findings (Petek, 2001; Flock, 2016;

Table 2. Effects of moulting practice and length of egg storage on internal egg quality traits during the post-moult laying period (Mean ± SEM).

Groups	Yolk diameter, mm	Yolk height, mm	Albumen length, mm	Albumen width, mm	Albumen height, mm	Yolk color score	Haugh unit
Moulting (M)							
Control (C)	44.3 ± 0.31	17.4 ± 0.14	104.2 ± 1.37	82.3 ± 1.12	6.27 ± 0.15	10.9 ± 0.61	73.0 ± 1.45
Moulting (M)	44.9 ± 0.32	18.4 ± 0.15	106.4 ± 1.39	81.7 ± 1.14	6.63 ± 0.16	11.2 ± 0.62	74.2 ± 1.48
Storage Length (SL)							
Fresh	42.4 ± 0.52^{c}	18.6 ± 0.25^{a}	96.8 ± 2.30^{b}	75.7 ± 1.88^{c}	8.54 ± 0.25^{a}	10.9 ± 0.10^{b}	88.0 ± 2.44^{a}
15 days	43.5 ± 0.56^{bc}	18.4 ± 0.26^{a}	103.2 ± 2.43^{b}	80.2 ± 1.99^{bc}	6.79 ± 0.26^{b}	11.2 ± 0.11^{ab}	75.2 ± 2.59^{b}
30 days	43.4 ± 0.58^{bc}	19.4 ± 0.27^{a}	98.9 ± 2.54^{b}	81.4 ± 2.06^{ab}	6.01 ± 0.28^{c}	10.9 ± 0.11^{b}	68.7 ± 2.69^{b}
45 days	46.9 ± 0.52^{a}	17.8 ± 0.24^{b}	111.2 ± 2.30^{a}	83.1 ± 1.88^{ab}	5.86 ± 0.25^{c}	11.0 ± 0.10^{ab}	70.9 ± 2.44^{b}
60 days	44.3 ± 0.53^{b}	16.9 ± 0.25^{c}	110.4 ± 2.34^{a}	86.3 ± 1.91^{a}	5.77 ± 0.26^{c}	11.3 ± 0.10^{a}	70.5 ± 2.49^{b}
75 days	47.2 ± 0.56^{a}	16.4 ± 0.26^{c}	111.3 ± 2.44^{a}	85.3 ± 1.99^{a}	5.72 ± 0.28^{c}	11.3 ± 0.11^{a}	68.5 ± 2.59^{b}
Moulting x Storage length							
Cx0	42.7 ± 0.72	18.4 ± 0.34	99.6 ± 3.14	78.3 ± 2.56	8.00 ± 0.35	10.9 ± 0.14	86.4 ± 3.34
Cx15	42.4 ± 0.82	17.4 ± 0.40	98.4 ± 3.63	80.9 ± 2.96	6.84 ± 0.40	11.2 ± 0.16	79.4 ± 3.86
Cx30	43.5 ± 0.77	18.8 ± 0.36	97.8 ± 3.36	83.9 ± 2.74	5.38 ± 0.38	10.9 ± 0.15	62.6 ± 3.57
Cx45	46.0 ± 0.77	17.4 ± 0.36	114.1 ± 3.36	83.3 ± 2.74	5.59 ± 0.38	10.9 ± 0.15	69.8 ± 3.57
Cx60	43.6 ± 0.77	16.7 ± 0.36	105.4 ± 3.36	81.9 ± 2.74	5.98 ± 0.38	10.9 ± 0.15	73.0 ± 3.57
Cx75	47.4 ± 0.74	15.8 ± 0.35	109.8 ± 3.25	85.3 ± 2.64	5.81 ± 0.35	10.9 ± 0.14	67.5 ± 3.45
Mx0	42.1 ± 0.77	20.0 ± 0.36	94.0 ± 3.36	73.1 ± 2.74	9.08 ± 0.38	10.9 ± 0.15	89.5 ± 3.57
Mx15	44.5 ± 0.74	19.4 ± 0.35	108.0 ± 3.25	79.5 ± 2.65	6.75 ± 0.36	11.1 ± 0.14	70.6 ± 3.45
Mx30	43.2 ± 0.86	18.9 ± 0.40	100.0 ± 3.80	78.8 ± 3.09	6.64 ± 0.42	10.9 ± 0.17	74.8 ± 4.03
Mx45	47.7 ± 0.72	18.1 ± 0.34	108.4 ± 3.14	82.8 ± 2.56	6.14 ± 0.35	11.2 ± 0.14	72.9 ± 3.34
Mx60	45.0 ± 0.74	17.2 ± 0.35	115.4 ± 3.25	90.7 ± 2.65	5.55 ± 0.36	11.7 ± 0.14	67.9 ± 3.45
Mx75	47.1 ± 0.83	17.1 ± 0.39	112.8 ± 3.63	85.3 ± 2.96	5.64 ± 0.40	11.6 ± 0.16	69.5 ± 3.34
ANOVA							
M	n.s	0.001	n.s	n.s	n.s	0.001	n.s
SL	0.001	0.001	0.001	0.002	0.001	0.029	0.001
MxSL	n.s	n.s	n.s	n.s	n.s	n.s	n.s

n.s. non-significant

a-c; a-b; within the same columns, values with different superscripts were found significantly different for moulting and storage period.

Hy-Line Technical Update, 2019), as expected, the application of moulting improved the post-moult egg weight and egg weight related parameters such as egg breaking strength. But there was a decrease in egg shell thickness when the storage period was longer than regular. In another study, it was reported that moulting programs had a significant effect on the post-moult egg weight but there were no significant differences among control and moulting treatments for shape index, specific gravity, shell strength (Aygün & Yetişir, 2014). The bird fed with alfalfa hay for moulting had a greater egg weight, whereas pumice stone did not show any significant difference between the egg quality traits of moulted layers (Son et al., 2022).

Eggshell quality is very crucial in commercial production because of the economic losses of cracked or damaged eggs which account for 6% to 8% of total egg production (Hamilton et al., 1979). Egg breaking strength and shell thickness are the most important indicators of egg shell quality. Besides the total number of eggs, the shell quality also determines the number of saleable table eggs that a hen will lay in a lifetime period. In this study, there were no significant differences between the moulting and non-moulting groups for the egg shell thickness. But the shell thickness of eggs was significantly decreased with a prolonged storage period. During storage, shell thickness values of the eggs reduced from 36.6 to 29.6 mm. As a result of decreasing egg shell thickness, the breaking strength of egg shells also significantly decreased with a prolonged storage period. The shell breaking strength in the group fed with the moult induced-diet was significantly higher than that in the control birds fed normal layer diet ($P < 0.02$).

When the eggs collected from both moulted and non-moulted hens were analysed there was a significant variance in breaking strength values ($P < 0.02$). The egg shell of hens from the moulting group looked stronger than the eggs of hens from the non-moult control group. The length of the storage period also significantly influenced the breaking strength of eggs ($P < 0.05$). If we compare the shell strength of eggs in different storage period groups, eggs in all groups had a similar value until 45 days of storage. The eggs in the longer storage period than 45 days had a statistically weaker egg shell. The breaking strength value was the greatest on day 0 (31.6 N), and the lowest on day 75 (25.5 N). The breaking strength values measured in all interactive groups (moulting x storage period) ranged from 24.7 N to 32.1 N. In practice, there is no universal standard for the egg breaking point. But in general, a breaking point of 3.5 kgf (1 kgf to 9.80665 N) or higher has a strong, good enough and healthy shell that will endure most of the shipping and transportation vibrations or shocks that eggs endure (Mikesell, 2021). According to this reference, eggs in all groups had a weak breaking point. A greater breaking strength value signifies a stronger egg shell, which is ideal as the shell provides a protective barrier for the egg contents. In this study, there were no significant effects of moulting practice and length of the cold storage period on the shape index of eggs. The shape index value of all eggs in the groups was found to be between acceptable values of 72–76 (Sarıca & Erensayın, 2009).

All internal egg quality traits measured in this study were affected significantly by the length of the storage period. Similarly, to the findings of Aygün and Yetişir (2014), there were no significant differences for the post-moult albumen height and Haugh units (HU) of eggs among moulted and non-moulted hens. Otherwise, there was a distinct decrease in the HU of eggs after 15 days of storage and it remained stable after this time throughout the storage. This was concurrent with the findings of Grasshorn et al. (2016) who reported that Haugh units decreased with increasing storage duration. Jones and Musgrove (2005) showed that the HU initially was 88.00 and decreased to 68.50 by day 75. At the same time, the decline in albumen height and the HU resembles the results reported by Silversides and Villeneuve (1994). According to USDA-Agricultural Marketing Service guidelines (USDA, 2000), the white of eggs is described as firm (AA grade eggs) if they receive a score of 72 HU or higher and reasonably firm (A grade eggs) if they receive a score of 60–72 HU (Hisasaga et al., 2020). In this study, the average HU values of eggs from moulted and non-moulted hens can be classified as AA grade eggs. In general, the egg grade after 15 days of storage declined to A grade. Similar to our findings, Jones et al. (2002) reported a decline to grade A in HU values at 6 weeks in control eggs for a study conducted at refrigerated temperatures. Kralik et al. (2014) showed that the storage period was significantly affected ($P < 0.05$) albumen height and HU values of eggs. The decline of the Haugh units can also be considered an indicator of reduced lysozyme activity (Trziszka, 1994), known as an important agent to protect the egg contents against microbial contamination. The Haugh unit is an indicator of albumen quality (Haugh, 1937) and albumen quality is recognized as one of the major factors in measuring egg quality (Obianwuna et al., 2022). In another study, Hassan (2013) showed that storage length affected all egg quality traits and refrigerated eggs were able to maintain their quality comparable to the fresh eggs. In another study, Hamidu et al. (2017) reported that table egg quality as measured by the Haugh unit was not affected by oil preservation but quality decreased with increasing storage duration. In that study, it was found that within the first 28 days of storage the changes in egg weight, Haugh units and air cell size were not critical if eggs were kept at 6°C, but there was a rapid loss of quality at storage temperatures of 15°C and 22°C, especially in the Haugh unit. Foreman et al. (2023) reported that the eggs from the moulted Sasso breeder hens had a higher albumen

height and haugh unit. In our study, there were no significant differences for the albumen height of eggs but a decrease was observed with the prolonged storage period, as expected (Quan et al., 2021). The average albumen heights of the eggs from moulted and non-moulted hens were 6.63 mm and 6.27 mm whereas the height was found to be 8.54 mm in fresh eggs and and 5.72 mm in eggs stored 75 days. In agreement with our study, Son et al. (2022) reported that albumen height of eggs varied within 6.07–6.43 in commercial laying hens. Another research showed that albumen height decreased from 7.05 to 4.85 mm compared to day 1 and week 10 of the extended cold storage (Jones & Musgrove, 2005).

In this study, the increase in yolk height and yolk colour score of post-moult hen eggs was found to be similar with the previous observations of Attia et al. (1994). The yolk colour scores in all groups were found to be greater than those reported by Son et al. (2022) who found these to be between 7.50 and 8.40 in commercial laying hens. The yolk height was increased in the moulting group because of the greater egg weight of post-moult layer hens while it was significantly decreased due to the length of egg storage, especially longer than 45 days of storage. The yolk colour score of the eggs was found to be statistically different in storage length groups. The improvement of yolk parameters after moulting may be due to rejuvenation of laying hen reproductive organs, as well as in broiler breeders (Brake & Thaxton, 1979; Berry & Brake, 1985; Verheyen et al., 1987). In general, yolk colour is brightening with increasing hen age and genotype has a significant effect on egg yolk colour (Nolte et al., 2021).

Conclusion

A whole grain barley-based diet is a high fibre diet and may be useful as a non-fasting moult feed. But palatability and nutritional value of this feed are comparatively low. Supplementation of a high fibre diet with different enzymes may improve feed digestibility and nutrient availability of this moult diet. Non-fasting moulting treatment used in this study increased external egg quality by increasing the egg weight and breaking strength. Almost all internal egg quality values deteriorated with the prolonged storage period. Egg grade has been declined from AA to A after 15 days of storage. Eggs that need to be stored for a long time should be stored under the conditions recommended by the regulations and should be consumed in a short period as much as possible.

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Machine Milking Ability of Ewes of Tsigai, Improved Valachian, Lacaune Breeds and Their Crosses: Udder Morphological Traits and Milking Characteristics

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Abstract. *Good and homogenous udder morphology of dairy ewes is desirable for good milkability, udder health and animal welfare, especially if machine milking is in use. We evaluated the udder morphology and milkability of 286 ewes of eight genotypes (Tsigai, Improved Valachian, Lacaune breeds and 5 types of their crosses). The aim was to obtain an overview of the most important parameters to be evaluated for breeding dairy sheep breeds. The following traits were evaluated on a 9-point linear scale: udder depth, cistern depth, teat position, teat size, udder cleft, udder attachment and general udder shape. Selected parameters that characterise the milk yield and milkability of ewes were also recorded in individual control measurements: amount of machine milked in 30 and 60 seconds, machine milk yield, machine stripping, total milk yield and percentage thereof. Linear evaluation and precise udder measurements showed that Tsigai and Improved Valachian ewes had smaller udders with smaller cisterns and a better teat position than Lacaune ewes. The proportion of machine stripping (PMS) was best (lowest value) in the Improved Valachian ewes (26.0%) among the purebred breeds, followed by the Tsigai ewes (27.2%), and it was found to be highest in the purebred Lacaune ewes (36.3%). PMS was significantly influenced by teat size ($r = 0.177$ and 0.113, respectively; $P < 0.001$), udder attachment ($r = -0.205$; $P < 0.001$) and general udder shape ($r = -0141$; $P < 0.001$). From the study, it could be concluded that in Slovakia it will be necessary to additionally use data from linear udder evaluation (mainly udder depth, teat position, teat size and udder attachment) for breeding of dairy sheep breeds.*

Introduction

Udder morphology is an important trait to consider in both dairy and meat sheep production systems (Martinez et al., 2011; Pourlis, 2011; Pourlis, 2020; Kapusi et al., 2015; Ivanova and Raicheva, 2019; Seker et al., 2022, 2024). Udder and teat characteristics can affect milking ability (whether milked by hand, machine or lambing), disease incidence (such as mastitis) and production levels (both milk and meat) (Rovai et al., 2008; Hassoun et al., 2016; Knuth et al., 2021; Dzidic et al., 2019, 2022).

The morphology of the ewe's udder has been well studied in dairy breeds (Marie-Etancelin et al., 2005; Kominakis et al., 2009; Casu et al., 2010; Gelasakis et al., 2012; Ivancia et al., 2023), and in recent years interest has also increased in breeds reared primarily for meat production (Purfield et al., 2017; Suliman et al., 2021). Reasonably full, spherical and deep udders are often preferred as an indication of good milk production (Pieramati et al., 2011), but only up to an intermediate level: udders that become too deep are more difficult to milk, more difficult for lambs to access and often more prone to injury. The attachment of the udder at the top and the bottom to the abdomen should be wide and strong (De la Fuente et al., 1996; Fernández et al., 1997). Other examples include teat placement and size. The preferred teat placement and angle in dairy systems allow easier access for mechanical milking while still allowing lambs to suckle. In meat systems (Martinez et al., 2011; Karakus et al., 2024), the main priority is to allow lambs to suckle, but other features such as protection from the elements are sometimes also considered important. Teat size is also an important characteristic to consider, as teats that are too large or too small may not fit into the mechanical milking cup or may affect the ease with which lambs can latch on to the teat.

The evaluation of udder morphology can be carried out by both direct measurements and subjective scoring of various morphology related traits using linear scoring systems (Dzidic et al., 2004). A number

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of different scoring systems have been developed, focusing on both udder- and teat-related traits that are easy to score and repeatable, such as: udder depth, udder attachment, teat placement, teat angle and teat shape, to name a few examples (Milerski et al., 2019). In most dairy sheep breeding programmes, the four udder traits that are considered most important are udder depth, udder attachment, teat angle and teat length (De la Fuente et al., 1996; Casu et al., 2010; Gelasakis et al., 2012).

Selection for milk yield alone leads to a deterioration in udder morphology (Bruckmaier et al., 1997), so it is important to include udder morphology traits in dairy sheep breeding programmes to prevent this. Some udder traits have also been found to be phenotypically and genetically related to milking performance and udder health; therefore, improvements in udder morphology will also help to improve milking performance and reduce the incidence of mastitis in livestock.

The impact of poor udder morphology can be significant in both dairy and meat sheep systems. Problems associated with poor morphology and milking systems include slow parlour throughput, injury due to changes in milking pressure or over milking, and infection. Similarly, in meat production systems, poor udder morphology prevents lambs from suckling properly, which affects their growth rate and can result in injury and infection to the ewe. When udder morphology is more favourable, ewes are less susceptible to infection, remain in the flock longer, culling and replacement rates are reduced and production levels in terms of milk and meat are improved (Rovai et al., 2008).

The aim of this work was to obtain an overview of the most important parameters to be evaluated for breeding dairy sheep breeds. Selected traits of linear evaluation of udder and milkability of ewes of the Tsigai, Improved Valachian, Lacaune breeds and their crosses were analysed to find out to what extent the selected traits of milk production and milkability depend on udder morphology.

Materials and methods

The udder morphology of ewes of 3 purebred breeds (Tsigai (T), Improved Valachian (IV) and Lacaune (LC)) and 5 types of their crosses (IVxLC (37.5% LC), IVxLC (50% LC), IVxLC (75% LC), TxLC (50% LC), TxLC (75% LC)) was measured during the milking period of 2002–2004 (number of ewes = 286; some ewes were measured several times).

We scored the following traits on a 9-point linear scale:

- Udder depth (UD-LA) – or udder height – measured as the distance between the posterior insertion and the base of the udder;
- Cistern depth (CD-LA), which is the distance between the base of the udder and the bottom of the cistern;
- Teat position (TP-LA), which is the angle of the teat attachment to the udder;
- Teat size (TS-LA), which is the length and diameter of the teat;
- Udder cleft (UC-LA), which is the depth of the groove between the udder halves;
- Udder attachment (UA-LA)–, which is the circumference of the udder attachment to the abdominal wall;
- General udder shape (GSU-LA), which is the overall shape of the udder, including its width and height.

We also recorded selected parameters that characterise the milk yield and milkability of ewes in individual control measurements. We studied the following parameters (in mL): amount of milk milked by machine within 30 and 60 seconds (MY30s; MY60s), machine milk yield (MMY), machine stripping (MS), total milk yield (TMY) and percentage of machine stripping (PMS), proportion of MY30s in TMY (PMY30s) and proportion of MY60s in TMY (PMY60s).

To analyse the primary data of all the variables (583 measurements for each parameter), we used the linear model with fixed effects, taking into account the factor genotype (8 levels), parity (3 levels), control year*period of milking (6 levels) and days in milk as a covariate. Partial correlation coefficients were calculated on the residuals after data adjustment by the above-mentioned linear model of covariance analysis. The SAS statistical package (SAS/STAT, 1999-2001), GLM and CORR procedures were used for the calculations.

Results

All parameters studied (Table 1) were significantly influenced by genotype ($P < 0.001$). As seen in Table 1, most parameters were also significantly influenced by the effect of parity. According to the linear evaluation, ewes in the third lactation had significantly greater udder depth (5.57 points), depth of the cistern (5.32 points), larger teats (4.90 points) and a more horizontal position of the teats (5.48 points) than ewes in the first lactation (4.51; 4.89; 4.27, respectively; $P < 0.05$ to 0.001). Ewes in the first lactation had better milk ejection (based on the amount and proportion of milk milked in 30 and 60 seconds) and lower PMS than ewes in the third lactation ($P < 0.01$ to 0.001).

When comparing purebred ewes of T, IV and LC breeds, LC ewes had the greatest udder depth (UD-LA = 6.19), followed by IV (4.55), and the lowest depth was found in T ewes (3.68; Table 1). The differences were highly significant ($P < 0.001$). On the other hand, the worst teat position was in LC ewes (TP-LA = 5.76), followed by IV ewes (4.58), and the best position was in T ewes (4.52). The differences between T and LC ewes were highly significant ($P < 0.001$). Teat size was significantly larger in IV

Table 1. Estimates of mean values (LSM) of selected morphological and functional traits of udder in sheep in dependence on their genotype. No = 583 measurements for each parameter

Trait	Genotype							
	IV	IVxLC (37.5% LC)	IVxLC (50% LC)	IVxLC (75% LC)	T	TxLC (50% LC)	TxLC (75% LC)	LC
UD-LA	4.55	4.37	5.76	5.76	3.68	5.18	4.86	6.19
UD (mm)	13.70	14.10	17.46	16.96	12.22	15.05	15.22	18.52
CD-LA	4.08	4.65	5.76	5.13	4.11	5.82	5.43	5.98
CD (mm)	1.91	2.16	3.50	2.94	1.59	2.91	2.33	3.36
TP-LA	4.58	4.83	5.60	5.49	4.52	5.95	5.18	5.76
TP-st.	40.99	42.65	45.79	45.94	40.06	50.51	41.95	46.27
VC-LA	4.94	5.15	4.66	4.68	4.14	4.54	4.35	4.50
TS (mm)	3.73	3.75	3.50	3.56	3.38	3.39	3.63	3.47
UC-LA	5.07	5.08	5.58	4.83	4.82	4.99	3.82	4.32
UA-LA	5.61	5.83	5.64	5.71	4.95	5.49	4.86	5.33
GSU-LA	5.24	5.67	5.83	5.89	4.15	5.68	5.34	5.73
MY30S	231.7	194.6	240.09	230.70	183.7	233.1	220.2	243.7
MY60S	305.0	273.1	351.4	343.4	210.9	288.7	321.6	345.0
MMY	307.1	279.7	378.4	371.3	211.2	302.2	361.1	355.8
MS	100.3	107.8	136.5	130.5	79.4	114.9	95.2	180.5
TMY	407.4	387.6	514.8	501.8	290.6	417.1	456.3	536.4
PMS	26.0	35.7	27.5	28.8	27.2	28.2	23.5	36.3
PMY30S	58.1	45.8	49.2	46.6	64.5	58.4	51.0	45.2
PMY60S	73.6	63.3	68.4	66.8	72.6	70.0	71.1	62.0

Abbreviations: Tsigai (T), Improved Valachian (IV), Lacaune (LC), Udder depth (UD), Linear assessment (LA), Cistern depth (CD), Teat position (TP), Teat size (TS), Udder cleft (UC), Udder attachment (UA), General udder shape (GSU), Teat angle (TA), Teat length (TL), Machine milk yield milked within 30 seconds (MY30s), Machine milk yield milked within 60 seconds (MY60s); Machine milk yield (MMY), machine stripping (MS), Total milk yield (TMY) and Percentage of machine stripping (PMS), Proportion of MY30s in TMY (PMY30s) and Proportion of MY60s in TMY (PMY60s).

ewes (TS-LA = 3.73) than in LC (3.47) and T ewes (3.38; $P < 0.001$). The crosses IV x LC and T x LC with 50% and 75% genetic content had larger udders, larger udder cisterns but a worse teat position.

LC ewes had the highest TMY (536.4 mL; Table 1) but only the fourth best MMY (355.8 mL). However, it was higher than in purebred T ewes (290.6 mL) and IV ewes (407.4 mL). Among the purebred breeds, the proportion of machine stripping (PMS) was lowest in IV ewes (26.0%), followed by T ewes (27.2%), and highest in purebred IV ewes (36.3%). The lowest value for PMS is an advantage of the breed, as a low value for this trait is expected.

The proportion of milk milked within 30 and 60 seconds from TMY was highest in T ewes (64.5% and 72.6%, respectively), followed by IV ewes (58.1% and 73.6%, respectively), and lowest in LC ewes (45.2% and 62.0%, respectively).

Table 2 shows that udder depth was highly significantly correlated with machine milk (r = 0.296 and 0.314, respectively) and total milk yield (r = 0.465 and 0.518, respectively; $P < 0.001$) as well as with the amount of milk milked within 30 and 60 seconds. The proportion of machine stripping was highly significantly influenced by teat size (r = 0.177 and 0.113, respectively; $P < 0.001$). The larger the teat, the higher the proportion of machine stripping. Teat position had no effect on PMS in our experiment. In contrast, both milk yield (MMY, TMY) and milkability (MY30s, MY60s, PMS) were highly (significantly) dependent on udder attachment and general teat shape ($P < 0.001$) (Table 2). The better the teat attachment, the lower the PMS (r = –0.205; $P < 0.001$) and the better the general udder shape score, the lower the PMS (r = –0.141; $P < 0.001$). Our results show that the improvement of the native breeds T and IV by the breed LC increases not only the udder size but also the milk production (MMY and TMY) in the created crosses. However, it is mainly the teat position that deteriorates in combination with larger udder cisterns. Traits related to milkability (PMS, PMY30s, PMY60s) are slightly worse in the crosses than in purebred T and IV ewes, with the worst being in the LC breed.

