Veterinarija ir Zootechnika

Volume 79(2)
2021

Veterinarija ir Zootechnika

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Volume 79(2)
2021

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.

Issues per Year – 4. From 2016 - 2.

The Guide for Authors can be found of the journal's website https://vetzoo.lsmuni.lt/directions-to-authors

Submission of Manuscripts

The manuscripts are registered in the Editorial Office of LSMU VA "Veterinarija ir zootechnika" after filling the Manuscripts Registration Form (*Supplement 1*).
One copy of the manuscript should be addressed to the Editorial Office or e-mailed to the following address: evaldas.slyzius@lsmuni.lt
The manuscripts are refereed (*Supplement 2*) at least by two members of the Editorial Board or appointed referees. The authors are expected to take into consideration the remarks and before the final submission make correction or give motivated explanations of discount of the remarks. The amended paper should be sent back to the Editorial Office in two weeks after the submission of reviews to the author.
If two reviews are negative the article is rejected. If one review is negative, the article should be reviewed by additional referee. If the review of the third referee is positive, the final decision is made by the Editorial board.
The final corrected manuscript should be submitted to the Editorial Board together with the signed letter that authors have approved the paper for release and in agreement with its publication (*Supplement 3*).
The paper should include reference that scientific research has been carried out in accordance with the EU and Lithuanian legal acts.

Publication Information:

„VETERINARIJA ir ZOOTECHNIKA"
A scientific journal and the Official Organ of the Veterinary Academy,
Lithuanian University of Health Sciences (LSMU VA).

ISSN 1392-2130 (Print)
ISSN 2669-2511 (Online)

evaldas.slyzius@lsmuni.lt

Layout Rūta Atie
Printed by UAB "Igmovila", Laisvės al. 38A-12, LT-44240 Kaunas, Lithuania. Edition of 10 copies.

Veterinarija ir Zootechnika

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Volume 79(2)
2021

Veterinarija ir Zootechnika

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Volume 79(2)
Pages 1–99
2021

CONTENTS

Comparative Investigations on Omega-3 Fatty Acids Treatments on Physicochemical Properties of Meat from Rabbits with Experimentally Induced Visceral Obesity

Zhenya Ivanova[1], Boycho Bivolarski[1], Stefan Ribarski[2], Natalia Grigorova[1], Ekaterina Vachkova[1], Ivan Penchev Georgiev[1]

[1]Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Bulgaria

[2]Department of Morphology, Physiology and Animal Nutrition, Faculty of Agriculture, Trakia University, Bulgaria

Keywords: *castrated rabbits, diet restriction, krill and fish oil, physicochemical properties of meat.*

Abstract. *The effect of omega-3 polyunsaturated fatty acids (PUFA) on meat physicochemical properties were investigated in rabbits with experimentally induced obesity by castration. forty-two male New Zealand White rabbits were divided into seven groups (n = 6): Group A – castrated, fed a full-diet and treated with krill oil; Group B – castrated, fed a full-diet and treated with fish oil; Group C – castrated, fed a full-diet and untreated; Group D – non-castrated, non-treated, fed a full-diet; Group E – castrated, fed a restricted diet (50%) and treated with krill oil; Group F – castrated, fed a restricted diet (50%) and treated with fish oil; and Group G – castrated, fed a restricted diet (50%) and untreated. experimental rabbits were 3-month-old and received feed for fattening rabbits. Krill and fish oil were applied per os as gelatin capsules containing 600 mg omega-3 PUFA over 60 days. During the processing of carcasses, individual samples of Longissimus Lumborum muscle (LL) and Semimembranosus muscle (SM) were collected. The results from the present experiment showed that castration and full-diet feeding of rabbits had beneficial effects on the quality of meat lipids and proteins. The castration of rabbits receiving a full diet had a most pronounced effect on fat accumulation in LL meat. The opposite tendency was demonstrated in rabbits with diet restriction in both studied muscles: statistically, significant differences were observed between treated and untreated rabbits. The higher fat content of LL and SM was associated with the omega-3 PUFA supplementation on the one hand and with diet restriction on the other. At the same time, castrated rabbits fed with a full diet exhibited statistically significantly higher protein content of LL and SM.*

Introduction

Rabbit farming is a branch of animal husbandry producing various valuable nutritional products. The most crucial role in this aspect is rabbit meat due to its low-fat content. Its low cholesterol, but high macro -, trace elements (calcium, potassium, phosphorus, sodium, cobalt, iron, copper and zinc) and B group vitamins content have determined the dietetic properties of the rabbit meat. In addition, it is a low-energy product of animal origin, as 100 g of rabbit meat provide only 160–170 kcal vs 195–380 kcal for beef and 260–330 kcal for pork meat (Bivolarski, 2012). Therefore, the world scientific community pays particular attention and spends a considerable amount of financial and human resources in the detailed study of effects of genetic, physiological, nutritional and other factors on growth, development, metabolism and meat quality in rabbits (Bivolarski et al., 2011; Hou et al., 2020; Ivanova, 2015; Maetrens et al., 2008; Palazzo et al., 2020; Ouyed et al., 2008; Vachkova, 2008).

Correspondence to Zhenya Ivanova, PhD, Department of the Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000, Stara Zagora, Bulgaria, Phone: +35942699628; E-mail: jen_s@abv.bg

Rabbits increase their weight until 100–120 days of age. Therefore, this period of intensive growth is most appropriate from an economic point of view for feed conversion per unit weight gain. As the age of rabbits advances, the feed expenditure increases substantially and is the highest after four months of age (Marinov et al., 2009).

The effect of feed restriction on physicochemical properties of rabbit meat depends on the level of restriction, feed quality, the duration of feeding, the age of animals. In rabbit farming practice, feed restriction is applied from 10 to 35 days, with 40%–90% of the total diet (Bivolarski, 2012; Bovera et al., 2008; Di Meo et al., 2007; Ivanova, 2015). Restricted feeding of rabbits results in stunted growth, and after return to regular feeding, the animals do not attain the necessary body weight. Consequently, compensatory growth and its effect on live body weight depend on the intensity and duration of feed restriction (Chodova et al., 2013). The authors concluded that the rabbits could not regain their body weight more prolonged and more considerable feed restriction. In contrast, moderate feed restriction did not affect them.

It is acknowledged that the early castration of male animals increases the slaughter yield, the amount of

lipids in fat depots and increases meat fat content. In addition, the meat of castrated male rabbits had a higher water-holding capacity (WHC) and higher pH values (Ribarski et al., 2013). Most of the studies on the effects of castration on physicochemical properties of meat were performed in rams, boars and bulls, whereas data in rabbits are scarce (Ribarski et al., 2013). The meat of castrated male rabbits and females is more tender and leaner than intact males (Lebas et al., 2000).

The purpose of the present study was to investigate the effect of omega-3 polyunsaturated fatty acids on the physicochemical properties of the meat of rabbits with experimentally visceral obesity (castration) on a complete- or restricted diet.

Material and methods

The experiments were conducted with 42 male New Zealand White rabbits, divided into seven groups (n = 6): Group A – castrated, fed a full-diet and treated with krill oil; Group B – castrated, fed a full-diet and treated with fish oil; Group C – castrated, fed a full-diet and untreated; Group D – intact, fed a full-diet and untreated; Group E – castrated, fed a restricted diet (50%) and treated with krill oil; Group F – castrated, fed a restricted diet (50%) and treated with fish oil; and Group G – castrated, fed a restricted diet (50%) and untreated. experimental rabbits were 3-month-old and received feed for fattening rabbits (Table 1).