Table 2. Residual correlations among traits of milkability and linear assessment and measures of udder in sheep

Trait	MY30S	MY60S	MMY	MS	TMY	PMS	PMY30S	PMY60S
UD-LA	0.227+++	0.251+++	0.296+++	0.355+++	0.465+++	0.052ns	–0.115++	–0.116++
UD-mm	0.182+++	0.265+++	0.314+++	0.429+++	0.518+++	0.079ns	–0.186+++	–0.145+++
CD-LA	0.148+++	0.156+++	0.153+++	0.77ns	0.184+++	–0.058ns	0.022ns	0.055ns
CD-mm	0.184+++	0.219+++	0.206+++	0.184+++	0.289+++	–0.028ns	–0.013ns	0.034ns
TP-LA	0.067ns	0.191+	0.094+	0.095+	0.139+++	–0.003ns	–0.043ns	–0.008ns
TP-st.	0.063ns	0.035ns	0.031ns	0.066ns	0.064ns	–0.019ns	0.034ns	0.016ns
TS-LA	0.125++	–0.137+++	–0.128++	0.134++	–0.049ns	0.177+++	–0.113++	–0.174+++
TS-mm	–0.95+	–0.148+++	–0.144+++	0.52ns	–0.107++	0.113++	–0.009ns	–0.106+
UC-LA	0.074ns	0.088+	0.079ns	–0.009ns	0.069ns	–0.057ns	0.032ns	0.062ns
UA-LA	0.345+++	0.363+++	0.334+++	–0.033ns	0.296+++	–0.205+++	0.124++	0.213+++
GSU-LA	0.402+++	0.396+++	0.383+++	0.114++	0.419+++	–0.141+++	0.095+	0.134++

Abbreviations: Udder depth (UD), Linear assessment (LA), Cistern depth (CD), Teat position (TP), Teat size (TS), Udder cleft (UC), Udder attachment (UA), General udder shape (GSU), Machine milk yield milked within 30 seconds (MY30s), Machine milk yield milked within 60 seconds (MY60s); Machine milk yield (MMY), machine stripping (MS), Total milk yield (TMY) and Percentage of machine stripping (PMS), Proportion of MY30s in TMY (PMY30s) and Proportion of MY60s in TMY (PMY60s).

Discussion

Mačuhová et al. (2008) studied the correlation coefficients between the parameters of udder traits and milking traits of the sheep breeds Tsigai, Improved Valachian and their crosses with Lacaune. Total milk yield and machine milk yield were significantly correlated with all udder traits and milking trait parameters except machine stripping and milk flow latency. Milk flow latency showed positive correlations with milking time and negative correlations with maximum peak flow.

The positive and significant correlation was also found between the teat position and cistern depth. The same effect was also observed between the teat angle and cistern depth (mm) in the flock of Mačuhová et al. (2008), and also in Manchega (Rovai et al., 1999) and East Friesian ewes (McKusick et al., 2000).

However, udders with deeper cisterns and larger teat angles may have a problem with cups falling off during milking (Labussiere, 1988) and an increase in stripping milk yield due to a part of the cisternal milk which is located below the opening into the teat canal and cannot be reached without machine stripping (Mačuhová et al., 2008; Bruckmaier et al., 1997; Carta et al., 1999). However, this may prolong the milking time and thus reduce the efficiency of machine milking. The ewes in the study by Mačuhová et al. (2008) were not high-yielding ewes, but positive correlations of total milk yield with cistern depth and teat position were observed in these sheep. This may indicate that further breeding for higher milk production may lead to deterioration of udder morphology (Mačuhová et al., 2008).

In a study by Beal et al. (1990), the correlation of average weigh-suckle-weigh and average MMY estimates of milk production with preweaning calf gain were high and similar (greater than 0.75) in beef cows. Inclusion of milk composition did not improve the multiple correlation of MMY-estimated milk production and calf gain.

Peris et al. (1996) studied the milkability of Murciano-Granadina dairy goats. As in sheep, milk flow and total machine milk (but not milking time) were influenced by parity, with second and third lactation goats having higher values. Positive correlations were found between daily milk yield and milk flow characteristics. Residual milk was positively correlated with machine stripping, but not with machine milk yield.

Rovai et al. (2008) found that Lacaune ewes had greater udder depth and cistern height, whereas Manchega ewes had longer and wider teats. Özyürek (2020) investigated the relationship between lactation traits, milk components and udder morphology in 34 Morkaraman and 32 Awassi sheep. While breed had a statistically significant effect on lactation milk yield and lactation length, age had a statistically significant effect on all lactation traits ($P< 0.05$). In contrast to Morkaraman, a positive correlation was found between udder circumference and both lactation milk yield and average daily milk yield, and also between udder length and both lactation milk yield and average daily milk yield in Awassi. There was a higher correlation between udder traits and lactation traits in Awassi than in Morkaraman (Özyürek, 2020). Similar to the findings of this study, Şeker et al. (2022) reported positive correlations between the lactation period and milk yield values of Awassi ewes. Except for the correlation coefficient between daily average milk yield and the lactation period in this study, all correlation coefficients calculated were found to be positive and statistically significant.

Arcos-Álvarez et al. (2020) found an acceptable correlation (r = 0.60) between udder measurements,

udder volume and daily milk yield in Pelibuey sheep. When direct measurements of milk production are not possible in practice, the measurement of udders and their volume could be a viable alternative to estimate milk yield production as an indirect method. Özyürek (2020) recommended new studies on the rate of milking from the alveolar and cistern areas in machine milking.

Conclusion

Linear evaluation and precise udder measurements showed that Tsigai and Improved Valachian ewes had smaller udders with smaller cisterns and a better teat position than Lacaune ewes. The proportion of machine stripping (PMS) was best in the Improved Valachian ewes (26.0%) among the purebred breeds, followed by the Tsigai ewes (27.2%), and highest in the purebred Lacaune ewes (36.3%). PMS was significantly influenced by teat size, udder attachment and general udder shape. It could be concluded that in Slovakia it will be necessary to additionally use data from linear udder evaluation (mainly udder depth, teat position, teat size and udder attachment) for breeding of dairy sheep breeds.

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Influence of Growth Retardation of Heifers on the Development, Production, Duration and Efficiency of Productive Lifespan of Dairy Cows

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Abstract. *It was established that the age at first calving of dairy cows, their milk production, reproductive traits, duration and efficiency of productive lifespan depend on the growth intensity of replacement heifers. Heifers with growth retardation in any period up to 1 year of age are characterized by an older age at first calving (by 4.7–7.9% depending on the age at which growth retardation was observed), lower live weight in the first year of life (by 4.1–24.4%), first lactation milk production (milk yield by 6.1–13.2%, milk fat yield by 7.4–13.1%, milk protein yield by 8.7–17.3%) and lifetime milk production (lifetime milk yield by 12.1–25.9%, milk fat + milk protein yields by 13.1–26.3%), as well as lower milk yield, milk fat and milk protein per one day of life, productive lifetime and lactation (by 8.6–21.1%) compared with animals without growth retardation. Animals with growth retardation are inferior to animals without growth retardation on the investigated traits, even after the subsequent elimination of retardation.*

Introduction

During the second half of the 20th century, significant progress was made in the genetic improvement of dairy cattle due to the selection based on animals' pedigree, production traits, and progeny testing (Brotherstone and Goddard, 2005). In recent years, the emphasis on the assessment of the genetic component has increased not only on the production traits of cows, but also on their growth, exterior, health, fertility, feed conversion efficiency, and survival in the herd (Egger-Danner et al., 2015; Stavetska, 2017), that is, the improvement of dairy cattle increasingly focuses on functional traits (Jenko et al., 2015). Functional traits characterize the milk production efficiency due to the reduction of its cost (Groen et al., 1997) and they are indicators of selection process effectiveness and dairy cattle welfare (De Vries and Marcondes, 2020). Boichard and Brochard (2012) and Fuerst-Waltl et al. (2016) believe that finding a balance between milk production and functional traits will make it possible to extend the lifespan, improve the type and strength of dairy cows.

An important component for creating high-performing dairy herds is targeted growth of replacement heifers. Productive and reproductive performances of dairy cows, their health, longevity and lifetime productivity depend on the quality of young animals (Osten-Sacken, 2005). The cost of raising dairy replacements is quite high and amounts to 15–20% of all expenses on a dairy farm (the first place is taken by cattle feed, and the second place is shared by raising heifers and wages). For example, in the USA, the cost of heifer rearing from their birth to calving is about $2300. Therefore, it is important in a dairy herd to provide the rearing of healthy heifers with optimal growth intensity while simultaneously reducing veterinary costs (Heinrichs, 1993).

The live weight of dairy cattle in the corresponding periods is gaining more and more economic value, as it is related to growth intensity of animals, reflects the conformity of the environmental conditions of the animal body, and determines the value of the carcass after culling and slaughter (Byrne et al., 2016). There is a correlation between the intensity of heifers' growth, their live weight at different age periods and future milk production. In some cases, the value of the correlation coefficient is up to +0.40 (Van Amburgh et al., 1998; James, 2001; Cooke et al., 2013; Polupan et al., 2018). The influence of live weight of heifers on their subsequent milk production is 8.21–42.87% depending on the age and number of lactations, but this relationship is mostly curvilinear (Sieber et al., 1988; Fedorovych, 2004). There is greater reliability between milk yield and live weight of heifers at 1–3 months of age, and it is practically absent at 12 months of age (Zabludovskyi and Golubchuk, 2002).

A direct genetic correlation has also been found between calf birth weight and their weight at first insemination (r = 0.31) (Yin and König, 2018), milk production, reproductive traits (Ghoraishy and

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Rokouei, 2013), as well as an inclination to mastitis and feet disorders (Brotherstone et al., 2007). Therefore, the correlation between live weight of heifers and other traits indicates the possibility of its use for early indirect selection to improve economically useful traits of cows.

The importance of raising dairy replacement heifers is confirmed by studies conducted in Australia. It has been established that heifers that reach the target live weight faster (85% of the average mature cow weight) are characterized by better development of reproductive organs. That is why they are mated earlier. These heifers dominate by live weight at first calving, milk production and productive lifetime. Primiparous cows with a 50 kg higher live weight had a higher milk yield by 1041 kg, milk fat yield by 38.5 kg, and milk protein by 42.5 kg over the first three lactations (Beggs and Jagoe, 2013).

Brickell et al. (2009) have reported that 14.5% of live-born dairy heifers did not remain in the herd until the first lactation; on average, 6.8% of heifers died or they were culled during the first six months of life. This causes significant losses for milk producers. Recently, Hyde et al. (2020) have found that mortality of dairy calves in the UK in the first three months of life is 6%, and that this rate has not changed much since the 1990s. Thus, over the last 30 years, this question has remained relevant.

Effective rearing of replacement heifers is determined by the compliance of their live weight with the breed standard in a definite age. However, in practice, some animals may lag behind in growth during the rearing period for various reasons like morbidity, insufficient or substandard feeding, violation of technology, etc. Genetic factors should not be excluded from this list. In the future, biological mechanisms of compensatory growth can contribute to the achievement of normal development of animals before the beginning of their reproductive and productive use (Busenko and Golub, 2010; Karapuz and Karapuz, 2010; Klimkovetskyi et al., 2020). However, according to the Chirvinsky-Maligonov law, during the period of growth retardation, the internal organs that develop most intensively during this period may suffer. Compensation for growth retardation may be incomplete and does not allow realizing the genetic potential for performance (Chirvinsky, 1949; Maligonov, 1968; Pelykh and Levchenko, 2012; Polupan, 2016; Klimkovetskyi et al., 2020). Thus, in the research of Klimkovetskyiet al. (2020), it has been established that growth retardation of heifers at an early age is compensated for more slowly. Under the conditions of live weight compensation before the beginning of reproductive use, growth retardation does not affect the milk production of primiparous cows, but productive lifetime and lifetime production of cows decrease (Klimkovetskyi et al., 2020).

This study aimed to investigate the effect of replacement heifers' growth retardation from birth to 12 months of age on their growth, performance, duration and efficiency of productive lifespan.

Material and methods

The research was conducted in a retrospective statistical experiment in the herd of Ukrainian Black-and-White Dairy and Holstein breeds in Breeding Station Terezyne, which is located in Kyiv region, Ukraine. The materials from electronic database Dairy Management System ORSEK were used. In total, 930 cows from their birth to culling were included in the study. To evaluate growth, milk and reproductive traits, duration and efficiency of productive lifespan, five groups were formed: control (without growth retardation) and four experimental groups with growth retardation at the age of 0–3, 3–6, 6–9 and 9–12 months. Growth retardation of heifers was considered to be less than 500 g of average daily gain for the corresponding three-month period.

Animal growth was studied by their live weight (kg) at the age of 6, 12 and 18 months of age and average daily gain (g) from birth to the age of 1.5 years; milk production – 305-day first and second lactations milk yield (kg), milk fat concentration (%) and yield (kg), milk protein concentration (%) and yield (kg); reproductive traits – age at first calving (days), calving-conception interval (days), reproductive capacity coefficient (%),which is defined as a number of days per year (365) divided by calving interval.

A retrospective analysis of duration and efficiency of productive lifespan of cows was carried out according to our methodology (Polupan, 2010, 2014). In particular, lifespan, productive lifetime, total lactation length (days), lifetime number of lactation, lifetime milk yield, fat yield and protein yield (kg), lifetime, productive life and total lactation daily milk yield (kg), daily milkfat and daily milk protein yields (g) of cows were studied.

The calculations were performed by methods of parametric statistics (Osadcha and Shanaieva-Tsymbal, 2022) with the software package Statistica 12.0 (Fetisov, 2018). The investigated characteristics in the groups were estimated by calculating the arithmetic means ($\overline{x}$) and their standard errors (± S.E.). The level of statistical significance of the difference in group means ($d = \overline{x}_2 - \overline{x}_1$) was determined by calculating the reliability criterion Student $t_d = \frac{d}{S.E._d} = \frac{(\overline{x}_2 - \overline{x}_1)}{\sqrt[2]{S.E._1^2 + S.E._2^2}}$ by its further comparison with standard values. The reliability of the results was compared with three standard levels of statistical significance with their designation $P < 0.05$, $P < 0.01$, $P < 0.001$.

Results

By comparing the means between the groups, it was established that heifers of all experimental groups had lower live weight at 6, 12, and 18 months of age compared with heifers of the control group (Table 1).

Table 1. Growth and reproductive traits of cows with growth retardation in different rearing periods (Mean ± S.E.)

Parameters 0–3		Groups with growth retardation in period, *age interval in months*				Group without growth retardation (control)
		3–6	6–9	9–12		
Number of cows		48	47	60	72	930
Live weight *(kg)* at the age, *months*	6	135 ± 1.5[c]	127 ± 1.4[c]	154 ± 2.4[c]	166 ± 2.3	168 ± 0.5
	12	261 ± 2.8[c]	259 ± 3.1[c]	252 ± 2.9[c]	267 ± 3.0[c]	288 ± 0.6
	18	371 ± 6.3[2]	376 ± 4.6[b]	375 ± 2.9[c]	370 ± 3.5[c]	392 ± 0.9
Average daily gain *(g)* at the age, *months*	0–3	451 ± 6.4[c]	589 ± 10.4[c]	652 ± 16.0[c]	702 ± 15.1	726 ± 3.1
	3–6	647 ± 15.4[c]	423 ± 9.2[c]	650 ± 13.9[c]	725 ± 12.2	707 ± 2.6
	6–9	666 ± 16.6	693 ± 23.2	410 ± 9.5[c]	674 ± 10.8	682 ± 2.6
	9–12	722 ± 19.1[c]	755 ± 19.5[c]	661 ± 13.9	435 ± 8.6[c]	640 ± 3.0
	0–12	622 ± 7.7[c]	615 ± 8.7[c]	593 ± 7.8[c.]	634 ± 8.0[c]	689 ± 1.7
	12–18	631 ± 24.6[a]	670 ± 25.3[c]	667 ± 15.6[c]	562 ± 15.3	569 ± 3.5
Age at first calving, *days*		865 ± 12.2[b]	872 ± 10.0[c]	891 ± 14.0[c]	877 ± 15.4[c]	826 ± 3.8
Calving-conception interval, *days*		150 ± 14.0[a]	141 ± 17.9[a]	180 ± 20.6	169 ± 19.4	181 ± 4.9
Reproductive capacity coefficient		0.887 ± 0.026	0.916 ± 0.027[a]	0.857 ± 0.029	0.871 ± 0.024	.847 ± 0.007

Note: as compared with control group [a] – $P < 0.05$; [b] – $P < 0.01$; [c] – $P < 0.001$.

In particular, the live weight of heifers with growth retardation at 0–3 months was lower compared with the control group by 21–33 kg or 5.4–19.7%, at 3–6 months by 16–41 kg or 4.1–24.5%, at 6–9 months by 14–36 kg or 4.4–12.5%, at 9–12 months by 2–21 kg or 5.4–7.3% (in all cases $P < 0.001$ or $P < 0.01$).

The average daily gain of heifers with growth retardation was lowest compared with the control group during the retardation period. Heifers with growth retardation from birth to 3 months had lower average daily gain in this period compared with the control group by 275 g or 37.9%, at 3–6 months by 284 g or 40.2%, at 6–9 months by 272 g or 39.9%, and at 9–12 months by 205 g or 32.0%. In all cases at the highest level of statistical significance of the reliability, the difference in means was observed. Despite a rather high average daily gain of heifers in other corresponding periods, which in many cases exceeded the average daily gain in the control group, the mechanisms of compensatory growth did not ensure the achievement of its level in the first year of postnatal growth, as in the analogue in the control group. Heifers of the experimental groups were inferior to the animals of the control group in average daily gain from birth to one year of age by 55–96 g or by 8.0–13.9%. Significantly higher growth intensity of heifers of most experimental groups after one year of age also did not provide full compensation of growth retardation to 18 months of age.

Heifers of breeding age with growth retardation in different periods of the first year of postnatal growth were characterized by a slightly older age at first calving (by 39–65 days) compared with animals of the control group ($P < 0.001$ or $P < 0.01$). At the same time, the primiparous cows in experimental groups had a shorter calving-conception interval (by 1–40 days) and, as a result, a higher reproductive capacity coefficient (by 0.010–0.069).

Despite the older calving, the primiparous cows of experimental groups with growth retardation in different rearing periods showed less 305-day milk yield and milk fat and protein yields compared with the control group (Table 2).

The greatest decrease of milk production was noted for groups with growth retardation after weaning and attaining puberty phases. In the group with growth retardation in the period of 3–6 months, the decrease in milk yield compared with the control group was 880 ± 206.6 kg or 13.2% ($t_d = 4.26$, $P < 0.001$); at 6–9 months, it was 557 ± 163.6 kg or 8.4% ($t_d = 3.40$, $P < 0.001$). Milk fat yield was lower respectively by 32.7 ± 7.80 kg or 13.1% ($t_d = 4.19$, $P < 0.001$) and 22.1 ± 6.27 kg or 8.9% ($t_d = 3.52$, $P < 0.001$), milk protein yield by 37.7 ± 6.78 kg or by 17.3% ($t_d = 5.56$, $P < 0.001$) and 24.8 ± 5.85 kg or by 11.4% ($t_d = 4.24$, $P < 0.001$). Milk protein concentration in the groups with growth retardation was lower by 0.06–0.16% ($P < 0.001$) than in the control group.

As a result, the negative effect of growth retardation during different periods of the first year of postnatal growth does not reveal a prolonged effect on 305-day second lactation. Second lactation cows with growth retardation in the periods of 0–3 and 3–6 months were characterized by even slightly higher milk yield compared with the control group (+46–224 kg). The only exception was cows with growth retardation in 9–12 months, which had significantly lower milk yield (by 664 ± 230.4 kg, $t_d = 2.88$, $P < 0.01$), milk fat yield (by 25.9 ± 8,82 kg, $t_d = 2.94$, $P < 0.01$) and milk protein yield (by 25.7 ± 7.77 kg, $t_d = 3.31$, $P < 0.001$).

At the same time, growth retardations in different periods of the first year of heifer rearing have a prolonged effect on the efficiency of productive lifespan of cows (Table 3).

Table 2. Milk production of cows with growth retardation in different rearing periods (Mean ± S.E.)

Parameters 0–3 3–6			Groups with growth retardation in period, *age interval in months*				Group without growth retardation (control)
			6–9	9–12			
Number of cows			48	47	60	72	930
First lactation	milk yield, *kg*		6237 ± 198.9	5762 ± 200.8^{c}	6085 ± 156.2^{c}	6178 ± 163.8^{b}	6642 ± 48.6
	milk fat	%	3.69 ± 0.012^{c}	3.75 ± 0.014	3.72 ± 0.013	3.72 ± 0.009^{b}	3.74 ± 0.003
		kg	230.2 ± 7.49^{a}	216.0 ± 7.57^{c}	226.6 ± 5.98^{c}	230.2 ± 6.14^{b}	248.7 ± 1.87
	milk protein	%	3.13 ± 0.012^{c}	3.15 ± 0.012^{c}	3.17 ± 0.016^{c}	3.23 ± 0.013^{c}	3.29 ± 0.003
		kg	195.6 ± 6.47^{c}	180.8 ± 6.58^{c}	193.7 ± 5.61^{c}	199.5 ± 5.56^{b}	218.5 ± 1.65
Second lactation	milk yield, *kg*		7386 ± 290.9	7208 ± 308.3	7020 ± 350.7	6498 ± 221.2^{b}	7162 ± 64.3
	milk fat yield, *kg*		269.8 ± 10.85	266.7 ± 12.09	261.5 ± 13.51	243.9 ± 8.46^{b}	269.8 ± 2.49
	milk protein yield, *kg*		232.9 ± 9.50	227.1 ± 10.21	226.7 ± 11.59	211.2 ± 7.45^{c}	236.9 ± 2.19

Note: as compared with the control group ^{a} – $P < 0.05$; ^{b} – $P < 0.01$; ^{c} – $P < 0.001$.

Table 3. Duration and efficiency of productive lifespan of cows with growth retardation in different rearing periods (Mean ± S.E.)

Parameters 0–3		Groups with growth retardation in period, *age interval in months*				Group without growth retardation (control)
		3–6	6–9	9–12		
Number of cows		48	47	60	72	930
Duration, *days*	lifespan	2012 ± 113.7	1913 ± 86.9	1945 ± 64.0	2116 ± 79.5	2064 ± 21.3
	productive lifetime	1147 ± 114.9	1041 ± 87.8^{a}	1054 ± 66.8^{b}	1239 ± 83.2	1243 ± 21.9
	total lactation	1008 ± 96.3	934 ± 78.4^{a}	932 ± 57.7^{b}	1091 ± 71.6	1108 ± 18.7
Lifetime number of lactation		3.06 ± 0.259	2.70 ± 0.187	2.72 ± 0.167	3.14 ± 0.210	3.04 ± 0.054
Lifetime production, *kg*	milk yield	18 592±2025.4	16 698±1603.1^{b}	16 191±1125.7^{c}	19 191±1397.3	21 840±340.0
	milk fat yield	685 ± 76.4	619 ± 59.8^{b}	602 ± 42.3^{c}	717 ± 52.3	821 ± 15.1
	milk protein yield	592 ± 68.0	531 ± 52.8^{c}	526 ± 37.9^{c}	626 ± 46.4^{a}	723 ± 13.3
	milk fat + protein yields	1277 ± 144.3	1150 ± 112.6^{b}	1139 ± 81.0^{c}	1343 ± 98.7	1545 ± 28.3
Daily milk yield per cow, *kg*	lifespan	8.3 ± 0.45^{b}	8.1 ± 0.48^{c}	7.9 ± 0.36^{c}	8.5 ± 0.36^{c}	9.9 ± 0.11
	productive lifetime	16.2 ± 0.67^{a}	15.8 ± 0.68^{b}	15.7 ± 0.53^{c}	15.8 ± 0.55^{c}	17.8 ± 0.15
	total lactation	18.0 ± 0.68^{a}	17.5 ± 0.72^{b}	17.5 ± 0.54^{c}	17.6 ± 0.53^{c}	19.7 ± 0.15
Daily milk fat and protein yields per cow, *g*	lifespan	569 ± 32.2^{c}	554 ± 33.5^{c}	553 ± 26.2^{c}	591 ± 25.2^{c}	701 ± 7.6
	productive lifetime	1103 ± 45.9^{b}	1084 ± 46.7^{c}	1090 ± 38.4^{c}	1102 ± 38.8^{c}	1262 ± 10.7
	total lactation	1230 ± 46.8^{b}	1199 ± 49.9^{c}	1217 ± 39.2^{c}	1225 ± 37.5^{c}	1394 ± 11.0

Note: as compared with the control group ^{a} – $P < 0.05$; ^{b} – $P < 0.01$; ^{c} – $P < 0.001$.