Krill and fish oil were given – *per os* as gelatin capsules containing 600 mg omega-3 polyunsaturated fatty acids (PUFA) over 60 days. At the end of the experiment, the animals were euthanised in compliance with current normative documents and the rules of the Animal Ethics Committee of the Trakia University. After carcass dissection, samples from *Longissimus Lumborum* muscle (LL) and *Semimembranosus* muscle (SM) were collected and treated by methods described by Pozahariskaja et al. (1964). The samples were first cooled at 2°C and stored for 24 h. Afterwards, meat pH was measured with a pH meter Consort C532. The pH meter was equipped with a combined penetrating electrode for meat analysis and calibrated with standard solutions (pH 4.0 and pH 7.0). The samples for pH determination were localised on the right T5 level for LL and in the middle third of the muscle for SM (Blasco et al., 1992).

The water-holding capacity (WHC) of muscles was determined as described by Grau and Hamm (1953).

Total protein, fat, ash and water (moisture) contents were analysed by classical methods of general chemical analysis of cooled meat (Vashin et al., 1999).

The statistical analysis of results was done using Statistica V.7.1 for Windows (StatSoft Inc., USA). Descriptive statistics were used to calculate means and standard errors of means. The effect of the group on studied parameters was evaluated with a one-way analysis of variance (ANOVA). The significance of the differences was determined by the post hoc LSD test and set as ($P < 0.05$).

Results

Physicochemical properties of LL meat from the four groups of rabbits fed a full diet are shown in Table 2. The moisture content was the lowest in Group C – 69.99 ± 0.69%. It was statistically significantly different from the other three groups (A, B and D). The opposite tendency was established for protein, fat, dry matter, pH and WHC of meat, which was substantially higher in Group C than in the other groups. The highest ash content was - in Group C, but it was statistically significantly different only compared with Group A. An interesting tendency was established for meat fat content. It was the highest in Group C (4.64 ± 0.40%) and Group B (3.11 ± 0.59%). Fat content was statistically significantly different between Group C and all other groups and Group B vs Groups C and D.

SM's physicochemical analysis did not reveal inter-group differences concerning fat and ash contents. Like in the case of LL, moisture content was the lowest in Group C (72.73 ± 0.45%) and statistically significantly different from the other groups. The values also differed considerably between the groups treated with krill and fish oil (A vs B) and Groups C and D (Table 3). SM protein content was the highest in Group C (23.40 ± 0.36%) and significantly different from average values recorded in Group B ($P < 0.04$)

Table 1. Ingredients and chemical composition of the diet in rabbits

Diet ingredients	Value	Diet ingredients	Value
Moisture (%)	11.00	Calcium (%)	0.80
Crude protein (%)	16.70	Total phosphorus (%)	0.65
Crude fat (%)	4.00	Sodium (%)	0.15
Crude fibre (%)	15.00	Metabolisable energy (kcal)	2500
Crude ash (%)	5.20	Vitamin A/retinol/ (IU/kg)	13000
Lysine (%)	0.65	Vitamin D3/cholecalciferol/ (IU/kg)	1600
Methionine (%)	0.32	Vitamin E/Dl-alpha Tocopheryl acetate/ (mg/kg)	60
Methionine (%) + Cysteine (%)	0.63	Copper (mg/kg)	10