The most significant decrease in the duration of lifespan, productive lifetime and total lactation, as well as lifetime number of lactation was noted in groups with low average daily gains in the period from 3 to 9 months. In particular, cows with growth retardation at the age of 3–6 months compared with the control group had a lower lifetime number of lactation by 0.34 ± 0.195 (t_d = 1.74, $P < 0.1$), and at 6–9 months by 0.32 ± 0.176 (t_d = 1.82, $P < 0.1$). In the duration of a lifespan, this difference was 151 ± 89.5 (t_d = 1.69, $P < 0.1$) and 119 ± 70.3 (t_d = 1.69, $P < 0.1$) days, respectively; in the duration of a productive lifetime, this difference was 202 ± 90.5 (t_d = 2.23, $P < 0.05$) and 189 ± 70.3 (td = 2.69, $P < 0.01$) days; in the duration of a total lactation duration, this difference was 174 ± 80.6 (t_d = 2.16, $P < 0.05$) and 176 ± 60.7 (t_d = 2.90, $P < 0.01$) days.

In all experimental groups with growth retardation, a decrease of lifelong milk production indicators was noted. The highest losses in milk production were noticed for groups with growth retardation at 3–9 months of age. Cows with low average daily gains at the age of 3–6 months compared with the control group had less lifetime milk yield by 5142 ± 1638.8 kg (t_d = 3.14, $P < 0.01$), and at 6–9 months by 5649 ± 1175.9 kg (t_d = 4.80, $P < 0.001$), and lifetime milk fat + protein yields by respectively by 395 ± 116.1 kg (t_d = 3.40, $P < 0.001$) and 406 ± 85.8 kg (t_d = 4.73, $P < 0.001$). Losses in daily milk fat and protein yields per cow with growth retardation at 0–3 months of age were 132 ± 33.1 g (t_d = 3.99, $P < 0.001$), at 3–6 months – 147 ± 34.4 g (t_d = 4.27, $P < 0.001$), at 6–9 months – 148 ± 27.3 g (t_d = 5.42, $P < 0.001$), and at 9–12 months of age – 110 ± 26.3 g (t_d = 4.18, $P < 0.001$). Therefore, the growth retardation of heifers at all periods of the first year of postnatal growth causes a significant decrease in the efficiency of a productive lifespan. More significant losses of milk production were observed due to reduced average daily gains after the milk feeding and attaining puberty phases (up to 9 months of age).

Discussion

Literary sources report on the causes that lead to calves' growth retardation and ways to eliminate them. The intensity of calves' growth reflects the compliance of feeding with the needs of the animal's body. If animals were fed unbalanced diets, were not supplied with nutrient needs and suffered from stress or diseases, growth retardation was usually observed (Roland et al., 2016; Shivley et al., 2018). Growth retardation has also been associated with underfeeding during the milk feeding phase, according to the long-established industry standard to restrict milk feeding of calves (Khan et al., 2007; Palczynski et al., 2020), low intake of concentrates by calves at the weaning, regrouping calves more than twice before weaning and a low incidence risk of milk fever (< 5%) (Tautenhahn et al., 2020). A high rate of calf morbidity and, as a result, growth retardation are associated with increasing use of antibiotics and a noticeable increase in antimicrobial resistance (World Health Organization, 2014). Costa et al. (2019) believe that under industrial intensive technology, improving animal welfare, in particular keeping calves in pairs or groups, rather than individually, increasing the amount of milk in the milking phase of rearing to avoid suffering calves from hunger, and using local anesthesia during dehorning would help reduce growth retardation of calves.

Wathes et al. (2014) believed that optimal average daily gain of dairy replacement heifers during the rearing period was 750 g. Soberon et al. (2012) reported it to be 660–820 g, Van Amburgh et al. (1998) found that it was 400–800 g before puberty, while Lytvynenko (2010) and Poslavska et al. (2016) recorded 650–700 g during the entire rearing period, including700–800 g at the age of 0–6 months, 600–700 g at 6–12 months, and 550–600 g at 12–24 months. If average daily gain is lower, heifers reach puberty and age at first calving later (Sumner and Keyserlingk, 2018). The current study found that the average daily gain of heifers in the control group was 716 g up to 6 months of age, 661 g at the age of 6–12 months, and 560 g at 12–18 months. This result is in line with what has been described by Van Amburgh et al. (1998), Lytvynenko (2010) and Poslavska et al. (2016). The average daily gain of heifers with growth retardation was lower by 32.5–40.2%, depending on the age of the calves.

The optimal age at first calving in dairy cattle varies from 23 to 25 months (Do et al., 2013; Wathes et al., 2014). Cook et al. (2013) and Wathes et al. (2014) called the target age at first calving for dairy cattle to be 24 months. Under these conditions, optimal economic efficiency in dairy farming can be achieved due to high lifetime fertility of cows, high survival rates, and high milk production, compared with heifers with an older age at first calving. At the same time, Kalińska et al. (2019), Haworth et al. (2008) and Frejlach et al. (2015) reported that the highest first lactation yield, lifetime milk yield, lifetime milk fat and milk protein yields were produced by cows between 24 and 28 months of age at first calving and even later. The age of cows at the first calving in the current study in the control group was within these limits – 27 months. Cows of all groups with growth retardation during the rearing period were 28–29 months at the age at first calving. The oldest age at first calving observed in cows with growth retardation was 6–9 months, that is, during the period of intensive puberty.

Rational rearing of replacement heifers is an important factor that determines the subsequent milk production of cows. Numerous studies have established the dependence of milk production on live weight of animals during their rearing period (Bazeley et al., 2016; Bondarchuk, 2016; Heinrichs et al., 2017). According to Shuliar et al. (2020), in the herd of the Ukrainian Black-and-White dairy breed, newborn heifers with 32–33 kg of live weight,166–175 kg at the age of 6 months, 281–290 kg at 12 months, and 381–390 kg at 18 months were characterized by higher milk production (milk yield, milk fat and protein yields, and their total amount). Similar results were obtained in our study in the control group.

Prishedko et al. (2017) have reported that higher milk production was associated with higher average daily gain. According to the results of the current study, Holstein cows with a higher average daily gain up to 18 months of age prevailed in 305-day first lactation milk yield by 1093.0 kg (28.06%, $P < 0.001$), and milk fat yield by 40.60 kg (28.93%, $P < 0.001$). It is obvious that animals in the control group and with an earlier age at first calving (27

months in the current study) were characterized by higher milk production. It was established that 305-day first lactation milk yield in the groups of cows with growth retardation, compared with the control group, was lower on average by 576 kg (8.6%), milk fat yield by 23.0 kg, and milk protein yield by 26.1 kg. The milk protein concentration in groups with growth retardation was lower compared with control by 0.06–0.16%. It was in line with a statement of Van Amburgh et al. (1998), who noted that milk yield of primiparous cows with low average daily gain during the rearing period was lower by 10–40%.

Earlier in our research, it was found that duration and efficiency of a productive lifespan of cows depends on growth intensity of heifers (Siryak et al., 2021). In particular, it was established that the highest duration and efficiency of a productive lifespan was observed in cows with live weight at the age of 6 months higher than 160 kg with an average daily gain up to 6 months above 700 g. In this study, live weight and average daily gain within these limits were found in cows in the control group and with growth retardation at the age of 9–12 months. Cows of these groups had a higher productive lifetime by 94–200 days, lifetime milk yield by 1923–4324 kg, lifetime milk fat yield by 84–194 kg, lifetime milk protein yield by 82–148 kg, and lifetime milk fat + protein yields by 167–305 kg.

Consequently, growth retardation of heifers had a negative effect on their rearing efficiency, reproductive traits, milk production and lifetime efficiency. The exceptional importance of the period from birth to 9 months of age in dairy heifers rearing was highlighted. This is in line with research results of Klimkovetskyi et al. (2020).

Conclusions

It was established that growth retardation of heifers in different periods from birth to 12 months of age had irreversible effects on productive traits of cows. Lower growth intensity, milk and reproductive performance were found in groups with growth retardation from birth to 9 months of age, that is, during milk feeding and attaining puberty phases. Cows with growth retardation were characterized by lower live weight at the age of 1 year (on average by 28 kg) and 1.5 years (by 19 kg), older age at first calving (by 50 days), lower first lactation milk yield (by 576.5 kg), milk fat yield (by 22.9 kg) and milk protein yield (by 26.1 kg), as well as lower lifetime milk yield (by 4172 kg), lifetime milk fat yield (by 165 kg), lifetime milk protein yield (by 154 kg) and milk fat and milk protein per one day of life (by 134 g), productive lifetime (by 167 g) and lactation (by 176 g) compared with the control group.

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Investigations on Effect of Bacillus *Licheniformis* BL11 Probiotic Formula on Antimicrobial Resistance in Commensal Poultry *E. Coli* Isolates

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Key words: *resistance, antimicrobial drugs, probiotics, commensal Escherichia coli, poultry*

Abstract. *The aim of the present study was to investigate phenotype resistance profiles and some genetic determinants in resident E. coli bacteria isolated from broilers whose ration was supplemented with a probiotic formula containing a Bacillus licheniformis BL11 strain (Huvepharma, Belgium).*

For bacteriological examination, cloacal swabs were collected at various intervals throughout the study: from day-old, 14-day-old and 28-day-old chickens. A total of 300 swabs were collected for bacteriological examination: 150 from control and 150 from probiotic-supplemented chickens. The total number of E. coli strains isolated from control and experimental broilers was 214: 107 strains from the control group and another 107 from birds that received a probiotic with the feed.

Among E. coli isolates from day-old broilers in the experimental group, the highest resistance rate was observed against gentamicin (69.0%), followed by that against ampicillin and amoxicillin/clavulanic acid (52.4%). Escherichia coli isolated from probiotic-supplemented broilers at 14 days of age demonstrated statistically significantly higher rate of resistance ($P \leqslant 0.05$) against cefotaxime (51.6%) and ceftazidime (38.7%) compared with isolates from the control group (12.0%). Also, the prevalence of E. coli strains resistant against amoxicillin/clavulanic acid in supplemented broilers (35.5%) was insignificantly lower than the respective rate in control chickens (48.0%). At 28 days of age, the resistance against ciprofloxacin in poultry E. coli isolates in probiotic-fed broilers was significantly higher ($P \leqslant 0.01$) than the resistance rate in non-supplemented birds (85.3% and 52.7%, respectively). The resistance to ampicillin among the isolates from experimental broilers was statistically significantly more common ($P \leqslant 0.01$), i.e., 82.3%, as well as against third generation cephalosporins (44.1%, 41.2%). The genetic analysis of resistance in commensal E. coli isolates revealed the presence of bla $_{CTX\text{-}M\text{-}1}$, tetA and QnrS genes. In conclusion, we should note that in our study related to the use of Bacillus licheniformis BL 11 strain, a probiotic formula in broilers, no basic differences were observed both in terms of the prevalence of resistance to chemotherapeutics and in terms of economic indicators in the broilers in the control and experimental groups.

Introduction

The spread of resistance to antimicrobial drugs is a serious public health concern of our time. Resistant bacteria isolated from animals and the genetic determinants carried by them may be transferred to people via various mechanisms, direct contact, contaminated food, and from the environment (Thorsteinsdottir et al., 2010; Dolejska et al., 2013; Huijbers et al., 2014; Aun et al., 2021; Rousham et al., 2021). The use of third generation cephalosporins and fluoroquinolones in intensive livestock operations (poultry farming, pig farming) is a subject of thorough monitoring and analysis due to their critical importance in the treatment of severe bacterial infections in humans (Costa et al., 2011). According to Costa et al. (2011), the administration of enrofloxacin in farm animals may increase resistance rates to other classes of chemotherapeutics as well. Szmolka et al. (2013) has mentioned that the broader use of fluoroquinolones in poultry farming is a recognized prerequisite for the increased prevalence of resistant *E. coli* bacteria. Furthermore, the transfer of resistant animal *E. coli* isolates producing extended spectrum beta-lactamases is also deemed as a risk for the therapy of some human bacterial infections (Leverstein van Hall et al., 2011; Dahms et al., 2015), having in mind that poultry meat is very often contaminated with such strains (Overderest et al., 2011; Kluytmans et al., 2013; Ceccarelli et al., 2019). According to the criteria of the WHO (2017) and OIE (2015), the aminoglycosides, third generation cephalosporins, macrolides and penicillins are defined as critically important chemotherapeutics for human and veterinary medicine.

The acknowledged necessity about restriction of the use of antimicrobial drugs in farm animals implies the investigation of alternative products for infectious diseases control, animal welfare improvement, selection of more resistant breeds, etc. (Gadde et al., 2017).

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Probiotic products based on members of *Bacillus* spp. are resistant and can be stored for a long time due to specificity of spore forming bacteria (Gao et al., 2007). They possess an inhibitory effect on microbial pathogens due to production of organic acids, reducing the gastrointestinal pH. Cladera-Olivera et al. (2004) discussed the hypothesis that the production of bacteriocins was one of the important features of bacilli in probiotic products, influencing gastrointestinal microbial pathogens in poultry. Also, they support the gastrointestinal microbiota via competitive exclusion, improve the activity of digestive enzymes, reduce the amount of toxic products, ammonia and aflatoxins, produce antimicrobial substances and, last but not least, modulate the immune system by promoting the production of secretory immunoglobulins A (Xu et al., 2012; Latorre et al., 2017; Mingmongkolchai and Panbangred, 2018). These features of probiotic formulas with *Bacillus* spp. reduce the possibility for emergence of infections and mortality rates in poultry, along with improvement of feed conversion. Rajput et al. (2020) affirmed that probiotics containing *B. licheniformis* strains may alleviate oxidative stress and influence gene expression associated with lipid metabolism. As outlined by Mingmongkolchai and Panbangred (2018), some representatives of the genus *Bacillus*, e.g., *B. clausii*, *B. cereus*, *B. subtilis*, *B. licheniformis* possess plasmids conferring antimicrobial drug resistance that may be transferred into the gastrointestinal tract of birds. This gave a reason to EFSA experts (2015) recommending a preliminary testing of strains in probiotic formulas for their sensitivity to various classes of chemotherapeutics.

The aim of the present study was to investigate phenotype resistance profiles and some genetic determinants in resident *E. coli* bacteria isolated from broilers whose ration was supplemented with a probiotic formula containing a *Bacillus licheniformis* BL11 strain (Huvepharma, Belgium).

Material and methods

Birds, rearing conditions and treatment

The experiment was performed in a poultry plant in South Bulgaria. The farm had two production facilities. In each facility, 17 500 day-old unsexed Ross 308 chicks were housed with free access to feed and water. The chickens in both facilities received the same feed from the first day of life until slaughter. Three types of compound feeds were fed: starter, grower and finisher; their composition is presented in Table 1. Halofuginone hydrochloride was added to the starter and the grower as coccidiostat. The drinking water was supplied via nipple drinking systems. The results of the investigation of water quality are presented in Table 2. During the experiment, broilers were not treated with chemotherapeutics. The two groups were reared under equal conditions. The environmental conditions in facilities (ventilation rate, heating, lighting and relative humidity) were in line with technological norms for the hybrid (Aviagen, 2018). The chickens were reared on the floor on ground straw bedding. During the experiment, the birds had free access to fresh water and feed. At the beginning of the study, the ambient temperature was 32°C, then it gradually decreased to 22°C, and was maintained at that level until the end. The relative humidity in

Table 1. Composition of broiler chicken feed (%)

Ingredients	Starter (0–17 days)	Grower (18–27 days)	Finisher (28 day to slaughter)
Maize	23	20	26
Wheat	36	34	31
Maize gluten meal	4	1.5	
Soybean meal	29	27	22
Sunflower meal		6	7
Sunflower oil	3.00	6.75	5.50
L-valine	0.035	0.03	0.06
Lysine	0.39	0.34	0.26
Methionine	0.29	0.28	0.28
Threonine	0.16	0.13	0.12
Limestone	1.16	1.19	1.05
Monocalcium phosphate	1.16	0.66	0.54
Sodium chloride	0.17	0.17	0.19
Sodium hydrogen carbonate	0.33	0.33	0.32
Mineral vitamin premix	0.55	0.55	0.55
Vitamin C	0.01	0.01	0.01
Coccidiostat – halofuginone hydrochloride	0.05	0.05	

Table 2. Results from the laboratory analysis of drinking water

Parameter	Units	Test result*	Reference values
Calcium	mg/L	108.72	150
Manganese	µg/L	< 10.00	50
Nitrates	mg/L	27.10	50
Nitrites	mg/L	< 0.05	0.50
Hydrogen ions activity (pH)	pH	7.35	6.5–9.5
Electroconductivity	µS/cm	847.00	up to 2000
Cations – ammonium	mg/L	< 0.06	0.50
Turbidity	FNU	0.14	up to 1.5
Free chlorine residual	mg/L	0.321	0.3–0.4
Enterococcus spp.	cfu/100 mL	< 1	0
Escherichia coli	cfu/100 mL	< 1	0
Clostridium perfringens	cfu/100 mL	< 1	0
Coliforms	cfu/100 mL	< 1	0
Total viable counts	cfu/mL	23	up to 100
Total viable counts	cfu/mL	15	up to 20
Salmonella spp.	detectable / undetectable in 250 mL	not detected	undetectable

* The water sample for laboratory analysis was collected from the last nipple of the drinking system.

facilities was 60–70%. A 6-hour dark period was provided for the night, and light intensity was subject to dawn-to-dusk light control. The health of birds was monitored three times per day.

The birds in the experimental group received a probiotic containing Bacillus licheniformis BL11. The probiotic was applied via drinking water (100 g per 20 000 birds, with a dose of *B. licheniformis* in sachet $1.6X10^6$ cfu/mL). The probiotic treatment was done twice: from day 1 to day 7 of age and from day 20 to day 26. The birds from the control group were not treated with the probiotic.

Samples and bacteriological examinations

Cloacal swabs were collected from broilers at 1, 14 and 28 days of age. For each age period, 50 swabs were collected from control broilers and another 50 swabs from probiotic-supplemented broilers. The total number of samples was 300 (150 from control birds and 150 from experimental birds). For primary isolation of *E. coli*, swabs were inoculated on McConkey agar and incubated at 37°C for 24 hours. The algorithm of *E. coli* identification included cultivation on Kligrer iron agar (Himedia, India), IMViC test (production of indole, methyl red test, Voges-Proskauer test, growth on Simmons citrate agar) and a kit for identification of enterobacteria (Erba Lachema, Czech Republic).

Tests of *E. coli* sensitivity to antimicrobial drugs

The sensitivity of *E. coli* isolates to chemotherapeutics was initially determined by the disk diffusion method using the following antibiotic disks: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefotaxime (10 µg), ceftazidime (5 µg), gentamicin (10 µg), tetracycline (30 µg), and ciprofloxacin (5 µg) (Himedia Biosciences, India). Minimum inhibitory concentrations (MIC) of chemotherapeutics were determined by the E-test, (Hi Comb™, Himedia, India). For evaluation of the inhibitory effect of clavulanic acid on some ESBL producers, gradua-ted MIC strips loaded with combinations of *cef-tazidime+clavulanic acid* (0.064–4 µg/mL) and *cefotaxime+clavulanic acid* (0.016–1 µg/mL (Hi Comb™ , Himedia, India) were used. *Escherichia coli ATCC 25922* was used for control of the methods for phenotypic determination of antimicrobial resistance of *E. coli* strains. The interpretation of results was done on the basis of epidemiological cut-off values (ECOFFs). Categorization of MIC_{90} was done by determining the cumulative percentage of resistant isolates.

Genetic tests

The extraction of DNA from pure cultures was done with DNeasy Blood Tissue kit (Qiagen, Germany). The identification of genes conferring resistance to beta-lactams ($bla_{CTX\text{-}M\text{-}1}$,$bla_{SHV,}$ catalog number BBAR00377A and catalog number BBAR00387A), tetracyclines (*tetA*, catalog number BBAR00449A) and ciprofloxacin (*qnrS*, catalog number BBAR00441A) was performed with commercial Microbial DNA qPCR assay kits, Qiagen, Germany. The thermal profile of the protocol included an initial denaturation step at 95°C for 10 min, 40 cycles with initial denaturation at 95°C for 15 sec and annealing/elongation at 60°C for 2 min. Amplification reactions were done in a Stratagene Mx3000P qPCR system (Agilent Technologies, USA).

Economic performance

During the experiment, the live body weight was

determined on 1, 14 and 28 days of age on 1003 randomly selected broiler chickens from each group. The average daily weight gain (ADG) was determined for the periods 1–16, 17–36 and 1–36 days. For the same periods, the average daily feed intake (ADFI) and feed conversion rate (FCR) were calculated. The European Production Efficiency Factor (EPEF) for these periods was calculated using the formula:

EPEF = [(livability, % × BW, kg) / (age, days × FCR)] × 100.

Statistical analysis

The statistical analysis of sensitivity rates of isolates to chemotherapeutics was done with GraphPad Instat 3.

The parameters of economic performance in both groups were subjected to the following tests: Kolmogorov-Smirnov test for normality of sample distribution; descriptive statistics with calculation of arithmetic mean, standard error of the mean, minimum and maximum values; and the t-test for independent samples for between-group comparison. The minimum level of statistical significance was $P < 0.05$. The calculations were done with the statistical software MedCalc v 10.2.0.0, Belgium.

Results

The total number of *E. coli* isolates from the control and experimental group of broilers was 214: 107 from control birds and another 107 from probiotic-supplemented birds, and from the remaining 86 samples no bacteria belonging to the species *Esherichia coli* were isolated.

Tables 3, 4 and 5 present the results of the prevalence of resistance in poultry *E. coli* isolates in both groups at 1, 14 and 28 days of age.

The highest resistance rates among *E. coli* isolates from control day-old broilers were shown against gentamicin (86.9%), ampicillin (67.4%), tetracycline (60.9%) and ciprofloxacin (52.1%). For isolates from the experimental birds, the resistance against gentamicin was also the most frequent (69.0%), followed by resistance against ampicillin and amoxicillin/clavulanic acid (52.4%). The prevalence of tetracycline-resistant strains was lower as compared with the control group (19.0%). *E. coli* isolates from the control group which were resistant to cefotaxime were 6.5% vs 7.1% among isolates from the experimental group. A lower rate of resistance

Table 3. Resistant *E. coli* isolates from day-old broilers

	Control group (n = 46)		Experimental group (n = 42)	
Chemotherapeutics	Resistant *E. coli* isolates (n/%)	CL	Resistant *E. coli* isolates (n/%)	CL
Ampicillin	31/67.4*	53.4÷79.9	22/52.4*	37.5÷67.0
Amoxicillin/clavulanic acid	21/45.6	31.7÷59.9	22/52.4	37.5÷67.0
Cefotaxime	3/6.5	1.3÷15.2	3/7.1	1.4÷16.5
Ceftazidime	0	-	1/2.4	0÷9.0
Gentamicin	40/86.9*	75.9÷94.4	29/69.0*	54.5÷81.8
Tetracycline	28/60.9***	46.5÷74.3	8/19.0***	8.8÷31.9
Ciprofloxacin	24/52.1	37.9÷66.1	20/47.6	32.9÷62.5

Legend: $P < 0.05$*; $P < 0.01$**; $P < 0.001$ ***

Table 4. Resistant *E. coli* isolates from 14-day-old broilers

	Control group (n = 25)		Experimental group (n = 31)	
Chemotherapeutics	Resistant *E. coli* isolates (n/%)	CL	Resistant *E. coli* isolates (n/%)	CL
Ampicillin	17/68.0	49.8÷84.4	23/74.2	57.6÷87.8
Amoxicillin/clavulanic acid	12/48.0	29.0÷67.2	11/35.5	19.9÷52.9
Cefotaxime	0	-	16/51.6	32.4÷70.6
Ceftazidime	3/12.0*	2.4÷27.2	12/38.7*	20.9÷58.1
Gentamicin	12/48.0	29.0÷67.2	19/61.3	41.8÷79.0
Tetracycline	21/84.0**	67.4÷95.4	14/45.2**	26.5÷64.6
Ciprofloxacin	17/68.0	49.8÷84.4	26/83.9	67.5÷95.3

Legend: $P \leqslant 0.05$*; $P \leqslant 0.01$**; $P \leqslant 0.001$***

Table 5. Resistant *E. coli* isolates from 28-day-old broilers

	Control group (n = 36)		Experimental group (n = 34)	
Chemotherapeutics	Resistant *E. coli* isolates (n/%)	CL	Resistant *E. coli* isolates (n/%)	CL
Ampicillin	19/52.7**	36.6÷68.5	28/82.3**	67.9÷93.0
Amoxicillin/clavulanic acid	11/30.5	16.7÷46.2	15/44.1	28.1÷60.8
Cefotaxime	4/11.1**	3.0÷23.2	15/44.1**	28.1÷60.8
Ceftazidime	4/11.1**	3.0÷23.2	14/41.2**	25.4÷58.9
Gentamicin	21/58.3	42.1÷73.6	19/55.8	39.1÷71.8
Tetracycline	24/66.7	50.7÷80.8	27/79.4	64.4÷91.0
Ciprofloxacin	19/52.7**	36.6÷68.5	29/85.3**	70.3÷95.0

Legend: $P \leqslant 0.05$*; $P \leqslant 0.01$**; $P \leqslant 0.001$***

against ceftazidime was observed in poultry from the experimental group (2.4%).

The prevalence of resistance to cefotaxime (51.6%), respectively to ceftazidime (38.7%), in *E. coli* isolates from 14-day-old experimental broilers statistically significantly exceeded ($P \leqslant 0.05$) the proportion of resistant isolates from the control group (12.0%). The tetracycline resistant strains from control chickens (84.0%) were significantly more prevalent ($P \leqslant 0.01$) than those isolated from the supplemented experimental group (45.2%). Higher yet statistically insignificant rates of resistance in both groups were demonstrated against ampicillin (68.0%, 74.2%), gentamicin (48.0%, 61.3%) and ciprofloxacin (68.0%, 83.9%). The percentage of *E. coli* strains resistant against amoxicillin/clavulanic acid in the probiotic-treated group was insignificantly lower (35.5%) as compared with isolates from control birds (48.0%).

By day 28 of age, the prevalence of ciprofloxacin-resistant *E. coli* isolates from experimental broilers exceeded significantly ($P \leqslant 0.01$) that of control strains (85.3% vs 52.7%). Furthermore, the spread of resistance against ampicillin (82.3%) and third generation cephalosporins (44.1%, 41.2%) among isolates from experimental birds was substantially higher. In both groups, high resistance rates were found out against gentamicin (58.3%, 55.8%) and tetracycline (66.7%, 79.4%).

Tables 6 and 7 present the minimum inhibitory concentrations in *E. coli* isolates from control and experimental broilers.

Among *E. coli* isolates from both groups, the highest detected MIC_{90} values were for ampicillin and tetracycline (32 µg/mL). MIC_{90} values of 16 µg/mL were found for amoxicillin/clavulanic acid and tetracycline. MIC_{90} for third generation cephalosporins was 1 µg/mL for *E. coli* isolates from the control group, whereas MIC_{90} for ceftazidime in isolates from the experimental group was 2 µg/mL. In isolates from both groups, MIC_{90} for gentamicin was 2 µg/mL, and for ciprofloxacin, it was 1 µg/mL.

Table 8 presents phenotype resistance profiles and some of the genetic determinants of resistance in multi-resistant *E. coli* isolated from control and probiotic-supplemented broilers.

Among multi-resistant *E. coli* strains isolated from control broilers, the phenotype profile including resistance to ampicillin, amoxicillin/clavulanic acid and gentamicin (33.6%) was the most common one, followed by the profile determining resistance

Table 6. Minimum inhibitory concentrations in commensal *E. coli* isolates from the control group of broilers at 1, 14, and 28 days of age (n = 107)

	MIC_{90} µg/mL												
Chemotherapeutics	MIC_{90}	0.06	0.125	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	32			1	3	9	5	22*	39	18	7	3	
Amoxicillin/clavulanic acid	16		4		1	1	20	37*	21	14	9		
Cefotaxime	1.0		13	12	75*	7							
Ceftazidime	1.0		17	21	62*		7						
Gentamicin	4.0			24		10*	58	13	2				
Tetracycline	32					2	3	29*	42	17	11	3	
Ciprofloxacin	1		47	42*	18								

Legend: MIC thresholds are marked with asterisks

to gentamicin, tetracycline and ciprofloxacin (27.1%). *E. coli* isolates from the experimental group demonstrated a higher prevalence of multi-resistance to ampicillin, amoxicillin/clavulanic acid, cefotaxime and gentamicin (15.9%), as well as to ampicillin, cefotaxime, ceftazidime, gentamicin and ciprofloxacin (15.9%). The presence of the *bla* $_{CTX-M-1}$ gene but not of the *bla* $_{SHV}$ gene was confirmed in strains resistant to beta-lactams. Fifty strains resistant to ciprofloxacin (37%) carried the *Qnr*S gene.

Economic performance

Data about the economic performance of broilers are presented in Table 9. According to the results, the treatment with a probiotic containing *Bacillus licheniformis* BL11 strain improved ($P < 0.05$) growth performance in broilers (BW, FCR and EPEF) in comparison to the same indices in the control

Table 7. Minimum inhibitory concentrations in commensal *E. coli* isolates from the experimental group of broilers at 1, 14, and 28 days of age (n = 107)

	MIC$_{90}$ µg/mL												
Chemotherapeutics	MIC$_{90}$	0.06	0.125	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	32			2	1	1	2	28*	41	15	12	5	
Amoxicillin/clavulanic acid	16			7	4	4	2	42*	7	41			
Cefotaxime	1	2	2	37	32*	34							
Ceftazidime	2	3	1	17	57*	2	25	2					
Gentamicin	4.0		1	4	2	33*	55	12					
Tetracycline	32				4	4	6	44*	31	7	11		
Ciprofloxacin	1		32	21*	51	3							

Legend: MIC thresholds are marked with asterisks

Table 8. Phenotype resistance profile of *E. coli* isolates from broilers and genes conferring resistance to antimicrobial drugs

Genes conferring resistance to beta-lactams, tetracyclines and quinolones (n/%)				
Phenotype resistance profile of *E. coli* isolates	*bla* $_{CTX-M-1}$	*bla* $_{SHV}$	*tet*A	*Qnr*S
Control group (n = 107)				
T (20)	-	-	16 (14.9%)	
CIP (1)	-	-	-	-
AMP, AMC, G (36)	-	-	-	-
AMP, AMC, CIP (8)	-	-	-	2 (1.9%)
G,T, CIP (29)	-	-	27 (25.2%)	14 (13.1%)
AMP, T, CIP (15)	-	-	11 (10.3%)	3 (2.8%)
AMP, G, T (1)	-	-	-	-
AMP, CTX, CAZ, G, T, CIP (7)	7 (6.5%)	-	3 (2.8%)	1 (0.9%)
Experimental group (n = 107)				
AMP (3)	-	-	-	-
AMP, G (3)	-	-	-	-
AMP, AMC (7)	-	-	-	-
G, T (14)			4 (3.7%)	-
T, CIP (25)	-	-	9 (8.4%)	9 (8.4%)
AMP, G, CIP (2)	-	-	-	-
AMP, AMC, G, CIP (14)	-	-	-	3 (2.8%)
AMP, AMC, CTX, CIP (17)	11 (10.3%)	-	-	7 (6.5%)
AMP, CTX, CAZ, G, CIP (17)	17 (15.9%)	-	-	11 (10.3%)
AMP, AMC, CTX, CAZ, G, T (10)	10 (9.3%)	-	10 (9.3%)	-

Legend: AMP – ampicillin, AMC – amoxicillin/clavulanic acid, CTX – cefotaxime, CAZ – ceftazidime, G – gentamicin, T – tetracycline, CIP – ciprofloxacin

Table 9. Economic performance of chickens from control and experimental group (n = 1003)

	Control group	Experimental group	*P value*
Initial body weight, g	41.00 ± 0.08	48.00 ± 0.06	< 0.0001
BW 16 day, g	570.00 ± 0.69	580.00 ± 0.60	< 0.0001
BW 36 day, g	1886.00 ± 0.44	1811.00 ± 0.22	< 0.0001
ADG 1–16 days, g/ day	33.06 ± 0.04	33.25 ± 0.04	= 0.0008
ADG 17–36 days, g/ day	69.26 ± 0.23	64.79 ± 0.03	< 0.0001
ADG 1–36 days, g/ day	51.25 ± 0.12	48.97 ± 0.01	< 0.0001
FCR 1–16 days, g/g	0.872 ± 0.001	0.769 ± 0.001	< 0.0001
FCR 17–36 days, g/g	1.422 ± 0.001	1.560 ± 0.001	< 0.0001
FCR 1–36 days, g/g	1.631 ± 0.001	1.753 ± 0.001	< 0.0001
EPEF 1–16 days	400.96 ± 0.96	463.34 ± 0.88	< 0.0001
EPEF 17–36 days	673.74 ± 0.31	591.35 ± 0.15	< 0.0001
EPEF 1–36 days	310.08 ± 0.14	277.80 ± 0.07	< 0.0001
ADFI 1–16 days, g/ day	30.36	27.36	
ADFI 17–36 days, g/ day	136.30	144.06	
ADFI 1–36 days, g/ day	85.43	88.19	
Mortality 1–16 days, %	2.15	1.84	
Mortality 17–36 days, %	1.34	1.35	
Mortality 1–36 days, %	3.49	3.19	

Legend: BW – body weight; ADG – average daily weight gain; ADFI – average daily feed intake; FCR – feed conversion rate; EPEF – European production efficiency factor.

group during the starter stage (from days 1–14). During the grower stage (days 14–36) the broilers treated with the probiotic demonstrated reduced performance compared with controls. Thus, FCR of the experimental group was worse than that of control birds. The same trend was observed for the average daily feed intake and the average daily weight gain. For the entire duration of the trial (days 1–36), average values of economic parameters of controls were better than those in the experimental group. At the same time, the mortality rate of probiotic-supplemented broiler chickens was lower than the rates of the control group.

Discussion

According to Arif et al. (2021), probiotics based on *Bacillus* spp. increase the counts of spore-forming bacteria from this genus between days 21 and 35, and on the other side, reduce the counts of *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli* in the small intestinal compartment of broilers reared in farms with poor biosecurity parameters. Cladera-Oliveraet al. (2004) discussed the ability of *Bacillus* spp. representatives to produce bacteriocins, which inhibit the development of other bacterial species in the avian gut.

Some investigators discussed the emergence of multi-resistant *E. coli* strains from the resident microflora resistant against beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines even in newly hatched chickens (Baron et al., 2014; Olsen et al., 2017; Projahn et al., 2017; Roth et al., 2017; Saliuet al., 2017). In their opinion, birds become infected with resistant strains from eggshells and from the environment; a vertical transmission of such strains from breeding flocks is also possible.

Possibly, the spread of antimicrobial resistance among commensal *E. coli* is a multifactorial process including both the selective pressure from the application of these drugs in intensive livestock husbandry, as well as the spread of resistant bacterial clones, dissemination of plasmids conferring resistance to various classes of chemotherapeutics and, last but not least, the co-selection among resistant bacterial strains.

For example, the last report of EFSA (2023) on the prevalence of resistance among commensal *E. coli* from broilers in EU member states affirms that the resistance against ciprofloxacin was the most common (52.7%). The report discusses the fact that the proportion of *E. coli* isolates from broilers resistant to ciprofloxacin and cefotaxime from 2020 to 2021 was low (1%) while the proportion of strains resistant only against third generation cephalosporins was higher (7.1%). According to the analysis, multi-resistant strains were frequently encountered among commensal *E. coli* from broilers (37.7%). In Belgium, as reported by De Koster et al. (2021), the resistance to third generation cephalosporins and ciprofloxacin in resident poultry *E. coli* was high (70%) and co-

resistance was determined in 33.4% of strains. In Austria, Galleret al. (2021) observed a high incidence of resistance against third generation cephalosporins (cefotaxime 100%; ceftazidime 93.8%) and tetracycline (93.8%) but not against ciprofloxacin among commensal *E. coli* isolated from broilers. In the present study, multi-resistant strains including resistance against third generation cephalosporins were more prevalent among broilers from the experimental group (41.1%) at a lower rate than that reported by De Koster et al. and Galler et al.

As already acknowledged, beta-lactam antibiotics are among the most commonly used in human and veterinary medical practice, which creates prerequisites for colonization of the intestinal tract with extended spectrum beta-lactamase (ESBL) producing *E. coli* (Ferreira et al. 2022). The incidence of ESBL producing *E. coli* in the gastrointestinal tract of broilers is important for the spread of multi-resistant strains to people along the food chain, as well as among animals and in the environment (Dierikx et al., 2018; Subramanya et al., 2021). In the Netherlands, Van Hoek et al. (2018) found 30% of ESBL-producing strains among *E. coli* isolates from day-old chickens. According to the authors, as early as during the first 48 hours after population of premises with birds, the rate of ESBL producing *E. coli* strains increased without application of selective pressure. In their view, one of the factors determining the distribution of *E. coli* ESBL producers was the spread of specific clones ST88, ST10, ST58, ST155, and the horizontal transfer of such strains was discussed as a risk factor. A number of European researchers affirmed that beta-lactamases of the CTX-M-1 subtype were the most commonly encountered among resistant *E. coli* bacteria (Girlich et al., 2007; Chauvin et al., 2013; Dierikx et al., 2018; Apostolakos et al., 2019). In Germany, Laube et al. (2013) determined a broader prevalence of *bla* $_{CMY}$ (21.73%), followed by *bla* $_{SHV-12}$ (13.2%) and *bla* $_{CTX-M}$ (10.59%) genes in a similar survey on ESBL-producing isolates from broiler chickens. Furthermore, they reported a rather elevated percentage of ESBL producers (51%) in day-old chicks. Valentin et al. (2004) affirmed that, in Germany, *E. coli* strains from livestock and birds that produced beta-lactamases from the CTX-M-1 group were the most prevalent. Galler et al. (2021) presented data from Austria associated with the predominant spread of *bla* $_{SHV-12}$ (81.3%), followed by *bla* $_{CTX-M-1}$ (12.5%) among commensal *E. coli* isolated from broilers. In Portugal, Ferreira et al. (2022) also observed dominance of the *bla* $_{SHV-12}$ gene among commensal ESBL-producing isolates from broiler chickens. Saliu et al. (2017) reported the fact that the *bla* $_{CTX-M-1}$, *bla* $_{TEM-52}$, and *bla* $_{SHV-12}$ were the most common resistance genes in intensive livestock operations. Due to the genetic resemblance between ESBL-producing enterobacteria isolated from humans and broiler chickens, the authors suggested that broilers may be a primary reservoir of plasmids carrying the *bla* $_{CTX-M-1}$ gene. In our study, the resistance against third generation cephalosporins among commensal *E. coli* isolates was determined by the presence of *bla* $_{CTX-M-1}$, but not by the presence of *bla* $_{SHV}$.

In this study, strains resistant to cefotaxime and ceftazidime were more commonly isolated from broilers, supplemented with the probiotic formula. For example, 7.1% of isolates from day-old experimental broilers were resistant against cefotaxime, and 2.4% to ceftazidime, whereas in control chickens, the prevalence of cefotaxime-resistant strains was 6.5%, and none of the strains were resistant against ceftazidime. An interesting fact was that ampicillin-resistant (52.4%), amoxicillin/clavulanic acid-resistant (52.4%), and gentamicin-resistant (69.0%) strains from day-old probiotic-supplemented birds were also more numerous. During the next study period at 14 days of age, the prevalence of cefotaxime-resistant (51.6%) and ceftazidime-resistant (38.7%) *E. coli* isolates among experimental birds was also higher than that in controls. None of the commensal *E. coli* isolates from controls was resistant against cefotaxime and those resistant against ceftazidime were 12.0%. In 28-day-old birds, a variable resistance against third generation cephalosporins was noted: 44.1% and 41.2% of strains were resistant against cefotaxime and ceftazidime, respectively, whereas the resistance rates to these chemotherapeutics among control group isolates was once again lower: 11.1% for both drugs. In connection with these findings, the statement of Clemente et al. (2021) about the possible association of the broad spread of commensal *E. coli* poultry strains producing ESBL with some risk factors in industrial poultry farming co-selection of genes and mobile genetic platforms, plasmids, transposons, integrons resulting from the use of other chemotherapeutic classes, tetracyclines, sulfonamides and fluoroquinolones, may be cited. Saliu et al. (2017) also pointed out the environment as a factor influencing the spread of ESBL-producing enterobacteria in intensive poultry operations and discussed the effect of the ration on these processes. According to the investigations of Blaak et al. (2015), the spread of *E. coli* producing extended-spectrum beta-lactamases from poultry farms to the environment is an important risk factor for the transfer of such strains to people.

With respect to the prevalence of *E. coli* isolates resistant to aminopenicillins, a statistically significant reduction of strains resistant against amoxicillin/clavulanic acid was observed among isolates from the probiotic-supplemented group by day 14 of the experiment. The resistance rate among isolates from the experimental group was 35.5% vs 48.0% for isolates from untreated controls.

From the beginning of the trial to day 28, a tendency towards the increased number of tetracycline- and ciprofloxacin-resistant strains from the experimental group of birds was established. In strains from day-

old broilers, the prevalence of strains resistant against tetracycline was 19.0%, and against ciprofloxacin, it was 47.6%; by day 28 of the trial, the respective rates increased to 79.4% and 85.3%. The resistance to tetracycline and ciprofloxacin was conferred by the *tetA* and *QnrS* genes. Thus, our findings may be commented in the light of the statement of Ribeiro et al. (2023) that both among pathogenic and commensal *E. coli* isolates from broilers, the strains expressing resistance to ampicillin, tetracycline, ciprofloxacin, nalidixic acid and sulfamethazole-trimethoprim were the most numerous. Similar results were reported by Roth et al. (2019) affirming that, in some European countries, the highest resistance rates among commensal *E. coli* strains from broilers were those against ampicillin (91%), ciprofloxacin (90%), tetracycline (73%) and sulfonamides (60%).

Conclusion

The demonstrated broader prevalence of *E. coli* strains, resistant against third generation cephalosporins, ciprofloxacin and tetracycline, isolated from broilers receiving a probiotic formula was probably related to several specific factors, such as the exchange of certain bacterial clones, the presence of plasmids carrying the respective genetic determinants. Providing that scientific literature reports regarding the possible effects of alternative products of antibiotics on the spread of resistance among commensal intestinal microbiota are still few, the probiotic formula containing *Bacillus licheniformis* BL11 could possibly affect these events through the classic characteristics of probiotics, namely modulation of the immune system, antibacterial activity of probiotic strains and competitive exclusion. On the other hand, this product could also have a better effect in implementing strict control on biosecurity measures affecting the welfare of birds and the status of the environment. We should note that in our study, no basic differences were observed both in terms of the prevalence of resistance to chemotherapeutics and in terms of economic indicators in the broilers in the control and experimental groups.

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Growth Curve Analysis of Body Weight of Mixed-Sex Egyptian Native Geese (*Anser Anser Domesticus*) Using Three Nonlinear Mathematical Functions

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Abstract. *Three non-linear growth models (logistic, Gompertz and von Bertalanffy) were used to describe and estimate growth parameters of 10-week age-body weight relationship of mixed-sex Egyptian indigenous geese. The estimated asymptotic weight (A), scaling parameter (B), maturity index (K), inflection weight (Wi) and time (Ti) were respectively 3254.00 g, 13.15, 0.48 g/day, 1627 g and 5.37 weeks; 3895.00 g, 3.33, 0.25, 1431.99 g and 4.81 weeks; and 4559.00 g, 0.72, 0.17, 1350.81 g and 4.53 weeks for the logistic, Gompertz and von Bertalanffy growth function. The application of four goodness-of-fit criteria [coefficient of determination (R^2), root mean square error (RMSE), Akaike's information criterion (AIC) and Bayesian information criterion (BIC)] revealed that von Bertalanffy was the best fitting model describing age-body weight relationship of Egyptian native geese. This was premised on the fact that the von Bertanlanffy model had the highest R^2 (0.896) and lowest RMSE (327.05), AIC (8806.50) and BIC (5747.86) compared with the Gompertz (R^2: 0.897; RMSE: 328.70; AIC: 8813.05; BIC: 5754.40) and logistic (R^2: 0.891; RMSE: 338.90; AIC: 8841.71; BIC: 5783.07) models. The information provided in this study could be exploited for planning appropriate management practices and further genetic studies on improvement of Egyptian native goose for increased body weight.*

Introduction

The process of growth measured as body mass or body weight on a longitudinal time frame has often been summarized using mathematical equations fitted to growth curves or models (Onder et al., 2017). These models consist of functions which accept, on the basis of the reality of the biological growth of an animal, that the dependent variable has an estimated asymptotic value when the independent variable is at infinity (Narinc et al., 2017). Growth curve models provide a set of parameters that describe the growth pattern over time and estimate the expected weight of animals at certain ages (Lupi et al., 2015). The growth curve is represented mathematically as a function of age and live weight, covering all or part of the animal's lifespan (Echeverri et al., 2013) and these functions could display the summarized growth information in some indices, which may also have a biological interpretation (Ahmadi and Mottaghitalab, 2007).

The growth models have multifaceted applications in enhancing better understanding of the nexus between age and body weight of living organisms. These mathematical models have biological interpretations due to their ability to summarize a large quantity of data collected from body weight over time (Masoudi and Azarfar, 2017; Safari Alighiralou et al., 2017). Besides, growth curves are used to express the time-dependent nonlinear variation of live weight through mathematical functions and the generated equations can be used to predict the expected weight of a group of animals at a certain age (Kim et al., 2016). It is imperative to note that understanding growth patterns and associated growth curve parameters is important and they could serve as principal genetic tools for animal breeders in arriving at a logical conclusion on growth description and making informed decisions in developing appropriate animal improvement strategies.

There are many mathematical functions that have been applied to model the growth of poultry species and livestock in general. However, each model is unique with its own peculiar characteristics while some are modified forms of others. Among the growth curve fitting functions, the most widely used ones in modelling the age-body weight relationship of poultry are the three-parameter Gompertz, logistic, and von Bertalanffy models and the four-parameter Richards function. It is noteworthy that the most commonly used three-parameter models – logistic, Gompertz

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and von Bertalanffy – have fixed growth forms with the point of inflection at about 50%, 37% and 30% of the asymptote, respectively (Eleroglu et al., 2014). However, synthesis of empirical studies has revealed that the Gompertz function is the most widely used nonlinear regression equation in describing growth of poultry (Narinc et al., 2017).

Among African countries, the highest number of geese is found in Egypt, thus reflecting its acceptance and significant contribution to the menu and socio-economic activities of the populace. It is noteworthy that in spite of the highest population of geese in Egypt among African countries, there are currently no intensive or commercial geese farms in Egypt but production depends mainly on unimproved native geese which descended from Greylag goose (Makram, 2018) and small-size flocks reared by smallholder farmers around the upper, middle and delta regions of the Nile valley (Makram 2018; Makram et al., 2018).

Body weight is an important economic trait in farm animals. High premium is attached to it by livestock farmers (Oguntunji, 2017). This metric trait contributes significantly to the profit margin of livestock farmers, especially to livestock enterprises where the main target is market weight or dressed meat (Oguntunji, 2017). In view of the economic importance attached to this quantitative trait, different statistical methods have been applied by researchers to analyse and describe growth parameters such as feed intake, body weight gain, body weight, and feed conversion ratio among others.

In spite of abundant literature on growth modelling of poultry species, related empirical reports on the growth curve fitting of African indigenous waterfowls (ducks and geese) are not available. Therefore, the present study was conducted to describe the growth curve of Egyptian native geese using von Bertalanffy, Gompertz and logistic models.

Materials and Methods

Location of the experiment and management of experimental birds

The experiment was conducted in Fayoum governorate, Egypt on coordinate 29°18'35.82" N 30°50'30.48" E. Forty-five (45) day-old Egyptian indigenous gooslings were sourced from small holder farmers and were brooded on gravels under natural lighting.

From day-old to the fourth (0–4) week, the goslings were placed on 23% crude protein and 2900 Kcal/ME/kg feed supplemented with alfafa grass. The experimental birds were also fed 18% crude protein and 2900 KCal/ME/kg with alfafa grass between weeks 4 and 8. At the final phase of the experiment (weeks 8–10), the birds were fed feed containing 21% of crude protein and 3000 Kcal/ME/kg without grass supplement.

Data collection

The birds were wing-tagged at day-old for easy identification, and weekly body weight (BW) records of each bird were taken for 10 weeks using a sensitive digital scale calibrated to 2 decimal places. The BW measurement was taken at 7:00 hours before feeding. Besides, general handling of the birds during the experimental period and data collection followed the international best practices to reduce handling stress to the barest minimum.

Statistical procedures and data analysis

Three nonlinear growth functions: logistic, Gompertz and von Bertalanffy were fitted on the weekly body weight of experimental birds using the SPSS version 23 in order to identify the best growth function to describe the growth curve of native Egyptian geese.

The functional forms of the non-linear regression models are presented in Table 1.