Table 2. Physicochemical characteristics of *Longissimus lumborum* muscle

Parameters	Groups				Statistical significance among groups					
	Group A Mean ± SEM	Group B Mean ± SEM	Group C Mean ± SEM	Group D Mean ± SEM	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Moisture, %	74.44 ± 0.17	73.63 ± 0.13	69.99 ± 0.69	74.57 ± 0.20	NS	0.001	NS	0.001	NS	0.001
Protein, %	21.82 ± 0.32	22.12 ± 0.46	24.23 ± 0.41	22.25 ± 0.08	NS	0.001	NS	0.001	NS	0.001
Fat, %	2.86 ± 0.18	3.11 ± 0.59	4.64 ± 0.40	2.01 ± 0.23	NS	0.01	NS	0.01	NS	0.001
Dry matter, %	25.76 ± 0.27	26.37 ± 0.13	30.07 ± 0.71	25.40 ± 0.21	NS	0.001	NS	0.001	NS	0.001
Ash, %	1.08 ± 0.04	1.14 ± 0.04	1.20 ± 0.05	1.17 ± 0.02	NS	0.05	NS	NS	NS	NS
pH	5.55 ± 0.13	5.34 ± 0.06	6.63 ± 0.07	5.29 ± 0.04	NS	0.001	0.03	0.001	NS	0.001
WHC, %	19.52 ± 0.79	18.82 ± 0.33	27.36 ± 1.28	20.84 ± 1.17	NS	0.001	NS	0.001	NS	0.001

Table 3. Physicochemical characteristics of the *Semimembranosus* muscle

Parameters	Groups				Statistical significance among groups					
	Group A Mean ± SEM	Group B Mean ± SEM	Group C Mean ± SEM	Group D Mean ± SEM	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Moisture, %	74.68 ± 0.25	73.61 ± 0.12	72.73 ± 0.45	74.68 ± 0.29	0.04	0.001	NS	NS	0.03	0.001
Protein, %	21.71 ± 0.29	22.37 ± 0.38	23.40 ± 0.36	21.76 ± 0.35	0.01	NS	NS	0.04	NS	0.01
Fat, %	2.79 ± 0.16	2.90 ± 0.45	2.79 ± 0.27	2.49 ± 0.36	NS	NS	NS	NS	NS	NS
Dry matter, %	25.64 ± 0.20	26.40 ± 0.12	27.30 ± 0.45	25.49 ± 0.29	NS	0.001	NS	0.05	NS	0.001
Ash, %	1.14 ± 0.06	1.13 ± 0.06	1.11 ± 0.05	1.08 ± 0.08	NS	NS	NS	NS	NS	NS
pH	5.26 ± 0.06	5.44 ± 0.09	6.29 ± 0.09	5.58 ± 0.10	NS	0.001	0.03	0.001	NS	0.001
WHC, %	22.51 ± 0.28	19.88 ± 0.84	28.33 ± 2.29	17.54 ± 0.68	NS	0.01	0.03	0.001	NS	0.001

and Group D ($P < 0.01$). A similar difference was established between Groups A and B ($P < 0.01$).

The dry matter content of SM exhibited the same trend of change as LL dry matter. The highest average values among all groups were recorded in Group C (27.30 ± 0.45%). The WHC of SM was the highest again in Group C (28.33 ± 2.29%) and significantly different vs Group A ($P < 0.01$), Group B and D ($P < 0.001$). Also, another statistically significant difference was noted between Groups A and D ($P < 0.03$). The pH values of SM were the highest in Group C. They exhibited the same dynamics as the water holding capacity, i.e., considerable difference vs the other groups fed with a full diet and statistically significant differences between Groups A and D ($P < 0.03$).

It should be noted that there were less statistically significant differences in both studied muscles of rabbits on a restricted diet than in those fed with a full diet (Tables 4 and 5).

This tendency was most evident for protein and ash contents of SM muscles and moisture content, where the established differences were insignificant. The krill and fish oil treatments in rabbits provoked an increase in LL and SM fat content. The LL's fat content significantly differed between Groups E and G ($P < 0.01$). The moisture content of LL was the highest in Group G (75.25 ± 0.19%) and substantially different than Group E ($P < 0.05$). The WHC of the LL muscle was the lowest in Group G, exhibiting statistically, significant differences only vs Group F ($P < 0.02$). Comparing the pH values of LL, significant differences were observed between Groups E and F ($P < 0.01$) and between Groups E and G ($P < 0.03$). This parameter showed a similar pattern of change in SM as well.

It is interesting to note that the highest differences in WHC of studied muscles were established in SM. In Group G, the average WHC was the lowest in Group G (17.83 ± 0.54%) and significantly different ($P < 0.001$) from values in Groups E and F.