Accuracy of the growth curves

The accuracy of predictive growth models was determined using 4 goodness-of-fit statistics: coefficient of determination (R^2), root mean square error (RMSE), Akaike's information criterion (AIC) and Bayesian information criterion (BIC) according to Sanusi and Oseni (2020). Growth curve function with the lowest AIC, BIC and RMSE (Sanusi and Oseni 2020) and the highest R^2 (Lupi et al., 2015) was selected as the best growth function.

Results

Maturity weight (A)

The descriptive statistics of the body weight of the mixed-sex Egyptian geese are presented in Table 2. There was an increase in body weight from day old to the terminal age of the experiment. The estimated asymptotic weight was highest in the von Bertalanffy (4559.00 g) followed by the Gompertz (3895.00 g) but least in the logistic (3254.00 g) model (Table 3).

Table 1. The growth curve functions of logistic, Gompertz and Von Bertalanffy models

Model	Y_t	W_i	t_i
Logistic	$A(1+Be^{-kt})^{-1}$	A/2	(Ln.B)/k
Gompertz	$A\ exp(-Be^{-kt})$	A/e	(Ln.B)/k
Von Bertalanffy	$A(1-Be^{-kt})^3$	A(8/27)	(Ln.3B)/k

Y_t: body weight (g) of geese at t (week/day of age); A: the asymptotic weight (g) when time goes to infinity; B: scaling parameters (constant of integration); k: maturing rate (g/day); e: constanta (2.72); t: time (day); W_i: weight at inflection (g); t_i: time of inflection (week).

Table 2. Descriptive statistics of body weight (g) of mixed-sex geese (N = 45) at 0 to 10 weeks of age

Age (week)	Mean	SD (±)	CV (%)	Minimum	Maximum
0	97.78	12.73	13.02	75.00	120.00
1	239.67	69.53	29.01	150.00	400.00
2	512.00	129.39	25.27	235.00	675.00
3	731.78	199.08	27.20	435.00	1100.00
4	1321.33	423.20	32.03	630.00	1900.00
5	1560.00	413.15	26.48	800.00	2400.00
6	1840.00	397.23	21.59	1000.00	2600.00
7	2123.11	374.29	17.63	1350.00	2800.00
8	2392.33	406.48	16.99	1500.00	3100.00
9	2706.67	362.06	13.38	1800.00	3500.00
10	3077.00	366.23	11.90	2700	3950.00

SD: standard deviation; CV: coefficient of variation.

Table 3. The growth curve parameters for body weight of mixed-sex Egyptian native geese

Model	A (g)	B	K (g/day)	W_i (g)	t_i (week)	Iteration
Logistic	3254.00 ± 91.99	13.15 ± 1.02	0.48 ± 0.02	1627.00	5.37 (37.59 days)	7
Gompertz	3895.00 ± 190.20	3.33 ± 0.12	0.25 ± 0.02	1431.99	4.81 (33.67 days)	6
Von Bertalanffy	4559.00 ± 320.34	0.72 ± 0.02	0.17 ± 0.02	1350.81	4.53 (31.71 days)	5

A: the asymptotic weight (g) when times goes to infinity; B: scaling parameters (constant of integration); k: maturing rate (g/day); t: time (week/day); W_i: weight at inflection (g); t_i: time of inflection (week).

Scaling parameter (B)

The result of the scaling parameter (Table 3) was 13.15 in the logistic model, distantly followed by 3.33 in the Gompertz model and 0.72 in the von Bertalanffy model.

Maturing rate (K)

The result of the slope of the non-linear regression models (Table 3) indicated that the lowest K value was recorded for von Bertalanffy (0.17 g/d), followed by Gompertz (0.25 g/d) but highest in the logistic model (0.48 g/d).

Inflection weight (Wi)

Table 3 shows that the estimated body weight at inflection was 1350.81 g, 1431.99 g and 1627.00 g for the von Bertalanffy, Gompertz and logistic models, respectively.

Inflection time (Ti)

The time of inflection of the body weight followed the same trend as observed in weight at inflection; the shortest Ti (4.53 weeks, 31.71 days) was reported in the von Bertalanffy, intermediate in Gompertz (4.81 weeks; 33.67 days) but highest in the logistic (5.37 weeks, 37.59 days) model (Table 3).

Determination of the best-fitting model

The values of goodness-of-fit tests are presented in Table 4. Across the models, the R^2 value was 0.898 for von Bertalanffy, 0.897 and 0.891 for Gompertz and logistic models, respectively. Contrastingly, for other three goodness-of-fit criteria (RMSE, AIC, and BIC) used in this study, the lowest values were obtained for von Bertalanffy (B), followed by Gompertz (G) and logistic (L) models (RMSE: B-327.05, G-328.70, L-338.90; AIC: B-8806.50, G-8813.05, L-8841.71 and BIC: B-5747.86, G-5754.40, L-5783.07).

Growth curve description

All the nonlinear regression models fitted well the growth curve of Egyptian indigenous geese as reflected in the sigmoidal shape of the models (Fig. 1).

Table 4. The goodness-of-fit criteria for the growth curve models in mixed-sex Egyptian native geese

Model	R^2	RMSE	AIC	BIC
Logistic	0.891	338.90	8841.71	5783.07
Gompertz	0.897	328.70	8813.05	5754.40
von Bertalanffy	0.898	327.05	8806.50	5747.86

R^2: coefficient of determination; RMSE: root mean square error; AIC: Akaike's information criterion; BIC: Bayesian information criterion.

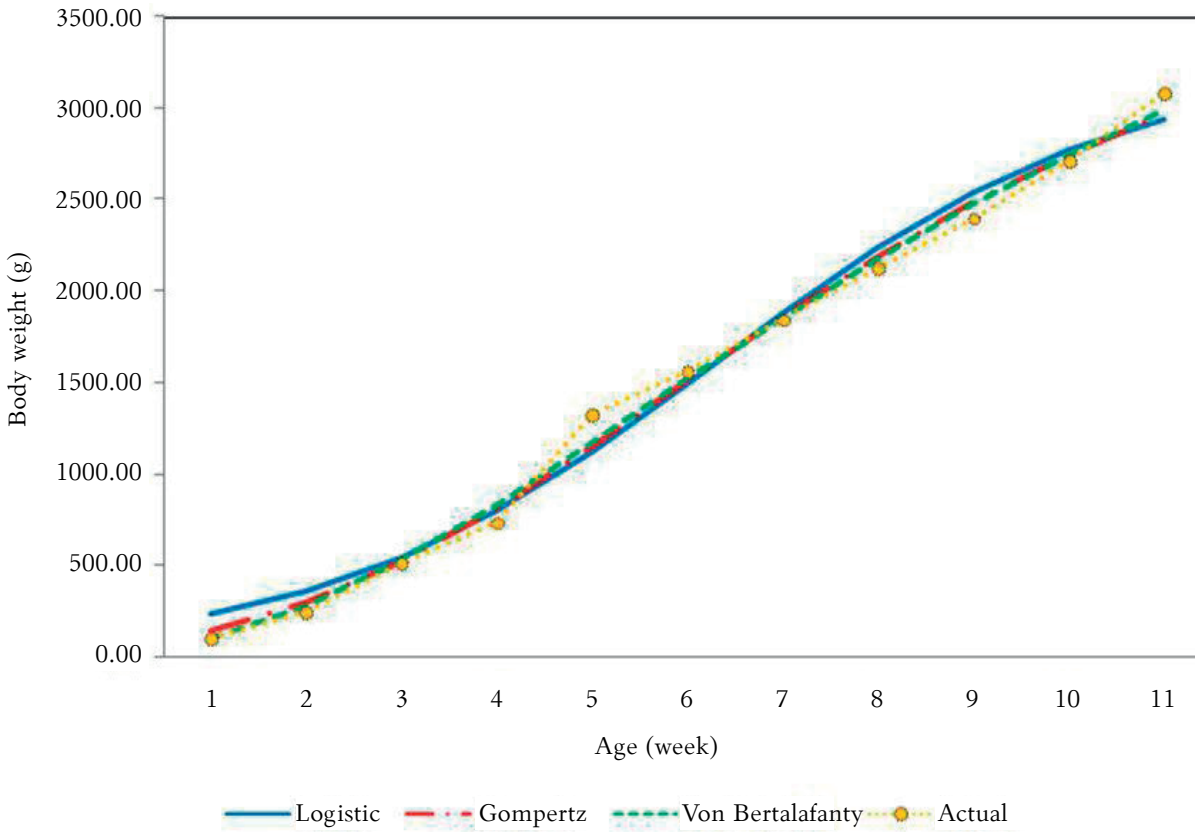

Fig. 1. The growth curve of body weight in mixed-sex goose based on the logistic, Gompertz and von Bertalanffy models

Discussion

Maturity weight (A)

The trend of asymptote in the present study whereby the asymptotic weight was highest in the von Bertalanffy model intermediate in Gompertz but lowest in the logistic model is consistent with the trend reported for the three growth functions in related studies involving mixed-sex and non-mixed sex geese breeds (Ibtisham et al., 2017; Onder et al., 2017; Karadavut et al., 2022; Liu et al., 2022).

Furthermore, the range of the asymptotic value reported for the three growth curve models in this study (3254.00–4559.00 g) was much higher than the values reported for Turkish native geese (logistic: 92.02 g, Gompertz: 169.37 g, and von Bertanlanffy: 207.84 g) reared in different environmental enrichments (Karadavut et al., 2022). However, it compares favourably with 3,425 g, 3,840 g and 4,181 g reported for the logistic, Gompertz and von Bertalanffy model, respectively in indigenous Chinese geese (Liu et al., 2022). Conversely, the range of estimated A obtained for these growth models was lower compared with the values reported by Cyril et al. (2021) for mixed-sex Slovakian breeds of geese (Landes: 5332.51 g; Pomeranian: 6186.14 g and Steinbacher: 5048.27 g) using the Gompertz model.

The range of the values of asymptotic weight clearly indicated the possible highest average body weight of Egyptian local geese reared for 10 weeks. The A parameter indicates mature weight or asymptotic weight or the potential final weight of the animal over time (Cak et al., 2017). It is worth emphasising that A does not imply the heaviest body weight attained by the individual, but it indicates the average weight of matured animal independent of short term fluctuation in weight due to temporary environmental effect (Gbangboche et al., 2008).

Furthermore, in studies involving growth curve modelling of poultry, synthesis of empirical reports unequivocally demonstrated that the asymptotic weight was influenced by myriads of genetic and non-genetic factors such as line (Karabag et al., 2017), genotype (Ibtisham et al., 2017; Cyril et al., 2021), management system (Karadavut et al., 2022), sex (Onder et al., 2017), and model type (Onder et al., 2017) among others. The observed influence of interplay of genotype and environment on maturity weight is not unexpected because body weight is a polygenic quantitative trait and is responsive to both genetic and diverse environmental stimuli. For instance, Karabag et al. (2017) reported an increase of 60.9% and 33.2% in mature weight parameter (A) of high body weight and low body weight line of Japanese quails, respectively, compared with the control line using Richards function after 11 generations of divergent selection for 5-week body weight.

Scaling parameter (B)

The range of the integration constant reported for the three non-linear growth models (0.72 to 3.33) was within the range reported for geese in various studies

(Ibtisham et al., 2017; Cyril et al., 2021; Liu et al., 2022).

Similar to the result herein reported, it is noteworthy that in most geese growth curve studies involving Gompertz, logistic and von Bertalanffy models, the logistic function consistently had the highest B value followed by Gompertz but lowest in the von Bertalanffy model (Ibtisham et al., 2017; Onder et al., 2017; Liu et al., 2022) except in a recent report (Karadavut et al., 2022) on local Turkish geese where the scaling parameter was lowest in the logistic model, intermediate in von Bertalanffy but highest in the Gompertz model.

The scaling parameter is established by the initial body weight at birth/hatch and relates weight at hatching (hatching, t = 0) to asymptotic weight (Sanusi, 2018). This parameter is model-dependent and can influence the asymptotic time when the asymptotic age is reached (Sanusi, 2018). Similarly, Gbangboche et al. (2008) corroborated this submission that parameter B indicates the proportion of the asymptotic mature weight to be gained after birth and is established by the initial values of weight. Nevertheless, it is worthy of note that this growth parameter does not have biological meaning but is related to the time interval between birth to maturity (Cak et al., 2017).

The lack of biological meaning of the scaling parameter is consistent with the fact that the estimated B values (0.72 to 13.35 g) for the three growth functions are inconsistent with the ideal day-old body weight of gooslings irrespective of the parental genotype (i.e. local, exotic or hybrid). For instance, goosling body weight at day old has been reported to be in the range of 90 to 94 g (Onder et al., 2017) and 79.58 to 114.98 g (Ibtisham et al., 2017) in recent studies, and 75–120 g in the present study (Table 2).

Maturing rate (K)

Similar to the trend of results of K values reported herein, reports of related studies on indigenous Turkish (Onder et al., 2017; Karadavut et al., 2022) and Chinese (Liu et al., 2022) geese breeds using the von Bertalanffy, Gompertz and logistic models also indicated that the parameter K values followed same trend.

It is noteworthy that irrespective of the breed, sex, management and growth functions used in previous studies, the range of estimated maturity rate reported for the growth functions in this study (0.17–0.48 g/d) was higher than the range of 0.031 to 0.071 and 0.00252 to 0.000655 reported for indigenous Chinese (Liu et al., 2022) and Turkish (Karadavut et al., 2022) goose breed, respectively. In similar vein, the maturity rate of 0.25 g/day reported for Gompertz model in this study is five-fold higher than 0.05 g/day documented for three Slovakian native geese breeds (Cyril et al. 2021) using the Gompertz model.

It is worth emphasizing that for the three non-linear regression models, values of parameter K were higher than 0, thus implying a relative growth rate from hatch to maximum growth (Sanusi and Oseni, 2020). The maturity index of growth rate estimates the relative rate at which the asymptotic value is reached (Sanusi, 2018), represents the rate of maturity of animal and indicates the growth velocity in reaching the asymptotic weight from the initial weight (Lupi et al., 2015). The highest value of K implies earlier maturity; thus, the higher the K value, the faster the animal reaches or attains the mature weight and low values indicate animals with a delayed maturity or those that tend to mature more slowly (Lupi et al., 2015).

The highest K value estimated for the logistic growth function implies that growth of geese described by this growth model would have the fastest growth rate and would reach maturity age earlier than those described with the Gompertz and von Bertalanffy models. Therefore, it is noteworthy that this growth curve parameter is an important economic trait because of its direct effect on mature body weight and inflection weight of animals. In view of this, geese that attain the estimated maximum growth rate (K) at earlier ages can be selected for breeding, since this growth parameter is moderately heritable (Kaplan et al., 2016). This submission is consistent with an earlier submission of Kopuzlu et al. (2014) that parameter K describes the earliness of maturing and offers a unique trait to evaluate animals, and the relationships between size and productivities.

Inflection weight (Wi)

The comparison of the trend of Wi reported for Egyptian local geese in this study with similar studies indicated that Wi values followed the same trend in indigenous Chinese (Ibtisham et al., 2017; Liu et al., 2022) and Turkish (Ibtisham et al., 2017; Onder et al., 2017) breeds of geese.

It is noteworthy that the inflection weight of 1431.99 g reported for the Gompertz model was much lower than the range (1855.98 g to 2274.38 g) reported for three mixed-sex Slovakian geese breeds (Cyril et al., 2021), using the Gompertz model. However, it was higher than 1413 g reported for Magang goose (Liu et al. 2022) and 1263.66 g reported for Sichuan White goose (Liu et al., 2017) using the Gompertz model.

Furthermore, the highest Wi (1627.00 g) reported for the logistic model was lower in contrast to 1712 g and range 2033 g to 2452 g reported for Magang female geese (Liu et al., 2022) and native Turkish breed of geese (Onder et al., 2017), respectively, but higher than 1321.7 g reported for Sichuan White goose (Liu et al., 2017). The weight at inflection for the von Bertalanffy model (1350.81 g) in this study is comparable to 1343.81 g reported for mixed-sex Chinese Sichuan White goose (Liu et al., 2017).

Inflection time (Ti)

The comparison of the estimated Ti values with similar studies indicated that the estimated Ti for the von Bertalanffy model (4.53 weeks; 31.71 days) in the present study was much lower compared with

5.05 weeks reported for Sichuan white goose (Liu et al., 2017), but higher than 25.86 days and 21.60 to 22.50 days documented for Magang geese (Liu et al., 2022) and Turkish native geese (Onder et al., 2017), respectively. In similar vein, the estimated inflection time for the logistic model (5.37 week) compared favourably with 5.0–5.34 weeks reported for native Chinese geese breeds (Qu et al., 2017) but higher than the range (4.10–4.30 weeks) estimated for Wanxi white (Wang et al., 2014**)** and Sichuan white (Liu et al., 2017) geese.

A critical look at the estimated Wi and Ti for Egyptian native geese in the present study reveals that it takes a shorter period (31.71 days) for geese to reach the highest Wi (1350.81) using the von Bertalanffy model but takes extra two and six days for Gompertz (Ti 33.67 days; Wi 1431.99 g) and logistic (Ti 37.59 days; Wi 1627g) growth functions, respectively, to reach inflection weights.

The inflection point indicates the period with the fastest growth rate, after which the growth rate will gradually slow down (Ibtisham et al., 2017). The inflection age, i.e. the age at maximum instantaneous relative growth rate can be used to predict the market age (Guo et al., 2016). Early maturity age is an economic trait in livestock enterprise. Early-maturing animals have propensity to reach adult and market weights earlier than the late maturing ones. In addition, the early maturing animals tend to reach puberty earlier and commence reproductive activities earlier than late maturing ones, thus, producing more progenies in their lifetime with attendant higher economic returns compared with late maturing ones.

It is noteworthy that empirical studies on estimated genetic parameters for growth models in poultry are sparse; nevertheless, reports of diverse growth curve studies of poultry species such as turkey (Aslam et al., 2011) and quails (Kaplan et al., 2016; Karabag et al., 2017) have unequivocally demonstrated that the reported growth curve parameters (A, B and K) and inflection points (I_A and I_W) are heritable and could be altered via selection. In view of this, sound understanding and exploitation of growth curve parameters and inflection points would be of invaluable assistance in selection and improvement of the understudied Egyptian native geese.

Determination of the best-fitting model

Based on the values of the coefficient of determination, it could be deduced that Bertalanffy was the best (having highest R^2) followed by Gompertz and logistic models.

It is noteworthy that the values (0.891–0.898) of R^2 for the three mathematical growth functions in this study were lower compared with 0.987 to 0.995 (Onder et al., 2017), 0.9504 to 0.9686 (Karadavut et al., 2022), 0.992 to 0.999 (Ibtisham et al., 2017), and 0.997 to 0.998 (Cyril et al., 2021) reported for different breeds of geese. The remote and immediate reasons for the trend are not clearly understood. Nevertheless, the high R^2 reported for all the models indicated that all the models applied suitably fitted the growth data and that 89.10 to 89.80% variability in the body weight of Egyptian local geese was well explained by the three growth functions.

In growth curve studies, the goodness-of-fit helps to determine adequacy of a model in describing analyzed data and, because of its importance in choosing the best models, researchers often apply more than one model to arrive at the best goodness-of-fit tests in growth models, thus helping to choose the most appropriate model for the data analyzed (Sanusi, 2018) due to limitations of different goodness of-fit criteria.

For instance, R^2 and adjusted R^2 have been reported not representing a good metric for assessing the performance of nonlinear models since they do not account for the number of parameters amongst others; hence, it was proposed that they should not be used in isolation, but in combination with other goodness-of-fit algorithms (Archontoulius and Miguez, 2015). In view of this, the decision to choose the best fit model could not be based on the R^2 alone due to the aforementioned limitations and its limitation in not penalizing over-parameterization (Sanusi and Oseni, 2020) but would be based on agreement with other goodness-of-fit criteria.

Nevertheless, it is noteworthy that the application of other goodness-of-fit criteria corroborated R^2, identifying the von Bertalanffy model as the best growth-fitting model followed by Gompertz while the logistic model was the poorest. This conclusion was hinged on the fact that the goodness-of-fit test values reported for the von Bertalanffy model were highest for R^2 but lowest for RMSE, AIC and BIC. Therefore, based on the highest R^2 and lowest RMSE, AIC and BIC values reported for the von Bertalanffy model compared with Gompertz and logistic models, it can be concluded that von Bertalanffy was the best growth model describing the growth curve of Egyptian local geese. In similar vein, the von Bertalanffy function was identified as the best fitting model in related studies using similar growth models in Turkish native (Onder et al., 2017) and Chinese Magang (Liu et al., 2022) breeds of geese.

The von Bertalanffy model being the best fitting growth function in this study is not unexpected. Growth fitting curves with flexible inflection points such as Richards, Morgan and von Bertalanffy have been identified to describe nonlinear growth curves better than Gompertz and logistic models with fixed inflection points (Porter et al., 2010). Similarly, Zuidhof (2005) reported that the sigmoidal models with a flexible point of inflection predicted carcass part weights better than Gompertz with a fixed point of inflection. However, synthesis of empirical studies on growth curve of geese revealed that studies adjudging von Bertalanffy as the best model were few but Gompertz and logistic (Liu et al., 2022) and

Richards (Ibtisham et al., 2017) were mostly adjudged best predictive models.

Growth curve description

It is noteworthy that the growth functions overestimated the growth of geese from hatch (0 day) to 4 weeks of age but slightly underestimated the growth between 4 and 6 weeks of age. Furthermore, they perfectly fitted the curve between weeks 6 and 7 but slightly overestimated the growth again after week 7. Nevertheless, the differences between the observed and estimated values were negligible but higher in the logistic model than in others.

A recent report by Safari et al. (2021) has adduced poor fitting of growth curves with a fixed or little flexible inflection point to the dependency of their inflection points on the weight at sexual maturity. A possible principal factor contributing to superior fitting of data of growth of Egyptian local geese by the von Bertanlanfy function could be attributed to its lower inflection point (31.73 days) compared with curves with fixed inflection points (Gompertz – 33.67 days; logistic – 37.59 days). This submission was accentuated by earlier reports of Schulim-Zeuthen et al. (2008) that the Schumacher equation with a flexible inflection point described the growth curve better, and its superior fitting of the growth model was linked to its inflection point at an earlier age compared with the Gompertz and logistic models.

Conclusion

The study demonstrated that the three mathematical growth functions explained well the variability in the age-body weight relationship of Egyptian local geese. However, putting into consideration the values of goodness-of-fit tests, the von Bertalanffy was the best growth-fitting curve model based on its highest R^2 and lowest RMSE, AIC and BIC compared with Gompertz and logistic models. The estimated growth parameters and growth descriptors could be exploited by geese farmers in making informed decision on feeding strategies and exploration of the growth parameters and descriptors by animal breeders in genetic improvement of Egyptian indigenous geese.

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Competing interests

The authors declare that they have no potential conflict of interest.

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Use of Grape Marc Flour Supplementation in Laying Hens' Diet on Laying Productivity, Egg Quality and Biochemical Parameters

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Key words: *laying hens; grape marc flour; egg quality; yolk MDA level.*

Abstract*: The present study was carried out to evaluate the effect of grape marc flour addition to the laying hens' diet on their egg production, egg quality, total yolk lipids and yolk total fatty acid composition, cholesterol content in the yolk and blood serum as well as on the yolk lipid oxidation. An experiment was conducted with a total of 90 laying hens (at 40 weeks old) from Lohmann Classic Brown breed, randomly divided into three groups, 30 hens in each (3 replications x 10 layers per group). The diet of the experimental hens was supplemented with 1% and 3% of grape marc flour. The trial duration was 48 days. Grape marc flour addition to the laying hens' compound feed did not significantly (P > 0.05) affect their live body weight, egg production, egg morphological properties as well as total yolk lipids content, total cholesterol content in the yolk and blood serum and yolk fatty acids composition. However, the egg yolk malondialdehyde (MDA) level during egg storage for 30 days at room temperature significantly decreased (P < 0.01) in comparison with control eggs. The addition of grape marc flour has the potential to extend the shelf life of eggs.*

Introduction

Grape (*Vitis vinifera* L.) is one of the richest sources of phenolic and other antioxidant compounds among fruits (Kupe et al., 2021). A large part of grape production is intended for the preparation of wines, juices, distillates and other products, generating by-products that could be used as ingredients for the development of new products as well as components in animal and poultry diets (Shirahigue et al., 2010). In this way, environmental pollution due to the accumulation of these residues is also prevented (Devesa-Rey et al., 2011; Christ and Burrit, 2013; Fontana et al., 2013). The wine industry generates substantial quantities of waste, such as grape marc, discarded clusters, seeds and sediments. In fact, pomace represents about 20–30% of the original grape weight (Dwyer et al., 2014). In the Balkan countries, there is a long-standing tradition for production of grape marc distillates after the winemaking process (Lukic et al., 2011). After the distillation process, the solid residue from grape obtained is called spent grape marc (Graça et al., 2018). Globally, the annual production of grape marc (GM), the residue of skins, seeds and stems remaining after making wine, has been estimated to be approximately nine million tons (Moate et al., 2020). A number of authors have reported the positive effect of the addition of grape pomace in the diet of ruminants (Babău et al., 2019), equine (Kollathova et al., 2021), rabbits (Bonzaida et al., 2021), laying hens (Kara et al., 2016; Mirghelenj et al., 2017; Olteanu et al., 2019), and quails (Froes et al., 2018). Sahin et al. (2008), Jung et al. (2011), and Zang and Kim (2014) explain this fact by the antioxidant action of polyphenols (catechin, epicatechin, procyanidin and anthocyanidin) which are contained in grape pomace. Malosini et al. (1993) conducted an experiment with heavy lambs receiving 30% and 60% of grape marc in their diet. The authors recommended that this by-product be added in limited quantities, because its higher intake leads to a decrease in digestibility. In fact, according to Wu et al. (2022), grape marc can replace 20% of the control ration to maintain sheep productivity, health, and environmental sustainability. Moate et al. (2020) observe a reduction of methane emissions but at the cost of decreased milk production when dairy cows are fed grape marc. There are no documented studies on the effect of using grape marc in the laying hens' diet on their egg productivity, egg quality and egg fatty acid profile. The aim of the current scientific work is to determine how the addition of a grape marc meal to the laying hens compound feed can affect egg performance, egg morphological properties, total yolk lipids, total yolk fatty acid profile, blood serum and yolk cholesterol contents, as well as yolk lipid oxidation.

Materials and methods

This experiment complies with Directive 2010/63/EU on the protection of animals used for scientific purposes, and the experimental procedures have been approved by the Bulgarian Animal Ethics Committee in accordance with Bulgarian Veterinary Law (2011) on the protection of animals used for experimental and other scientific purposes and relevant provisions

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Council Directive 86/609/EEC (Permission for using agricultural animals for scientific purpose, N177 obtained on the base of Protocol N33/18.06.2015).

The study was carried out in the Poultry Experimental Farm of the Institute of Animal Science – Kostinbrod, Bulgaria. A total of 90 Lohman Brown laying hens at the age of 40 weeks were randomly distributed into 3 groups (n = 30 hens/group): one control and two experimental (3 replications per group, 10 poultry in each replication). The layers from each replication were raised in separate boxes on a deep litter pen on a 16-hour lighting schedule. Water was supplied using nipple drinkers. The experiment duration was 48 days (14-day preparatory and 34-day experimental periods). During the preparatory period, the poultry from all the groups received compound feed for laying hens in the amount of 130 g/day/hen in order to eliminate the influence of the previous diet. During the experimental period, the hens received the same amount of this compound feed, whereas the diet of the experimental hens was supplemented with 1% (experimental group 1) and 3% (experimental group 2) of dried grape marc flour.

The ingredients and chemical composition of laying hens' diets are pointed in Table 1.

The total chemical composition of the diets and of the grape marc flour was determined as follows: moisture, crude protein, crude fat, and crude fibres by the conventional Weende analysis; the content of both Ca and P by AOAS (2007); ß carotene and licopene of the tested product by a method described by George et al. (2011); grape marc total polyphenol content after preliminary esterification by Folin-Ciocalteu method described by Blainski et al. (2013); fatty acids composition of grape marc lipid using HP 5890 II gas chromatograph equipped with flame ionization detector and type capillary column "Supelco" SP™-2390.

The pH values were measured using a pH meter Stirrer, type OP-951. The total antioxidant activity of grape marc was determined using the 2,2-diphenyl1-picrylhydrazyl (DPPH) method described by Petrova et al. (2016). The metabolizable energy of the diets was calculated according to Todorov et al. (2021). At the beginning and at the end of the trial, the live body weight of the poultry from the control and the experimental groups was measured. Daily laying intensity (in percent) was controlled throughout the trial. Thirty eggs from each group, laid within two consecutive days, were taken at the beginning and at the end of the experiment, and the following measurements were taken: the weight of the egg, yolk, albumen and eggshell with shell membrane (by balance with a precision of 0.001 g); egg shell thickness (mm) without the shell membrane (measured at three locations by a micrometer Ames 25EE with a precision of 0.0001 mm); Haugh units (by index meter); shape index (by index meter Van Dorn De Bilt N 72205-1); albumen index (determined by measuring the large and small egg diameters and albumen height

Table 1. Composition and nutritive value of laying hens' diets

Ingredients, %	Control	Experimental group 1	Experimental group 2
Wheat	65.04	64.54	63.04
Soybean meal	10.50	10.50	10.50
Sunflower meal	12.0	11.50	11.0
Sunflower oil	2.0	2.0	2.0
Spent grape marc	0.0	1.0	3.0
Limestone	4.30	4.30	4.30
Limestone (little rocks)	4.30	4.30	4.30
Mono calcium phosphate	0.6	0.6	0.6
Complex premix 6015*	1.25	1.25	1.25
Nutritive value			
Metabolizable energy, kcal kg^{-1}	2720	2720	2720
Crude proteins, %	16.72	16.72	16.72
Crude fat, %	3.31	3.31	3.31
Crude fibre, %	4.23	4.50	5.07
Ca, %	3.6	3.6	3.6
P, %	0.51	0.51	0.51
pH	6.11	6.34	6.33

* Complex premix contains: Mn (MnO): 120 mg/kg; Zn (ZnO): 110 mg/kg; Fe ($FeSO_4$): 140 mg/kg; Cu ($CuSO_4$): 18 mg/kg; I ($Ca(IO_3)_2$: 1.80 mg/kg; Se (Na_2SeO_3): 0.35 mg/kg; vitamin A (retinyl acetate): 9900 UI; vitamin D_3 (cholecalciferol): 3000 UI; vitamin E (DL-alpha-tocopherol): 30 mg/kg. It does not contain nutritive antibiotics, synthetic dyes and carotenoids or other stimulants.

using a caliper and calculated by the formula: I_{al} (%) = (h/[D+d]/2x100) where h is the height of the thick albumen (in mm); D is a big albumen diameter; and d is a small albumen diameter; yolk index (determined by measuring the yolk diameter and its height using a caliper and calculated by the formula: YI (%) = (h/d) x100) where h is height of the yolk and d is diameter of the yolk; egg yolk color (visually according to the Roche Color Fan). The content of Ca and P in the eggshell was determined according to AOAS (2007).

At the end of the treatment, 10 hens from each group were chosen randomly and blood samples were taken from *Vena cutanea ulnaris*. The content of total cholesterol in the blood serum was measured by commercial kits using biochemical analyser BioSystems (S. A. Costa Brava, Spain). At the end of the experiment, some lipid fractions of egg yolks of 10 eggs from each group were analysed. The total lipids were evaluated by the method of Bligh and Dyer (1959). The total cholesterol content in the yolk was determined by the method of Schoenheimer-Sperry modified by Sperry and Webb (1950). The fatty acid composition of egg yolk lipids was estimated by HP 5890 II gas chromatograph equipped with flame ionization detector and type capillary column "Supelco" SP™-2390 with a length of 60 m and an inner diameter of 0.25 mm after preliminary esterification. At the end of the trial, the lipid oxidation of egg yolk was examined by analyses of 6 eggs from each group as TBARS according to the method of Castellini et al. (2006). Oxidation products were quantified as malondialdehyde equivalents (mg MDA 100 g^{-1}). The results obtained in this study were statistically processed by EXCEL 2007, single factor, ANOVA program. All dates are presented as means with their standard errors (X ± SE).

Results

The chemical composition, antioxidant properties and pH value of dried grape marc flour used in our study are shown in Table 2.

Table 3 presents a fatty acid composition of the tested by-product.

Table 4 presents the data of live body weight, laying intensity and mortality of the hens from the control and the experimental groups.

The results on the content of calcium and phosphorus in the eggshell are presented in Table 6.

The profile of egg yolk fatty acids is presented in Table 7. The dietary supplementation included 3% of grape marc.

Discussion

In the scientific literature, the data about crude protein, crude fiber and crude fat of grape marc and grape pomace vary significantly according to the reports provided by different authors (Malossini et al., 1993; Mihna & Muhammad, 2017; Moate et al., 2020; Kolláthová et al., 2021). In general, the results presented here are within the range of values obtained by these authors. The differences in the chemical composition are probably due to many factors, including the variety and the color of grape and the different pressing processes associated with making red and white wines (Spanghero et al., 2009). As seen from Table 2, the grape marc used in our scientific work did not contain carotenoids – lycopene and ß carotene. The tested by-product had lower total content of polyphenols than the grape pomace described by Olteanu et al. (2019), and its total antioxidant activity was lower. The pH value of the tested supplement was 4.89, while the pH values of the feed at the beginning and the end of the experiment were within close range of 6.11, 6.34, and 6.33 for the control group, and experimental groups 1 and 2, respectively.

Grape marc flour is rich in oleic acid (20.3%) and linoleic acid (49.9%) (Table 3). Palmitic acid is the most common saturated fatty acid accounting for 16% of total fatty acids in grape marc. These findings are

Table 2. Chemical composition, total antioxidant capacity and the pH value of dried grape marc flour

Parameters	Grape marc flour
Moisture, %	6.42
Crude protein, %	12.64
Crude fat, %	8.04
Crude fibre, %	39.15
Ca, %	0.515
P, %	0.175
Total phenolic content, mg GAE/100 g	4.00
Total antioxidant activity, mmol TE/100 g	52.80
ß- carotene, µg/g	0.00
Licopene, µg/g	0.00
pH	4.89

GAE – galic acid equilent; TE – Trolox equivalent

Table 3. Fatty acid content of grape marc flour (given in percentage of the total amount of fatty acids)

Fatty acid	%	Fatty acid	%
Lauric ($C_{12:0}$)	0.1	Linoleic acid (n-6) ($C_{18:2}$)	49.9
Myristic ($C_{14:0}$)	0.5	α-Linoleic ($C_{18:3}$)	0.7
Pentadecanoic ($C_{15:0}$)	0.1	Arachidic ($C_{20:0}$)	0.7
Ginkgolic acid ($C_{15:1}$)	0.1	Eicosenoic ($C_{20:1}$)	0.3
Palmitic ($C_{16:0}$)	16.1	Heneicosanoic ($C_{21:0}$)	0.6
Palmitoleic ($C_{16:1}$)	0.7	Eicosadienoic ($C_{20:2}$)	0.1
Heptadecanoic ($C_{17:0}$)	0.1	Eicosatrienoic ($C_{20:3}$)	1.0
Stearic ($C_{18:0}$)	8.4	Arachidonic ($C_{20:4}$)	0.1
Oleic ($C_{18:1}$)	20.3	-	-
Monounsaturated fatty acids (MUFA)	21.4	Polyunsaturated fatty acids (PUFA)	52.0
Unsaturated fatty acids	73.4	Saturated fatty acids	26.6

Table 4. Live body weight (g), laying intensity (%) and mortality of laying hens (X ± SE)

Parameters \ Groups	Control	Grape marc flour (%)	
		1.0	3.0
Initial body weight (g)	1832 ± 31.90	1768 ± 31.64	1875 ± 29.70
Final body weight (g)	1930 ± 35.61	1902 ± 32.11	1925 ±3 0.84
Laying intensity (%), start of experiment	88.75 ± 3.26	87.33 ± 3.33	86.89 ± 4.15
Laying intensity (%), end of experiment	90.43 ± 1.35	90.57 ± 1.34	92.86 ± 1.01
Mortality (%)	0.00	0.00	0.00

Table 5. Egg morphological parameters of the hens from the control and the experimental groups (X ± SE)

Indices \ Groups	Control	Grape marc flour supplementation (%)		Control	Grape marc flour \supplementation (%)	
		1.0	3.0		1.0	3.0
	Start of the experiment			End of the experiment		
Egg weight, g	60.50 ± 0.77	57.64 ± 0.75	59.73 ± 0.74	64.94 ± 1.14	62.77 ± 0.83	64.53 ± 0.90
Albumen weight, g	38.76 ± 0.65	36.99 ± 0.53	37.45 ± 0.63	41.61 ± 0.89	39.59 ± 0.62	40.80 ± 0.73
Yolk weight, g	15.27 ± 0.23	14.57 ± 0.26	15.44 ± 0.20	16.31 ± 0.30	15.93 ± 0.22	16.01 ± 0.21
Shell weight, g	6.44 ± 0.09	6.20 ± 0.12	6.60 ± 0.11	7.24 ± 0.14	7.24 ± 0.11	7.60 ± 0.11
Shell thickness, mm	0.40 ± 0.004	0.39 ± 0.004	0.40 ± 0.004	0.40 ± 0.005	0.41 ± 0.003	0.42 ± 0.003
Haugh units	85.00 ± 0.84	83.07 ± 1.03	81.20 ± 1.18	84.00 ± 1.20	84.03 ± 1.17	84.03 ± 1.10
Shape index %	79.29 ± 0.53	79.42 ± 0.37	79.22 ± 0.49	78.90 ± 0.42	78.32 ± 0.46	78.87 ± 0.36
Albumen index %	10.72 ± 0.34	10.20 ± 0.36	9.43 ± 0.30	9.84 ± 0.40	10.31 ± 0.40	10.27 ± 0.39
Yolk index %	45.93 ± 0.64	43.44 ± 0.76	43.56 ± 0.44	43.67 ± 0.76	43.51 ± 0.68	42.46 ± 0.61
Yolk color (Roche)	4.18 ± 0.22	4.03 ± 0.15	4.10 ± 0.10	4.46 ± 0.22	4.30 ± 0.24	4.20 ± 0.25

in accordance with those of Moate et al. (2020). The essential linoleic acid has the highest proportion of polyunsaturated fatty acids (PUFA) in dried grape marc flour. Similar values of linoleic acid in grape pomace are reported by Ribeiro et al. (2015). The tested grape marc contains 21.4% of monounsaturated fatty acids (MUFA), 52% of polyunsaturated fatty acids (PUFA) and 26.6% of saturated fatty acids (SFA).

Laying hens' productivity and morphological properties of eggs

The live body weight of layers did not change significantly ($P > 0.05$) (Table 4). This parameter increased with 98 g, 134 g and 50 g for the control group and experimental groups 1 and 2, respectively, at the

Table 6. The content of calcium and phosphorus in the eggshell of laying hens from control and experimental groups (X ± SE)

Groups \ Indices		Calcium content (%)		Phosphorus content (%)	
		Start of the experiment	End of the experiment	Start of the experiment	End of the experiment
Control		34.80 ± 0.090	35.80 ± 0.080	0.120 ± 0.001	0.123 ± 0.001
Grape marc flour supplementation (%)	1.0	34.16 ± 0.100	35.38 ± 0.090	0.115 ± 0.002	0.116 ± 0.001
	3.0	34.64 ± 0.090	36.10 ± 0.100	0.125 ± 0.003	0.119 ± 0.002

Table 7. Fatty acid profile of lipids extracted from egg yolk (n = 6/group), (X ± SE)

Fatty acid	Control %	Grape marc flour supplementation 3%	Fatty acid	Control %	Grape marc flour supplementation 3%
$C_{6:0}$ Caproic acid	0.125 ± 0.25	-	$C_{18:2}$ (ω-6) Linoleic acid	8.05 ± 0.68	8.07 ± 0.97
$C_{8:0}$ Caprylic acid	0.1 ± 0.00	-	$C_{18:3}$ (ω-3) ϒ-Linoleic acid	0.22 ± 0.03	0.2 ± 0.025
$C_{10:0}$ Capric acid	0.1 ± 0.00	0.15 ± 0.05	$C_{20:0}$ Arachidic acid	0.1 ±0 .00	0.1 ± 0.00
$C_{12:0}$ Lauric acid	0.1 ± 0.00	0.15 ± 0.05	$C_{20:1}$ Eicosenoic acid	0.35 ± 0.022	0.37 ± 0.07
$C_{14:0}$ Myristic acid	0.46 ± 0.024	0.54 ± 0.04	$C_{21:0}$ Heneicosylic acid	0.13 ± 0.02	0.28 ± 0.087
$C_{14:1}$ Myristoleic acid	0.13± 0.33	0,125 ± 0.025	$C_{20:2}$ (ω-6) Eeicosadienoic acid	0.1 ± 0.00	0.12 ± 0.016
$C_{15:0}$ Pentadecylic acid	0.12 ± 0.02	0.12 ± 0.02	$C_{20:3}$ (ω-3) Eicosatrienoic acid	0.1 ± 0.00	0.1 ± 0.00
$C_{15:1}$ Ginkgolic acid	0.17 ± 0.05	0.15 ± 0.03	$C_{20:4}$(ω-6) Arachidonic acid	0.55 ± 0.11	0.50 ± 0.07
$C_{16:0}$ Palmitic acid	31.88 ± 0.72*	33.03 ± 0.70	$C_{22:0}$ Behenic acid	0.1 ± 0.00	0.16 ± 0.06
$C_{16:1}$ Palmitoleic acid	2.4 ± 0.15	3.12 ± 0.40	$C_{23:0}$ Tricosylic acid	0.13 ± 0.02	0.12 ± 0.02
$C_{17:0}$ Margaric acid	0.2 ± 0.00	0.18 ± 0.016	$C_{22:2}$(ω-6) Docosadienoic acid	0.25 ± 0.07*	0.43 ± 0.15
$C_{17:1}$ Heptaeceonic acid	0.125 ± 0.025	0.16 ± 0.024	$C_{20:5}$(ω-3) Eicosapentaenoic acid	0.43 ± 0.05	0.52 ± 0.12
$C_{18:0}$ Stearic acid	10.72 ± 0.75	10.00 ± 0.81	$C_{24:0}$ Lignoceric acid	0.18 ± 0.03	0.14 ± 0.02
$C_{18:1}$ Oleic acid	43.12 ± 1.21*	41.58 ± 1.035	$C_{22:6}$(ω-3) Docosahexaenoic acid	0.1 ± 0.00	0.13 ± 0.025
Saturated fatty acids	44.13 ± 1.45	44.6 ± 1.56	Monounsaturated fatty acids	46.2 ± 1.23*	45.4 ± 1.03
Unsaturated fatty acids	55.87 ± 1.44	55.4 ± 1.56	Polyunsaturated fatty acids	9.67 ± 0.83	10.9 ± 1.05*

Significance * $P \leqslant 0.05$

end of the trial. At the beginning of the experiment, the hens' laying intensity was as follows: 88.75% for the control group, 87.33% for experimental group 1, and 86.89% for experimental group 2, while at the end of the treatment, it slightly increased and the measured values reached 90.43%, 90.57%, 92.86% for the control group, and experimental groups 1 and 2, respectively. The differences between the groups were not significant ($P > 0.05$). At the end of the experiment, an increase in laying intensity was observed by 1.68%, 3.24%, 5.97% for the control group and experimental groups 1 and 2, respectively. Kara et al. (2016) obtained similar results when feeding a supplemented diet with 4% and 6% grape pomace for 12 weeks. Alm El Dein et al. (2017) established a significant increase of the laying intensity ($P < 0.05$) without a significant effect on the body weight by adding 1%, 2%, 3% and 4% of grape pomace (except for the level of 1%) to laying hens' diet. There was no mortality observed in all the groups during the treatment. Throughout the experiment, the poultry of all three groups consumed the diets with appetite and were in good health, lively, with good exterior and plumage.

The results reporting egg morphological properties of laying hens from the control and the experimental groups are presented in Table 5. The grape marc addition in doses of 1% and 3% did not affect significantly the weight of the egg, albumen, yolk and eggshell, as well as the shell thickness, Haugh units, shape albumen and yolk indexes. As far as we know, few studies are currently available about the dietary supplementation of grape pomace in layers and its impact on egg morphological parameters (Romero et al., 2022; Kara et al., 2016; Ozgan, 2008). Kara et al. (2016) included 4% and 6% of grape pomace in

layers' compound feed, but here no significant effects on egg quality were observed either. Romero et al. (2022) reported increasing the egg yolk color and Haugh units in the groups with the intake of grape pomace and extract. In contrast to findings, Ozgan (2008) reported an increase in the albumen index with the addition of 2% of grape pomace to laying hens' diets.

The inclusion of 1% and 3% of grape marc to the hens' diet did not have a negative effect on the content of calcium and phosphorus in the eggshell (Table 6).

In the commercial egg market, richer-colored yolks are in demand, and this characteristic depends exclusively on the compound feed. This is due to the fact that even though hens are not able to synthesize pigments, they are able to absorb between 20% and 60% of the diet pigments (Moura et al., 2011). In this study, the yolk color intensity into the groups varied in close range from 4.03 to 4.46 points on the Roche Color Fan both at the beginning and at the end of the trial ($P > 0.05$). This fact can be explained by the lack of carotenoids in grape marc used in our research. Froes et al. (2018) noticed an increase of yolk pigmentation density in quails' egg when feeding grape pomace supplemented diet (2%, 4%, 6%). According to the authors, this enhancement of yolk color is due to the anthocyanins' content in grape pomace.

Fatty acid composition of egg yolk

The dietary inclusion of 3% of grape marc reduced the proportion of MUFA in the yolk with respect to the control group (45.4% vs. 46.2%, $P = 0.05$) and increased the proportion of PUFA (10.9% vs. 9.67%, $P = 0.05$) (Table 7). The content of oleic acid decreased (41.58% vs. 43.12%, $P = 0.05$) as compared with the eggs of control hens. Romero et al. (2022) reported a reduction of SFA proportion in the yolk (31.9% vs. 32.9%, $P = 0.001$), a MUFA decrease (39.5% vs. 41.4%, $P < 0.001$), and a PUFA percentage decrease (28.9% vs. 25.7%, $P < 0.001$), as compared with the eggs of the control hens.

Yolk lipids and TBARS value

The content of total yolk lipids, total cholesterol in the yolk and blood serum as well as the lipid oxidation are presented in Table 8. There are no significant differences in regard to the total yolk lipids and the total cholesterol content in the yolk and blood serum between the groups ($P > 0.05$). The results obtained were in compliance with the experiment performed by Kara et al. (2016). Herber and Van Elswyk (1996) considered that the cholesterol in the egg yolk changed slightly or in many cases did not change at all under the influence of genetic, pharmacological or nutritive factors. As it can be seen from Table 8, dietary supplementation of grape marc in the doses of 1% and 3% significantly reduced MDA concentration in yolk after egg storage at room temperature ($P \leqslant 0.01$). This leads to an increase of eggs' shelf life. Similar results were found by other authors when adding grape pomace to the hens' diet (Brenes et al., 2010; Brannan, 2009; Banon et al., 2007; Lau and King, 2003; Pazos et al., 2004; Carpenter et al., 2007). These results can be explained by the antioxidant properties of the polyphenolic compounds contained in grape pomace and grape marc (Monteiro et al., 2021).

Conclusions

The dietary inclusion of grape marc flour in doses of 1% and 3% did not significantly change the body weight, laying intensity, egg morphological properties, the content of yolk lipids or the total cholesterol content in the blood serum and the egg yolk ($P > 0.05$). The addition of 3% of grape marc significantly decreased the content of oleic acid and significantly reduced the proportion of MUFA in the yolk. In addition, it significantly increased the proportion of PUFA as compared with the eggs of the control hens ($P < 0.05$). The use of grape marc in the hens' diet improved the shelf life of eggs. This is due to reduced concentration of MDA ($P < 0.05$) in egg yolk after storage of eggs at room temperature.