The correlation analysis of the results showed that the meat protein content of rabbits fed a full diet correlated positively with meat dry matter and pH in LL ($r = 0.82$ and $r = 0.70$) and SM ($r = 0.73$ and $r = 0.49$). A negative correlation was found between protein and moisture of both muscles ($r = –0.82$ and $r = –0.69$, respectively), between dry matter and moisture content ($r = –1.00$ and $r = –0.94$) and between moisture and pH ($r = –0.82$ and $r = –0.42$). There was also a positive correlation between dry matter and WHC ($r = 0.70$ in LL and $r = 0.26$ in SM), and between WHC and pH ($r = 0.78$ in LL and $r = 0.59$ in SM). In addition, in LL muscle, there

Table 4. Physicochemical characteristics of the *Longissimus lumborum* muscle

Parameters	Groups			Statistical significance among groups		
	Group E Mean ± SEM	Group F Mean ± SEM	Group G Mean ± SEM	E vs F	E vs G	F vs G
Moisture, %	74.62 ± 0.24	74.80 ± 0.19	75.25 ± 0.19	NS	0.05	NS
Protein, %	22.36 ± 0.18	22.48 ± 0.20	22.51 ± 0.14	NS	NS	NS
Fat, %	1.92 ± 0.10	1.58 ± 0.24	1.05 ± 0.23	NS	0.01	NS
Dry matter, %	25.40 ± 0.24	25.11 ± 0.21	24.75 ± 0.19	NS	0.04	NS
Ash, %	1.12 ± 0.07	1.04 ± 0.04	1.19 ± 0.07	NS	NS	NS
pH	5.20 ± 0.04	5.46 ± 0.09	5.40 ± 0.04	0.01	0.03	NS
WHC, %	19.1 ± 1.06	21.38 ± 1.13	18.00 ± 0.63	NS	NS	0.02

Table 5. Physicochemical characteristics of the *Semimembranosus* muscle

Parameters	Groups			Statistical significance among groups		
	Group E MEAN ± SEM	Group F MEAN ± SEM	Group G MEAN ± SEM	E vs F	E vs G	F vs G
Moisture, %	74.64 ± 0.13	74.50 ± 0.22	74.89 ± 0.30	NS	NS	NS
Protein, %	22.09 ± 0.19	22.07 ± 0.31	22.21 ± 0.18	NS	NS	NS
Fat, %	2.19 ± 0.11	2.29 ± 0.38	1.69 ± 0.25	NS	NS	NS
Dry matter, %	25.36 ± 0.13	25.55 ± 0.24	25.16 ± 0.31	NS	NS	NS
Ash, %	1.09 ± 0.07	1.19 ± 0.03	1.26 ± 0.08	NS	NS	NS
pH	5.31 ± 0.06	5.58 ± 0.05	5.49 ± 0.06	0.01	0.05	NS
WHC, %	22.52 ± 0.86	24.17 ± 0.91	17.83 ± 0.54	NS	0.001	0.001

were strong positive correlations between following the parameters: fat vs dry matter contents (r = 0.84), fat vs WHC (r = 0.56), fat vs pH (r = 0.65); protein vs WHC (r = 0.59); ash vs WHC (r = 0.44), dry matter vs pH (r = 0.82). The moisture content was strongly and negatively related to fat and WHC (r = –0.83; r = –0.72, respectively).

Fat contents of both muscles, in restricted rabbits, correlated significantly positively with dry matter (r = 0.75 and r = 0.66) and strongly negatively with moisture (r = –0.70 and r = 0.59); a negative correlation was also observed between dry matter and moisture (r= –0.98 and r= –0.99). Moreover, there was a significant negative correlation between LL moisture and WHC (r= –0.52) and LL ash vs WHC (r= –0.53).