Table 8. The effect of dietary grape marc on yolk lipids, yolk cholesterol, and lipid oxidation (X ± SE)

Groups / Indices	Control	Grape marc flour supplementation (%)	
		1.0	3.0
Total lipids g/100 g yolk	34.89 ± 0.42	36.10 ± 0.44	35.74 ± 0.33
Total cholesterol, mg/100 g yolk	1475.86 ± 37.86	1430.63 ± 44.96	1442.64 ± 20.06
Total cholesterol in blood serum, mmol/L	4.25 ± 0.38	4.23 ± 0.27	4.11 ± 0.27
Malondialdehyde (MDA), μg/g			
At the end of the experiment	0.48 ± 0.02	0.58 ± 0.04	0.60 ± 0.04
Storage 30 days in a fridge	0.85 ± 0.05	0.80 ± 0.05	0.84 ± 0.04
Storage 30 days at room temperature	4.15 ± 0.67	1.19 ± 0.08**	1.25± 0.07**

Significance by: ** $P \leqslant 0.01$

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LIVESTOCK PRODUCTION: RECENT TRENDS AND FUTURE PROSPECTS

Abstracts

A COMBINATION OF WHEAT CEREAL OUTER LAYER THERMO-MECHANICAL PROCESSING AND BIOCONVERSION FOR SUSTAINABLE VALUE-ADDED FEED STOCK PRODUCTION

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Wheat (*Triticum* spp.) is one of the most popular crops worldwide; however, the most popular part of wheat is the endosperm, and, until now, other layers have been used as low nutritional value feed stock. However, it should be pointed out that most of the bioactive compounds in cereals are concentrated in the outermost tissues. On the other hand, undesired compounds (mycotoxins, etc.) also occur in these fractions. For all these reasons, pre-treatment technologies to improve the properties of wheat bran (WPBP) are being studied, of which fermentation with selected lactic acid bacteria (LAB) strains is the most popular for WPBP valorization. Also, possible strategies for WPBP functionalization include extrusion. Extrusion causes many structural, physicochemical, and microbial transformations of stock, e.g., depolymerization of starch, denaturation of proteins, oxidation of lipids, decontamination of bacteria, etc., and these changes are influenced by extrusion process parameters. Until now, no study on the combination of extrusion and fermentation, especially with LAB strains which possess antimicrobial properties, has been published in the literature to date. The aim of this study was to evaluate the influence of combining extrusion and fermentation (with *L. casei* and *L. paracasei* strains) processes on the chemical and biosafety of WPBP. An extrusion experiment was performed by testing two different temperatures (115°C and 130°C) and three different speeds of extruder screw (16 rpm, 20 rpm, and 25 rpm). Very prospective results were obtained, and it was concluded that the appropriate extrusion parameters and LAB strain selection lead to higher formation of L(+) isomers and lower WPBP microbial contamination. Extrusion, as well as extrusion in combination with fermentation, reduces total biogenic amine content (on average, 2 times). The lowest mycotoxin concentration was found in $W_{ex130/screwspeed20}$ and $W_{ex130/screwspeed25}$ samples fermented with both LAB strains. Finally, the combination of extrusion and fermentation with *L. casei* and *L. paracasei* strains can be confirmed as a prospective innovative pre-treatment for WPBP, potentially capable of enhancing its composition and safety characteristics.

Keywords: wheat bran, extrusion, fermentation, mycotoxins, safety.

THE EFFECT OF DIFFERENT GENETICAL ARCHITECTURES FOR GENOMIC PREDICTION OF CARCASS FATNESS BY MACHINE LEARNING IN CATTLE

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Carcass fatness in cattle is an important phenotype in the consumer markets, and it plays a key role in metabolism of meat flavour properties including colour and tenderness. More recently, literature has emerged that offers findings about molecular markers (for instance, single nucleotide polymorphisms, SNPs,) in association with the phenotypes of interest termed as genomic prediction (GP) and genome wide association study (GWAS) [1]. This study set out to investigate the usefulness of various ML models for GWAS and GP of carcass fatness obtained by ultrasound technology. The dataset consisted of 1439 Nellore cows genotyped for 35237 single nucleotide polymorphism (SNP) for the phenotype of carcass fatness. Bayesian and machine learning (ML) analyses were based on the Bayesian ridge regression (BRR), Bayesian lasso (BL), Bayes A (BA), Bayes B (BB) and Bayes Cπ (BC), Bayesian least absolute shrinkage and selection operator (BL), reproductive kernel Hilbert space (RKHS), support vector machine (SVM), genomic best linear unbiased prediction (GBLUP) and elastic net (EN). EN, SVM and RF resulted in the lowest value of predictive ability of carcass fatness. The highest GP accuracy was obtained from GBLUP (0.9225). Linkage disequilibrium pruning resulted in a small variation on the accuracy results. Employment of pedigree and genomic kinship matrixes on the accuracies was also discussed.

Keywords: carcass fatness, cattle, genome wide association study, genomic prediction, machine learning.

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THE INVESTIGATION OF QUANTITATIVE GENETIC PARAMETERS OF CARCASS FATNESS IN *CATTLE*

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The main aim of this study was to investigate various genetic parameters for carcass fatness (CF) in cattle (n = 1439) located across three Brazilian states, born between 2009 and 2018 [1]. Ultrasound measurements of carcass fatness (corrected for age) were obtained by a private company.

Data were analyzed using Bayesian segregation analyses. Gibbs sampling was used to make statistical inferences on posterior distributions; inferences were based on single run of the Markov chain for each trait with 500 000 samples, with each 10th sample collected due to the high correlation among the samples [2]. Posterior mean (and SD) of major gene variance for CF was 8.7004 (1.5161), for additive gene effect, it was 3.7722 (0.0601), and for dominant effect, it was 7.0851 (0.2656). The highest posterior density regions for CF did not include zero which supported the evidence for a major gene.

Keywords: bayesian analyses, carcass fatness, major gene analyses, segregation analyses.

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THE INVOLVEMENT OF *IRS1* GENES IN MILK PROTEIN SYNTHESIS IN DAIRY GOAT MAMMARY CELL

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Goat milk has been consumed by humans since ancient times and it is an important source of nutrition for humans of all ages. The content of milk protein is the prodominant evaluation of milk qualitity. Milk protein synthesis is highly regulated by insulin and the gene of insulin receptor substrate 1 (*IRS1*) plays a central role in insulin signal transduction.

Material and methods. The gene expression of *IRS1* was reduced by RNA interference (RNAi) in goat mammary epithelial cells (GMECs) and evaluated by RT-qPCR. The content of casein was determined by ELISA. Comparative proteomic analysis was adopted to analyse the protein profile. Results. The content of casein in GMECs significantly changed when *IRS1* expression was reduced. Several differential expression proteins were identified.

Conclusion. The involvement of *IRS1* regulated the milk protein synthesis in GMECs.

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THE EFFECT OF DAIRY COW STATURE AND LIVE WEIGHT ON THE PRODUCTIVITY AND LONGEVITY TRAITS

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Dairy cow potential longevity exceeds 15 years, but in modern dairy farming, due to various internal and environmental factors, cattle lifespan has been reduced to little under 3 closed standard lactations. Although it is possible to breed exceedingly bigger animals, it comes with numerous negative effects, one of which is a shorter lifespan. In modern dairy farming, it is not recommended to have Holstein Black and White breed cows taller than 150 cm, as increased stature usually leads to leg problems, increased treatment costs and possible forced culling from herd, which increases the costs per animal and increases the demand of replacement heifers. The aim of our paper was to determine the effect of dairy cows' stature and live weight at the beginning of the first lactation on cow longevity and lifetime milk productivity. Analysed data were collected from the Latvian Data Centre milk recording records about 272 633 Holstein Black and White, Holstein Red and White and Red breed group dairy cows born in timespan form year 2010–2019. For study purposes, we distributed dairy cows in 4 separate groups, depending on their live weight and stature in the first 100 days of the first lactation. To characterize total cow milk productivity, we used energy corrected milk (ECM). All analysed dairy cows during the first standard lactation produced 7179.7 ± 4.05 kg ECM and showed a tendency to increase the productivity level until the third lactation (8358.3 ± 7.34 kg ECM), but in later lactations, cows produced significantly ($P < 0.05$) less (accordingly 8180.4 ± 9.18). The average lifespan in the analysed cow population was 1925.4 ± 1.31 days (approximately 5.27 years) with the average lifetime productivity of 24 352.3 ± 26.67 kg ECM (average per 1 life day 11.9 ± 0.01 kg ECM). Cows born in the timespan 2010–2019 demonstrated a strong tendency to gradually increase live weight and stature not only in the first^{t}, but also in the third lactation. In the meantime, the age at first calving decreased from 825.5 ± 0.80 days for cows born in the year 2010 to 766.3 ± 0.66 days for cows born in the year 2019. Different researchers explain this tendency with increased dairy cow growth potential, body capacity and metabolism. This combination of factors also gives a significant impact on the dairy cow milk productivity level [1]. Cows with live weight in the first lactation > 600 kg were characterized by a significantly ($P < 0.05$) shorter lifespan of 1758.8 ± 2.24 (approximately 4.8 years), but a higher lifetime milk productivity (24 757.9 ± 58.99 kg ECM) than in other studied groups, which could be explained by that fact that cows with higher live weight have a grater body capacity that can lead to increased dry matter intake and milk productivity. Cows with a bigger stature (above 151 cm) were characterized by a significantly shorter lifespan (1694.3 ± 2.40) and lifetime milk productivity (22 557.9 ± 61.34) that could be explained by the increased pressure on cow feet and claws, which leads to increased treatment costs and premature culling from herds.

Keywords: live weight, stature, lifespan, milk productivity.

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THE INFLUENCE OF THE BULL AND BULL LINE ON THE CONTENT OF FATTY ACIDS IN THE MILK OF BULLS' DAUGHTERS

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In recent years, increasing attention has been paid to improving the quality of dairy products. Therefore, the selection of animals according to the composition of milk fatty acids is of increasing interest. [1] The composition of milk fat has a significant influence on dairy products, shelf life and quality of milk processing. Unsaturated milk fatty acids have the greatest impact on human diet and health. [2] Myristic (C14:0) and palmitic acid (C16:0) are associated with cardiovascular disease due to increased blood cholesterol, and shorter chain saturated fatty acids (C4:0 C12:0) are associated with a positive health effect [3]. Milk producers are looking for methods to improve the physical and functional properties of cow's milk and to optimize the fat composition of milk for human health. Increasing knowledge of milk fatty acid synthesis, revealing the genetic background of milk fatty acids, may help to modify the fat composition of cow's milk [4].

The research was carried out in 2021–2022 at the Lithuanian University of Health Sciences, Department of Animal Breeding and Practical Training and Research Center of the Lithuanian University of Health Sciences. The milk samples of 263 cows of the Holstein breed bred in Lithuania were studied. The objective of this study was to focus on the genetic factors that affect the content of fatty acids in milk. In this study, cows were grouped according to the cow's father bull and the line of a bull. Statistical characteristics were calculated using statistical software SPSS 25.

There was no statistically significant impact on the fatty acids content in the milk of offspring's during the analysis of the influence of genetic factors on content of fatty acids, but the amount of fatty acids in the milk of the offspring of different bulls varies.

The biggest differences were detected in the content of unesterified fatty acids, preformed, saturated and unsaturated fatty acids in milk ($P > 0.05$). Meanwhile, the difference in the amount of fatty acids in the milk of the progeny of bulls of different lines was not significant. The most significant differences were in the amounts of non-esterified fatty acids, preformed, unsaturated fatty acids and mono-unsaturated fatty acids, and the lowest differences were found in the amounts of polyunsaturated and palmitic acids in milk ($P > 0.05$).

Keywords: cows, fatty acids, milk production, bulls.

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STUDY OF ANTIMICROBIAL EFFICACY OF TEAT DIP SOLUTIONS WITH POVIDONE IODINE AND CHLORHEXIDINE DIGLUCONATE AGAINST *P. ZOPFII* ALGAE ISOLATED FROM BOVINE MASTITIS MILK

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Prototheca zopfii is an environmental pathogen that causes chronic bovine mastitis. Reproduction of the pathogen in the mammary gland and regional lymph nodes causes granulomatous inflammation of the udder, damage to mammary parenchyma, atrophy of affected udder quarters, reduction of milk yield, and an increase in the count of somatic cells to 1 000 000/mL [1, 2]. *Prototheca* mastitis usually occurs due to contact with contaminated water sources or equipment [3]. There are no effective or approved treatment methods for *Prototheca* mastitis [4]. *Prototheca* survives pasteurization and is resistant to disinfection with chlorine compounds [5]. Frequent intramammary antibiotic infusions may contribute to the development of *Prototheca* infection in the udder [6]. It is difficult to eradicate the pathogen from the herd, infected cows are eliminated, and the only disease control measure is prevention.

The aim of this study was to determine in vitro sensitivity of *P. zopfii* isolated from bovine mastitis milk to post-milking teat dips *Profidip Iodine* and *Profidip Chlorhexidine*.

Research methods. The research was conducted in the Laboratory of Microbiological Research, Institute of Microbiology and Virology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences. *Prototheca zopfii* isolated from the milk of cows with chronic bovine mastitis was used. The research was carried out using the agar diffusion method. The activity of the teat dips was assessed in Sabouraud agar (BD DifcoTM, Franklin Lakes, NJ, USA), under aseptic conditions. Wells were formed in the inoculated medium in a Petri dish, each of which were filled with 100 µL of tested dips. The Petri dishes were incubated at 30°C. After 48 hours of incubation, antimicrobial activity was assessed by measuring the diameter of clear zones around wells.

Results. The study determined that both teat dips under investigation exhibited anti-*Prototheca* activity. *Profidip Chlorhexidine* inhibited the growth of *P. zopfii* strains, with an average clear zone at 19.1 ± 0.6 mm. *Profidip Iodine* was reliably more effective ($P < 0.05$), with an average clear zone at 31.4 ± 0.9 mm, and scanty algal growth on media.

Conclusion. Post milking teat dips *Profidip Iodine* and *Profidip Chlorhexidine* inhibit in vitro growth of *P. zopfii* strains. The teat dips exhibit different anti-*Prototheca* activity, with *Profidip Iodine* proving more effective ($P < 0.05$) against the algal cultures than *Profidip Chlorhexidine*.

Keywords: teat dip, P. zopfii, antimicrobial efficacy.

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A COMPARATIVE ANALYSIS OF THE PHYSICAL, CHEMICAL, AND SENSORY ATTRIBUTES OF CONVENTIONAL AND FREE-RANGE BROILER CHICKEN MEAT

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Investigating the physical-chemical traits and sensory qualities of conventional and free-range broiler meat was the aim of the current study. Broiler chicken carcasses from conventional and free-range broiler chickens were obtained from local suppliers. The analyses were performed at the Institute of Animal Rearing Technologies, Lithuanian University of Health Sciences (Kaunas, Lithuania). Evaluation of broiler chicken meat quality indices was performed after 48 hours of cooling at 4°C. The raw breast (*Pectoralis major*) meat was used for physicochemical characterization and sensory evaluation. Protein, fat, ash, dry meter content, pH, drip loss, water holding capacity, cooking loss, and color coordinates (CR-400, Minolta Camera, Osaka, Japan), measuring L* values of lightness, a* values for redness and b* values for yellowness were determined in broiler chicken breast meat. For sensory analysis, descriptive tests, and emotional expressions with Face Reader 6 software (Noldus Information Technology, Wageningen, Netherlands), 200 g of chicken breast meat was boiled at 100°C for 20 minutes. Ten participants were selected for the sensory analysis team. A one-way analysis of variance (ANOVA) was used to analyze the data. The Tukey test for comparison was used, with a 95% confidence interval of significance. Samples were prepared in triplicate for all the analyses done.

After examining the physical-chemical traits and properties of breast meat from conventional and free-range broilers, meat cooking losses were found to be statistically significant in both groups (difference between groups: 3.7% ($P < 0.05$); water holding capacity: 1.36% ($P < 0.05$)). Color indicators showed that the external surface of free-range broiler chicken breast meat was darker than conventionally reared meat. The study of sensory attributes revealed differences between samples in smell, color, tenderness, and juiciness. Consumers showed higher acceptability for free-range broiler meat in comparison with conventional. Patients for free-range broiler meat expressed higher "happy", "surprised", and "angry" emotions ($P \leqslant 0.05$)

Keywords: broiler chicken, free-range, conventional, meat quality, sensory attributes.

CONNECTIONS BETWEEN YOUNG HORSE PERFORMANCE AND TARSAL JOINT RADIOLOGICAL IMAGE MEASUREMENTS

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The study aimed to assess connections between tarsal dimensions and performance to understand changes connected with orthopedic status. Warmblood horses (1249 ± 114.6 days) on performance tests were x-rayed (19 stallions and 50 mares) and 14 measurements of the hock joint area were made (VetRayVision). The measurements were correlated with performance results: 3 basic body measurements, 3 conformation evaluations and performance traits (gaits and jumping: 2 in hand and 9 under saddle). The Pearson correlations ($P < 0.05$) of the tarsal measurements with basic body measurements were medium (0.3–0.6). The highest value was calculated between overall tarsal length and height at withers. Two significant correlations between conformation traits and the length of central/third tarsal bones were positive and low, while the others were low and negative (12 cases). The connections between measurements and movement in hand were significant for 17 cases from 28 possible and obtained low values (up to 0.35). The same level of correlation (0.10–0.38) was calculated for movement under rider (48 cases for 112 possible). Free jumping correlated only with one tarsal measurement (–0.25). Only one trait – rideability – was not connected with any tarsal measurement. The obtained results suggest that the meaning of the size of the tarsal joint is underevaluated by the judges' committees as the greater size of tarsal measurements is evaluated lower in the conformation evaluation.

Keywords: horse, tarsal joint, size measurement, performance.

ASSOCIATION BETWEEN DAIRY BULLS' FERTILITY INDEX AND COWS' CONCEIVING SUCCESS

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Ensuring optimal conditions can increase the chances of successful breeding and maintain a healthy and productive herd. The successful conceiving of cows involves various factors [1]. One of the ways to improve cows' fertility in the herd is to use the bulls' fertility index. The main trait of the fertility index is a non-return rate [2]. The aim of the study was to determinate how the fertility index is associated with a possibility to conceive from the first and the second insemination after spontaneous ovulation (AI) and timed artificial insemination (TAI). For this purpose, 243 healthy dairy cows with similar yielding (9000–10 000 kg per lactation), lactation (2–4) and BCS (3.75–4) were inseminated by different bulls with the fertility index –0.4, –0.1, 0.4 and 1.3. AI was performed after spontaneous oestrus when the standing reflex was determined (cows' oestrus was monitored 3 times per day for 15 min). TAI cows were inseminated by G7G protocol. Bulls with a 1.3 fertility index had a statistically significantly higher possibility to conceive cows after the first AI compared with bulls with a 0.4 fertility index. AI success between cows inseminated by bulls with different fertility indexes was not statistically significant in the second AI. Insemination success was highest when cows were inseminated after TAI by a bull with a fertility index 1.3. Cows inseminated by the mentioned bull had a 13.9% and 12.5% higher statistically significant possibility to conceive after TAI compared with cows inseminated by bulls with the fertility index –0.4 and 0.4, respectively. Simillar differences in the possibility to conceive were determined between bulls after the second TAI. In conclusion, a higher fertility index does not always mean better conceiving success. Bulls with a higher fertility index are more appropriate for insemination for cows after AI and TAI.

Keywords: fertility index, spontaneous, TAI, AI, conceiving.

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DISTRIBUTION AND POTENTIAL ROLE OF AQUAPORINS IN SPERM: LESSONS FROM A BOVINE STUDY. PRELIMINARY RESULTS

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In view of the need to search for new parameters assessing semen quality, allowing for a precise evaluation of male reproductive potential, studies are conducted with the main goal of identifying and analyzing the expression of aquaporins (AQPs) in bovine sperm. AQPs are small transmembrane proteins that, according to many researchers, may be involved in maintaining proper viability and motility of sperm [1, 2]. The latest data indicate that these proteins may also play an important role in the process of sperm cryopreservation [3]. The research presented here is conducted on cryopreserved semen samples from 20 healthy and sexually mature Polish Holstein-Friesian black and white bulls. The ejaculates were classified into two groups: high-quality semen (n = 10) and low-quality semen (n = 10), based on the evaluation of sperm concentration, motility, vitality, morphology, hypoosmotic swelling test and mitochondria activity. Using immunofluorescence (IF), a detailed localization of all AQPs potentially present in bovine sperm is being carried out. Subsequently, their expression will be analyzed using Western blot (WB), and the results will be verified using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). These analyses will be accompanied by measurements of selected metabolites (free amino acid and fatty acid profiles, biogenic amines, cholesterol, and malondialdehyde levels) both in sperm and seminal plasma. To date, the locations of AQP3, AQP7 and AQP8 have been identified and tentatively determined using IF and WB. Meanwhile, the presence of AQP1, AQP4, AQP5, AQP9 in bovine sperm was excluded. Currently, research on the search for a relationship between their expression and semen quality is ongoing. The presence of other aquaporins in bovine sperm, including AQP0, AQP11 and AQP12 is also verified.

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Keywords: water channel, male reproduction, sperm, potential biomarker.

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ANALYSIS OF MILKING INDICATORS OF DIFFERENT COW BREEDS

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Cow milking is considered as a laborious and time-consuming job at livestock farms [1]. The milking process represents one of the most important tasks on a dairy farm. On farms with conventional milking systems, it accounts for roughly a third of farm's total labor demand [2]. Increasing the milk flow rate at which milking is terminated can shorten milking time and increase milking efficiency [3]. It is important that milking speed-related traits present high heritability estimates for higher selection responses in breeding programs aiming to breed for more efficient and adapted animals for milking systems [4].

The aim of the study was to analyze the milking indicators of different cow breeds.

Materials and methods. In the study farm, cows are kept loose all year round, in a modern cold-type barn. During summer, the animals are not grazed, and they are fed a complete diet that meets their physiological needs. Cows are milked 2x20 side-by-side in a milking parlour. The milking data of 420 cows were collected, 140 of which were of the Lithuanian Red breed, 62 of the Swedish Red and White and 218 of the White and Red Holstein breeds. For the analysis of the data, statistical indicators were calculated for each evaluated trait (milk yield at milking, milking duration, average milk flow, maximum milk flow, milk yield in the first 2 minutes, milk flow in time intervals: 0–15 s, 15–30 s, 30–60 s, 60–120 s): arithmetic mean, mean error and statistical reliability of the data (P). The obtained results were considered statistically significant when $P < 0.05$.

Results and conclusion. We found that Lithuanian Red cows produced 0.51 kg more milk than White and Red Holsteins and 0.29 kg more milk than Swedish Red and White. The milk yield in the first two minutes of milking was 0.31 kg higher in the Lithuanian Red cows than in the Swedish Red and White cows and 0.38 kg higher than in the White and Red Holstein cows. The average milking time was 6.19 minutes. The milking time of the White and Red Holstein cows was 0.11 min longer than that of the Lithuanian Red cows ($P < 0.01$) and 0.17 min longer than that of the Swedish Red and White Holstein cows ($P < 0.01$). The average milk flow of Lithuanian Red cows was only 0.03 kg/min higher than that of Swedish Red and White and 0.11 kg/min higher than that of White and Red Holstein cows. The highest milk flow rate of the Lithuanian Red and Swedish Red and White breeds was 0.22 kg/min higher than that of the White and Red Holstein breed. The milk flow rates of the Lithuanian Red cows at the time intervals 0–15 s, 15–30 s, 30–60 s and 60–120 s were found to be 0.05, 0.23, 0.11 and 0.17 kg/min higher than that of the Swedish Red and White cows, and 0.04, 0.21, 0.21 and 0.2 kg/min higher than that of the White and Red Holstein cows. In conclusion, the Lithuanian Red cows on the farm produced more milk, milked faster and had higher milk flows.

Keywords: cows breed, milking duration, milk flow rate.

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ANALYSIS OF POPULATION STRUCTURE AND INBREEDING IN THE LATVIAN HEAVY WARMBLOOD HORSE POPULATION

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Latvian heavy warmblood horses (LSB) breeding program was developed in 2004. It is aimed at preserving this type, because the number of these horses is small and the status of the local endangered breed has been accepted. Conservation of the native population is important when thinking about genetic diversity today and in the future [1]. This study is relevant for those horse breeders who are working on the preservation program of the LSB horses breed in order to more efficiently plan their breeding work. The purpose of the study was to analyse the population structure and inbreeding of the LSB horse population.

The Latvian Horse Breeders Association provided the pedigree data of LSB horses. For LSB, the reference population included 257 females and 117 males that were alive at the time of data selection. For reference animals in the data set as many ancestor generations as possible were included. The software POPREP was used for the analysis of the population structure and the inbreeding coefficient.

In the LSB population, the average age of the breeding mares and stallions at the time of foal birth was between 8 and 15 years. The number of offspring raised for breeding varied from year to year, and during the last 10 years, the highest number of foals (24 foals) that were raised for breeding was in 2016.

In the LSB population, the average generation interval within the last 10 years was similar for stallions and mares (8.8 years on average). There were only 5 stallions in the LSB population, who produced 12–18 offspring during their lifetime; the contribution of the other stallions to the production of breeding animals was significantly lower. The largest number of offspring raised for breeding from one mare was 5 animals.

According to the pedigree analysis, the five generations' pedigree completeness was above 90% for animals born from 2000 and the first generation 100% completeness starting from 1985. Between 2000 and 2019, 374 animals were born, and the proportion of non-inbred animals in LSB population was only 9.09% (34 animals). The highest proportion of inbred animals (85.56 %) had an inbreeding coefficient up to 5%. There were some animals with inbreeding higher than 11%.

In conclusion, breeding organizations should control the LSB offspring inbreeding level and not mating related stallions and mares. The inbreeding coefficient can be decreased for the LSB population. Well-completed pedigree information will make it possible to control the level of inbreeding of the offspring.

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Keywords: inbreeding, horse population, heavy warmblood, population structure.

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DETERMINATION OF *TG5* GENE POLYMORPHISM AND ITS INFLUENCE ON PRODUCTIVITY TRAITS OF BEEF CATTLE REARED IN LITHUANIA

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Thyroglobulin (TG) is the precursor of T3 and T4 thyroid hormones, which have an important role in metabolic regulation and affect lipid metabolism [1]. The results of various scientific studies have shown that the *TG5* gene should be considered a functional and positional candidate gene that affects the accumulation of fat in the cattle body [2]. Thus, a single-nucleotide polymorphism located in the 5 untranslated region of this gene is used for marker-based selection aimed at increasing marbling. At present, scientists are actively studying the genetic determination of the meat production characters of cattle using DNA markers. This allows supplementing traditional breeding methods with the method of selecting animals based on desirable genetic markers. Thus, it becomes possible to significantly accelerate the selection process by improving the genetic potential of cattle breeds [3, 4]. The aim of this study was to investigate the prevalence of *TG5* gene polymorphism and to determine its influence on productivity traits of beef cattle reared in Lithuania. Cattle hair follicle samples were collected from 30 bulls of Angus, Limousin, Aubrac and Charolais breed. Hair samples and the data on productivity trait records were obtained from Šilutė control bulls feeding station. Bovine genomic DNA was extracted from hair follicles using Chelex DNA extraction method. Polymorphism of *TG5* locus was identified using a PCR-RFLP method. PCR product of *TG5* gene was digested with *BStx2I* restriction nuclease. Investigation of polymorphism of *TG5* gene showed that allele C (frequency – 0.617) and genotype CC (frequency – 0.533) were the most common in the analyzed population of beef cattle. Meanwhile, the heterozygous CT genotype was rarest, with a frequency of 0.167. Evaluating the observed and expected heterozygosity in the investigated group of animals, the observed heterozygosity was found to be lower than expected, indicating an insufficient amount of genetic diversity in the loci studied. The difference was statistically significant ($P < 0.01$). When calculating the influence of *TG5* gene polymorphism on cattle productivity traits, it was observed that this polymorphism had a statistically significant ($P < 0.05$) effect for many productivity traits. The data on the influence of genotypes showed that cattle of CC genotype had the higher live weight, hot carcass weight and carcass weight and the highest calculated averages of the three indicators (average daily weight gain/live weight/age in days; average daily weight gain/warm carcass weight/age in days; and average daily weight gain/ carcass weight/age in days) than animals of CT or TT genotype. The data was statistically significant ($P < 0.05$). In conclusion, the results showed that polymorphism of the *TG5* gene influences many productivity traits of beef cattle.

Keywords: cattle, *TG5* gene, polymorphism, PCR-RFLP.

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EVOLUTIONARY TRANSITION FROM CONVENTIONAL TO EAZA-RECOMMENDED METHOD OF *TENEBRIO MOLITOR* CULTIVATION

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This study focuses on enhancing the efficiency of cultivating and breeding mealworms (*Tenebrio molitor*) to serve as a nutritious animal feed, particularly beneficial for captive exotic animals. The research aimed at transitioning from a traditional cultivation approach, which relied heavily on visual assessments to determine mealworm developmental stages and sieving timing, to a protocol recommended by the European Association of Zoos and Aquaria (EAZA). The EAZA-endorsed method prescribes a fixed egg-laying period of 7–10 days and enforces strict controls on the age and size of mealworms at harvest. Despite maintaining consistent cultivation parameters such as relative humidity, ambient temperature, and nutritional regime across both methods, the transition required significant adjustments. Key changes included moving to a complete substrate replacement at the end of each ten-week cycle and initiating synchronized breeding cycles to avoid overlapping developmental stages, with only the beetles being transferred to new containers to facilitate egg-laying. However, achieving the EAZA standard of reducing the egg-laying time to a maximum of 10 days for optimal productivity is an ongoing objective. While collecting data for ten months and evaluating the amount of mature mealworms during the sieving process, a notable increase ($P < 0.05$) in larval yield under the EAZA guidelines compared with the traditional mixed-stage breeding approach was determined. Although full adherence to EAZA standards is yet to be achieved, our findings underscore the potential of these advanced mealworm cultivation and breeding techniques in improving animal feed production.

Keywords: *Tenebrio molitor,* mealworm beetle, output augmentation, breeding method.

DETERMINING THE OPTIMAL LACTATION PHASE OF A COW FOR CHANGING THE MILKING SYSTEM FROM CONVENTIONAL TO AUTOMATIC

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Several studies have proven that changing the milking system from a conventional to an automatic one has a positive effect on the milk yield of Holstein-Friesian cows [1]. One of the reasons for this increase in milk yield is the increased number of milkings performed by cows [2]. However, there are no reports which indicate the phase of lactation favorable for changing the milking system. The aim of the study was to determine the time of lactation for changing the milking system from conventional to automatic. The study included 191 cows from the Polish Holstein-Friesian breed in 7 different herds, where farms were equipped with an automatic Lely Astronaut A4 system. The milking data from these cows were collected for 4 years from 2010 to 2014. The statistical analysis of the data was performed by using the analysis of variance. The significant differences between the selected groups were established by using the Scheffe test (SAS software). Based on statistical analysis, it was seen that the optimal lactation phase in which the milking system was changed had a highly significant effect on the yield of primiparous milk. The study showed that in the primiparous group, the highest milk yield (10 125.6 kg) was found in those who experienced a change from conventional to automatic milking between the 101–200th day of lactation. On the other hand, the lowest milk yield was found in primiparous cows who started automatic milking after the 200th day of lactation (8540.6 kg). The study did not show the lactation phase in which the milking system was changed to the milk yield of cows in the second lactation. It should be emphasized, however, that the best effects were observed among cows that were milked from the middle (101–200 days) of lactation (10 278.0 kg).

Our findings have shown that the change of the milking system is more effective if the milking system is changed from a conventional milking system to an automatic milking system during the 101–200 days.

Keywords: automatic milking system, Holstein-Friesian, milk yield, lactation phase.

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HERITABILITY OF POLISH HOLSTEIN-FRIESIAN COWS' MILK ELECTRICAL CONDUCTIVITY RECORDED BY MILKING ROBOTS FOR INDIVIDUAL UDDER QUARTERS

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Some research results suggest that the electrical conductivity of cow's milk (EC) could be successfully used in breeding programs as an additional indicator of mastitis [1]. The electrical conductivity increases in milk produced by cows with mastitis due to damage to cells in the mammary tissue [2]. Roy et al. [3] have reported an increase in EC of milk samples from mastitis. The aim of the study was to estimate the daily heritability EC of milk from quarter milkings of Polish-Holstein-Friesian cows milked in barns equipped with milking robots.

A total of 608 984 daily records of EC for the first and the second lactation were related to 2432 cows from 23 herds. The EC heritability was estimated individually for each udder quarter, i.e. left front – LF, right front – RF, left rear – LR, right rear – RR. Univariate random regression model (RRM) and fourth-order Legendre polynomials for the regression on the number of milking days (from test day 5 to test day 305) were applied. The heritability was estimated using the Wombat package [4].

It was shown that the average EC of milk 6.92 mS. The value of the trait varied depending on the quarter of the udder and the day of lactation, ranging from 6.92 mS (RF, RR) to 6.93 mS (LF, LR). Daily heritability indicators of EC showed high variation during lactation and udder quarters. The heritability values ranged from 0.106 to 0.353 for LF, from 0.062 to 0.253 for LR, from 0.103 to 0.376 for RF, and from 0.113 to 0.381 for RR. It was recorded that the average EC heritability for four udder quarters and the entire first and second lactation was at a level of 0.191. The corresponding indices determined for individual quarters of the udder were: LF – 0.253, LR – 0.161, RF – 0.168, RR – 0.181.

Summing up the results of the research, it should be stated that the heritability of milk electrical conductivity changed depending on the analysed quarter of the udder. At the same time, these results suggest that the best response to selection in this respect may be expected in the case of the left front quarter.

Keywords: automatic milking system, Holstein-Friesian, electrical conductivity, heritability.

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THE VAGINAL MICROFLORA IN ANESTRUS PERIOD OF HEALTHY FEMALE DOGS

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Presence of bacteria, free or inside the epithelial cells, is frequently reported in vaginal smears of healthy dogs [1]. Depending on the cycle stage from 50% to 100% of clinically healthy dogs are characterized by a vaginal bacterial population, usually mixed, consisting of both aerobic and anaerobic microorganisms often opportunistic pathogens [2]. The treatment of pets with antimicrobials may affect the population of commensal bacteria and increase the risk for colonization of the urogenital tract by potentially pathogenic bacteria [3].

The aim of this study was to isolate microorganisms from the vagina of female dogs in the anestrus period and to evaluate the antimicrobial susceptibility.

Materials and methods. Samples from vagina were taken from 45 healthy female dogs in anaestrus period. The samples were cultured on differential-diagnostic and selective nutrient media. Microorganisms were identified according to biochemical and antigenic characteristics. The antimicrobial susceptibility was determined by the disk diffusion method using the Kirby-Bauer technique (Bauer et al., 1966). Nine antimicrobial agents were used for susceptibility testing. The P value of < 0.05 was considered to be significant.

Results. The predominant microorganisms isolated from female dogs in the anestrus period ($n = 45$) were Streptococcus spp. (24.3%), Staphylococcus pseudintermedius (21.95%) and Escherichia coli (21.95%). Bacteria were not isolated from four female dogs. In a comparison of the effects of antimicrobial agents of the female dogs in the anestrus period, cefovecin and enrofloxacin were found to be more effective than amoxicillin. Enrofloxacin was also more effective than erythromycin and vancomycin. The obtained results were statistically reliable ($P < 0.05$)

Conclusions. A wide variety of microorganisms may be isolated from a female dog's vagina in the anestrus period. Bacteria from Streptococcus genus, Staphylococcus pseudintermedius and Escherichia coli were the most common microflora in our investigation. The highest sensitivity was detected to cefovecin and enrofloxacin.

Keywords: female dogs, microorganisms, vagina, antimicrobial susceptibility.

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ISOLATION OF MICROORGANISMS FROM CANINE SKIN AND EVALUATION OF ANTIMICROBIAL RESISTANCE

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Various bacterial agents are found on canine skin and fur, which creates natural individual skin microbiota, but when skin barrier and immunity are conflicted, natural microbiota can become potentially pathogenic and make new pathways for new pathogenic microorganisms [1]. Microbiota, which is present in individual canine skin, "trains" an innate and adaptive immune system. Antimicrobial resistance is gradually increasing, so it is important to investigate antimicrobial resistance in cases of bacterial skin diseases to ensure targeted treatment [2]. The aim of this study was to isolate microbiota from canine skin and determine antimicrobial resistance.

Materials and methods. 36 canines were tested. According to clinical symptoms, dogs were divided into two groups: clinically healthy canines (n = 18) and canines which had clinical symptoms of pyoderma (n = 18). The swabs were placed in "Amies" transport medium and taken to the laboratory. The obtained samples were cultured in aerobic conditions on selective and differential nutrient media. Bacteria were identified according to biochemical and antigenic characteristics, and their antimicrobial susceptibility was determined by the disk diffusion method using the Kirby-Bauer technique [3]. The *P* value of < 0.05 was considered to be significant.

Results. The following strains of microorganisms were isolated from the healthy skin of dogs: *Staphylococcus warneri* (33.3%), *Bacillus* spp. (27.7%), and *Staphylococcus pseudinermedius* (5.5%). The most prevalent species from pyoderma cases were *Staphylococcus pseudintermedius* (38,8%) and *Staphylococcus aureus* (27.7 %). *Staphylococcus warneri* isolates from healthy canine skin were resistant to oxacillin (89.5%), trimethoprim-sulphamethoxazole (78%) and cefovecin (61.5%). In dogs with pyoderma symptoms, 89.5% of *S. pseudintermedius* strains were resistant to trimethoprim-sulphamethoxazole and oxacillin; 100% of *S. aureus* strains were resistant to oxacillin and fusidic acid.

Conclusions. Coagulase negative *Staphylococcus warneri* was the most prevalent in dogs with healthy skin. Coagulase positive *Staphylococcus aureus* and *Staphylococcus pseudintermedius* were predominant species in dogs with pyoderma. Most isolates from clinically healthy canine skin were resistant to oxacillin, trimethoprim-sulphamethoxazole and cefovecin, while those from canines with pyoderma were resistant to oxacillin, trimethoprim-sulphamethoxazole and fusidic acid.

Keywords: canine, skin, microorganisms, antimicrobial resistance.

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ULTRA-FREEZING EFFECT OF CRYOPRESERVED BOVINE SEMEN ON THE MOTILITY AND VIABILITY OF POST-THAW SPERMATOZOA

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Advancements like artificial insemination (AI) have significantly boosted genetic improvement and cattle production, with semen quality being essential for a successful AI [1]. Cryopreservation and cooled semen technology have been explored for improved semen transportation and insemination. However, the cost, maintenance and availability of liquid nitrogen, crucial for cryopreservation, underscore the need for alternative semen storage solutions [2].

In light of this, our investigation was carried out on the effects of varied storage temperatures and durations on motility and viability of the bull's semen. Cryopreserved semen samples were obtained from 6 bulls. For each bull, 6 doses were retained in liquid nitrogen (at –196°C) as a control group, while 6 doses were transferred to –75°C (ultra-freezer "Sanyo"). The study assessed 72 cryopreserved straws at distinct intervals: 1 day, 10 days, 30 days and 90 days. To obtain a comprehensive evaluation of motility, we calculated the average motility by combining the results from the subjective and objective motility (computer-aided semen analysis using Sperm Class Analyzer) assessments. This approach allowed us to consider both qualitative and quantitative aspects of sperm motility. Viability was assessed with the staining method (Eosin/Nigrosine) and the hypo-osmotic swelling test (HOST) to account for potential variations in dye penetration and staining efficiency, and to provide a more accurate representation of the spermatozoa viability after thawing.

For longer storage durations of 90 days, the results indicated that liquid nitrogen storage at –196°C showed significantly higher sperm motility and viability ($P < 0.05$), with values of 92.12% and 45.58%, respectively, compared with those at –75°C (73.74% motility and 25.08% viability). This suggests that for long-term preservation –196°C storage remains optimal.

However, interestingly, short-term storage for up to one month, the ultra-freezer storage at –75°C could serve as a viable and cost-effective alternative to –196°C storage. The motility rates were not significantly different ($P > 0.05$) with 78.68% at –75°C and 83.89% at –196°C. Moreover, the viability rates were comparable, with 41.5% at –75°C and 40.58% at –196°C, indicating that the quality of semen does not significantly diminish under these conditions.

Keywords: bull, semen quality, cryopreservation, ultra-freezing, artificial insemination.

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ANALYSIS AND COMPARISON OF QUALITY PARAMETERS OF CRYOPRESERVED SEXED AND UNSEXED BULL SEMEN

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Sexing of sperm can be achieved with more than an 85% accuracy using the flow cytometry method [1]. But a challenge of this is that the stress placed on the sperm has detrimental effects on the quality and viability of the sperm [2]. The objective of this study was to investigate the differences in quality and viability of sexed and unsexed sperm and the effect of the length of removal from cryopreservation.

For this study, three bulls were selected, and a sexed and unsexed sample of their semen was taken and cryopreserved in liquid nitrogen. The samples were removed and defrosted in a water bath maintained with a hot plate at 37°C. The first six samples, three sexed and three non-sexed, where removed after 10 seconds. They were then all tested for motility, viability, concentration, morphology, and the hypo-osmotic swelling (HOS) test. The next six samples were removed from the water bath after five hours and motility, viability, and HOS test were repeated.

In the sample defrosted for 10 seconds, the motility was found to be on average 40% higher in the sexed sample and 47.37% higher in the sexed sample for the five hour defrosted samples compared with the respective unsexed samples ($P < 0.05$). Viability was tested using eosin and nigrosin staining with the 10-second defrost being 25.36% more dead in the sexed sample and with the 5-hour defrost being 19.9% more dead in the sexed semen samples ($P < 0.05$). Concentration was calculated and found to be 75.73% more concentrated in the sexed sample ($P < 0.05$). Morphology was calculated by looking for the number of defects per 100 spermatozoa, and it was found that 60.61% less defects were in the unsexed sperm ($P < 0.05$). Lastly, the HOS test was performed with the 10-second defrost having 21.51% more spermatozoa dead in the sexed sample; in the 5-hour defrost, 1.55% more dead spermatozoa were found to be dead in the sexed sample ($P > 0.05$).

From all these results we can conclude that unsexed sperm has a higher viability than sexed sperm. A previous study found successful pregnancy rates to be 84.42% in unsexed sperm and 75.33% in sexed sperm [3]. From the parameters examined in this study, we can understand the reasons of the differences in viability.

Keywords: viability, cattle, sex sorted semen.

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TLR4, *MEF2A*, *MEF2C* AND *MAPK1* GENE EXPRESSIONS IN DAIRY CATTLE UDDER PARENCHYMA INFECTED WITH COAGULASE-POSITIVE STAPHYLOCCI

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Mastitis is a complex disease affected by many factors related to several pathways connected with each other or not connected. Coagulase-positive staphylococci (CoPS) are one of the main mastitic pathogens. Based on the previous studies, we selected four (*TLR4, MEF2A, MEF2C, MAPK1*) interconnected genes where protein products play essential roles during inflammation. TLR4 is involved in a migration of neutrophils to and from the mammary gland during mastitis. It also detects bacterial ligands, such as lipopolysaccharide (LPS) [1]. All genes from the MEF2 family are transcription factors which regulate the expression of immune-related genes. Moreover, MEF2A and MEF2C regulate apoptosis, while MEF2C is also involved in the formation of B cells. MEF2A and MEF2C perform some functions such as that cannot be performed by other proteins from this family [2]. MAPK1, however, plays a main role in the inflammatory response and in autoimmune diseases [3]. The aim of the study was to compare the expressions of the studied genes in dairy cow udder parenchyma from quarters infected with CoPS (n = 10) and those adjacent to them (AHCoPS, n = 10) to those derived from healthy cows (H, n = 10), wherein healthy udder quarters taken as a control were derived from the whole healthy udders.

A total of 30 samples of dairy cow udder parenchyma were collected just after slaughter. Animals were culled at the end of lactation (286 days, SD = 27) and showed either reproductive difficulties but had healthy udders or chronic or/and subclinical mammary gland inflammation that was recurrent and incurable. RNA was extracted from frozen tissue samples. Only RNA with the integrity number (RIN) > 7.0 were selected for further analysis. The RT-qPCR method was used to establish gene expressions. The variance analysis was conducted using the MIXED procedure in SAS (SAS/STAT, 2002-2012, v. 9.14).

The expression of *MEF2A* and *MAPK1* genes did not differ between the groups while the *TLR4* and *MEF2C* expressions were higher in CoPS and H than in AHCoPS. TLR family plays an essential role in pathogen recognition and activation of innate immunity mediating cytokine production; thus, such processes do not occur in AHCoPS but do in CoPS and H. The lowest expression of *MEF2C* in AHCoPS may mean that apoptotic processes are much lower than in CoPS and even H, and the protection of the AHCoPS quarter against infectious agents by producing defensive antibodies is lowered.

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Keywords: dairy cow, mastitis, adjacent quarters.

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CORRELATION BETWEEN SELECTED IMMUNE GENE EXPRESSIONS IN LIVER OF YOUNG CASTRATED BUCKS OF POLISH WHITE IMPROVED SUPPLEMENTED WITH *CURCUMIN-ROSEMARY* DRIED EXTRACT MIXTURE

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Expression profiles of cathelicidins (CATHs), acute phase proteins (APPs), and defensins (DEFs) can provide helpful information about the health state of goats [1, 2], e.g. after supplementation of the diet with pro-health substances. However, the synergic or antagonist activity of these proteins/peptides has not been studied yet. Therefore, the aim of the study was to analyze the effect of supplementation with the curcumin-rosemary dried extract mixture (a ratio of 896:19) on the correlations between APPs (serum amyloid (SAA)), haptoglobin (Hp), ceruloplasmin (Cp), C-reactive protein (CRP), alpha-1 acid glycoprotein (AGP), fibrinogen (Fb), α-lactalbumin (LALBA)), CATHs (capra hircus bacetnecin 3.4 (ChBAC3.4), cathelicidin 2 (BAC5), cathelicidin 3 (BAC7.5), cathelicidin 6 (MAP28), cathelicidin 7 (MAP34)), and DEFs (β-defensin 1 (GBD1), β-defensin 2 (GBD2)) gene expressions in the livers of young castrated bucks of Polish White Improved (PWI) breed. Two groups were distinguished: the control (CG), on the basal diet (n = 10), and the experimental (EG) (n = 10) with additive of 1.6 g/day/head of the mixture. The experiment lasted 124 days. At the start, bucks were 8 months of age with a live weight of 28.8 kg (± 4.9 kg) on average. A total RNA were isolated from their livers. The cyclophilin A (*PPIA*) and battenin (*CLN3*) were used as reference genes in the RT-qPCR method. The Pearson correlation was calculated (PROC CORR, SAS package). In the CG, relationships were found between *BAC7.5-GBD1* (0.74, $P = 0.04$), *BAC5-Hp* (0.74, $P = 0.04$), *BAC5-MAP28* (0.73, $P = 0.04$), *Hp-MAP28* (0.72, $P = 0.04$), *Fbα-Fbγ* (0.87, $P = 0.01$), *Fbα-Fbβ* (0.85, $P = 0.02$), and *Fbβ-Fbγ* (0.92, $P = 0.001$). The associations between gene expressions were much stronger in the EG than in the CG (all above 0.9). Moreover, several new associations appeared, especially with *MAP28* and *Fbs*. In opposite, the expression of *BAC5* in the EG was not correlated in any other gene. Much more and higher correlations between APPs, DEFs and CATHs gene expressions in the EG than in the CG may suggest that a supplementation with the studied mixture triggers the immune response in the livers of youngcastrated bucks of PWI breed, possibly causing mainly synergistic activity of their protein products.

Keywords: bucks, acute phase proteins, cathelicidins, defensins, correlations.

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COMPARISON OF THE NUTRITIONAL PROFILES OF EGGS PRODUCED BY LAYING HENS REARED UNDER INTENSIVE AND EXTENSIVE CONDITIONS

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Consumer demand for animal-based production has grown, emphasising product safety, quality, and animal welfare. Research has extensively explored the nutrition of laying hens in conventional, free-range, and organic systems [1]. However, the impact of outdoor access on egg-laying performance and quality traits remains inconclusive. So, the aim of this study was to perform a comparative analysis of the nutritional value of eggs from laying hens reared under extensive and intensive conditions. Eggs from domestically reared laying hens (extensive rearing with a known egg-laying period) and eggs purchased from a supermarket (intensive rearing of laying hens) were utilized for the research. The following indicators were measured in fresh and stored eggs (28 days at a refrigerator temperature of 4°C): egg height, yolk height, albumen height, Haugh unit, yolk colour intensity, eggshell thickness, yolk pH, albumen pH, dry matter (DM), fat, ash, and protein content in the yolk, as well as the sensory profile of fresh eggs. Comparing fresh and 28-day-old eggs from extensively and intensively reared laying hens, the extensive conditions resulted in higher essential indicators: fresh eggs were heavier by 24%, while stored eggs were heavier by 19% ($P < 0.05$). Extensively farmed fresh eggs showed a 7-point higher yolk colour intensity compared with intensive eggs, increasing by 8 points after storage ($P < 0.05$). The analysis of egg yolk colour characteristics using colour coordinates revealed that the yolk yellowness (b*) value was 27% higher in eggs from hens raised under extensive conditions than in intensively reared ones ($P < 0.05$). Extensively reared fresh eggs exhibited a 25% higher yolk weight, a 28% higher albumin weight, and a 57% higher albumin height compared with eggs from intensively reared hens ($P < 0.05$). Furthermore, the albumin weight of eggs stored for 28 days was 31% higher in eggs from extensively reared hens compared with eggs from intensively reared hens ($P < 0.05$). No significant differences were found in the chemical composition of eggs from laying hens raised under different conditions ($P > 0.05$). After the sensory profile assay, it was found that the intensity of the yolk smell was 10% more noticeable in the eggs of laying hens reared in extensive conditions compared with intensively reared eggs ($P < 0.05$). Eggs from extensively raised hens exhibited a 56% lower yolk colour uniformity compared with eggs from intensively reared hens, while the yolk fineness of eggs from intensively kept hens was 17% higher than that of eggs from extensively raised hens ($P < 0.05$). In conclusion, to provide a safe and high-quality alternative for egg consumption that meets acceptable standards, it is recommended to prioritize eggs from laying hens reared under extensive conditions.

Keywords: laying hens, rearing condition, egg quality, nutritional value.

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