Discussion

Rabbit meat quality depends on its physicochemical composition, is accordingly, it is significantly affected by any diet restriction. The meat's moisture, being a sum of free and bound water, is the primary muscle constituent and is influenced by the limited amount of feed (Ouhayoun and Zotte, 1996). Our results confirm that restricted feeding increased meat moisture content in both muscles, with a predominant manifestation of this tendency in castrated rabbits that did not receive krill or fish oil. In our view, this was because krill or fish oil treatments were necessary for rabbits whose diet was restricted, as evidenced by the negative correlation between fat and moisture contents of both muscles. The opposite tendency in moisture content was observed in rabbits fed with a full diet, where this parameter was the lowest in castrated untreated individuals. Fat content correlated negatively with moisture, but the most critical factor determining the low moisture content was the availability of needed energy

It should be noted that the water content of rabbit meat increased proportionally to the level of diet restriction. The moisture content of rabbits fed with a full diet was 62.3%, and in those which received 80% of the diet – 66.2% (Xiccato, 1999). Larzul et al. (2004) and Metzger et al. (2009) reported similar results, whereas Bernardini et al., 1994 affirmed that moisture correlated negatively to meat fat content. Therefore, rabbits fed a restricted diet stored less lipids in meat because of increased water content, as also shown by Xiccato (1999) found. The latter has established that more intensive diet restriction reduced meat fat within a broader range.

We found that in rabbits fed with a full diet, the lipid content in LL was higher than - in SM, which was most probably due to the specific biochemical processes in muscles with a different topographic location. The muscle fat content is essential for rabbit meat quality

(Hernandez, 2008; Blas and Wisewan, 2020), as it is primarily associated with – organoleptic properties of the product – its flavour and juiciness. Compared to meats of other species, rabbit meat is low in calories and contains less fat. Our results of lipid content in LL and SM, in full diet, fed rabbits, varied from 2.01% to 4.64% and were within the reference range reported by Hernandez and Gondret (2006) – from 0.6% to 14.4%. The slaughter age influences rabbit meat fat. Gondret et al. (1998) reported that intramuscular fat increased from 1.3% in rabbits, slaughter at 11 weeks of age. In our studies, the meat fat content of rabbits on a restricted diet was higher in SM than in LL. This reverse relationship compared to full diet-fed rabbits could be attributed to the higher LL moisture content. Despite this, the fat meat percentage in full diet-fed rabbits was substantially higher than in animals on a restricted diet. Our results are in line with those of Gondret et al. (2000), who demonstrated that rabbits with diet restriction had a considerably lower fat content in Biceps Femoris muscle, LL and SM than full dietfed rabbits. Similar results are also reported by Larzul et al. (2004). Opposite data have been established by Metzger et al. (2009), affirming that the restriction of rabbit diet did not influence the total muscle fat content of hindlimbs compared with full-fed animals. The changes in meat's fat content in restricted-fed rabbits were most probably related to the reduced enzyme activity of malate dehydrogenase and glucose-6-phosphate dehydrogenase, involved in the biosynthesis of fatty acids (Gondret et al., 1997; Zhong et al., 2021).

The lipids in rabbit meat are composed of 36,9% saturated fatty acids (SFA) (Zotte, 2000) and 34.6% of polyunsaturated fatty acids (PUFA) from the entire fatty acid content in hind limbs (Hernandez and Gondret, 2006). Fat amount, and quality adjustment in the diet could easily change this fatty acid ratio in meat. Still, high PUFA content could harm the oxidative stability of meat (Hernandez, 2008), which is a reasonable explanation for our results concerning LL fat content in full diet-fed rabbits. The values were the highest in Group C and statistically significant vs fat contents in Groups A, B and D. There were no significant differences in SM. Therefore, castration in full diet-fed rabbits had a most remarkable influence on fat deposition in LL muscle. The opposite tendency was noted in rabbits with diet restriction for both muscles: statistically significant differences were observed between treated and untreated rabbits. The highest fat content of LL and SM was associated with omega-3 PUFA supplementation on the one hand and restricted feeding on the other. Which could be because rabbits can include dietary fatty acids in adipose and muscle tissue lipids (Hernandez and Gondret, 2006). So far, no studies have investigated the level of diet restriction on the fatty acid composition and lipid content of rabbit meat. Here we showed that the lipid quality of the rabbit meat was affected by both castration and the amount of the diet. At the same time, the castration of male rabbits resulted in the deposition of more fat, which influences the visual evaluation of carcass fattiness (Ivanova, 2015).

Rabbit meat proteins are highly nutritional as they contain all essential amino acids (Bivolarski, 2012). The meat protein content is mainly influenced by dietary protein percentage. A significant reduction of muscle-growing was reported when growing rabbits were fed low-protein rations. When they returned to a full diet, meat protein was increased (Lebas and Ouhayoun, 1987). Our data indicated that LL and SM meat protein contents were significantly higher in castrated full diet-fed rabbits. (Ribarski et al. 2013) reported similar results. Concerning restricted feeding alone, the tendency towards the highest protein content of both muscles was preserved in the castrated group compared with the groups treated with fish or krill oil.

It should be noted that the differences in groups with diet restrictions were not statistically significant. Slightly higher meat protein content was reported in rabbits fed a restricted diet versus full-fed animals (Xiccato, 1999). If the ration contained excess proteins for its energy content, nitrogen retention could be slightly improved by dietary energy supplementation until the energy/protein ratio attains a given value. If this value is exceeded because of excess energy, it will reduce the body nitrogen (Fraga et al., 1983).

WHC is an important parameter indicating the capability of meat to retain tissue water. The full-fed rabbits have the highest WHC values in muscles. The opposite tendency was observed in rabbits fed a restricted diet, i.e., the lowest values were observed in Group G. The patterns of meat WHC changes demonstrated a better potential for tissue water retention than restrictively fed rabbits. The higher loss of water from the meat of rabbits with diet restriction was attributed to limited absorption of nutrients.

The rabbit farming industry has implemented alternative production systems, which substantially increased production costs and enhanced rabbits' welfare (Szendro, 2012). The utilisation of plastic floors, elevated platforms, dual-purpose cages, gnawing sticks, etc., provides a broader and more comfortable activity area, with less boredom and fewer behavioural problems. The surgery time (8–12 min per animal) and medication costs for castration of rabbits reared for meat consumption will result in serious expenditures which could be compensated only by selling high-quality meat. Therefore, the increasing demands for rabbit meat could be met by traditional rearing technologies and alternative systems producing high-quality meat for more exigent consumers, paying a higher price.

References

1. Bernardini Battaglini M., Castellini C., Lattaioli P. Rabbit carcass and meat quality: effect of strain rabbitry and age. Italian Journal of Food Science. 1994. T. 6. P. 157-166.
2. Bivolarski B. Reproductive biology, physiology and nutrition of rabbits. 2 M; Stara Zagora. 2012. 200 p.
3. Bivolarski B., Vachkova E., Ribarski S. Effect of weaning age upon the slaughter and physicochemical traits of rabbit meat. Veterinarski archiv. 2011. T. 81(4). P. 499-511.
4. Blasco A., Ouhayoun J., Masoero G. Status of rabbit meat and carcass: Criteria and terminology. Options Mediterraneennes-Series seminaries. 1992. T. 17.P. 105-120.
5. Bovera F., Di Meo C., Maron S., Vella N., Nizza A. Feed restriction during summer: effect on rabbit growth performance. Proceedings of the 9th World Rabbit Congress, Verona, Italy. 2008. P. 567–571.
6. Chodova D., Tumova E. The effect of feed restriction on meat quality of broiler rabbits: A review. Scientia Agriculturae Bohemica. 2013. T. 44(1). P. 55-62.
7. Dalle Zotte A. Main factors influencing the rabbit carcass and meat quality. Proceeding of the 7th World Rabbit Congress, Valencia, Spain. 2000. P. 507–537.
8. De Blas C., Wisewan J. Nutrition of the Rabbit. Cabi. (2nd Edit.). 2020. 325p.
9. Di Meo C., Bovera F., Marono S., Vella N., Nizza A. Effect of feed restriction on performance and feed digestibility in rabbits. Italian Journal of Animal Science. 2007. T. 6. P. 765–767.
10. Fraga J., De Blas C., Pérez E., Rodríguez M., Pérez J., Gálvez F. Effect of diet on chemical composition of rabbits slaughtered at fixed body weights. Journal of Animal Science. 1983. T. 56. P. 1097–1104.
11. Gondret F., Juin H., Mourat J., Bonneau M. Effect of age at slaughter on chemical traits and sensory quality of Longissimus Lumborum Muscle in the rabbit. Meat Science. 1998. T. 48(1-2). P. 181-187.
12. Gondret F., Lebas F, Bonneau M. Restricted feed intake during fattening reduce intramuscular lipid deposition without modifying muscle fiber characteristics in rabbits. Journal of Nutrition. 2000. T. 130. P. 228–233.
13. Gondret F., Mourot J., Bonneau M. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comparative Biochemistry and Physiology. 1997. T. 117B. P. 259–265.
14. Grau R, Hamm R. A simple method to determine how much water is retained in muscles. Naturwissenschaften. 1953. T. 40. P. 29-30.
15. Hernández P. Enhancement of nutritional quality and safety in rabbit meat. Proceeding of the 9-th World Rabbit Congress, 10-13 June, Verona, Italy. 2008. P. 1287–1299.
16. Hernández P., Gondret F. Rabbit meat quality. In: Maertens L, Coudert P (eds): Recent advances in rabbit sciences. Sciences. ILVO, Merelbeke, Belgium. 2006. P. 269-290.
17. Hou R., Zhang J., Chen T., Zhao W., Li L. Effects of dietary supplement of mulberry leaf (Morus alba) on growth and meat quality in rabbits. Indian Journal of Animal Research. 2020. T. 54(3). P. 317-332.
18. Ivanova Zh. Study the effect of omega-3 polyunsaturated fatty acids and antioxidants on some aspects of lipid metabolism and glucose homeostasis in rabbits with experimentally provoked obesity. PhD Thesis, Bulgaria, Stara Zagora. 2015. 192 p.
19. Larzul C., Thébault G., Allain D. Effect of feed restriction on rabbit meat quality of Rex du Poitou®. Meat Science. 2004. T. 67. P. 479–484.
20. Lebas F., Jehl N., Juih H., Delmas, D. Influence of the male rabbit castration on meat quality. 2/. Physico-chemical and sensory quality. Proceeding of 7th World Rabbit Congress, Valencia Spain. 2000. T. A. P. 607-612.
21. Lebas F., Ouhayoun J. Effect of dietary protein level, housing conditions and season on growth and slaughter traits of rabbits. Annales de Zootechnie. 1987. T. 36. P. 427–432.
22. Maertens L., Huyghebaer G., Delezie E. Fatty acid composition of rabbit meat when fed a linseed based diet during different periods after weaning. Proceedings of the 9-th World Rabbit Congress 10-13 June, Verona, Italy. 2008. P. 1381 – 1385.
23. Marinov B., Grigorov I., Gurov B., Peshev R. Raising Rabbits for Meat. Sofia, 2009. 334 p.
24. Metzger Sz., Szendró Zc., Bianchi M., Hullár I., Fébel H., Maertens L., Cavan, C., Petracci M., Radnai I., Biró-Németh E. Effect of energy restriction in interaction with genotype on the performance of growing rabbits: II. Carcass traits and meat quality. Livestock Science. 2009. T. 126. P. 221–228.
25. Ouhayoun J., Dalle Zotte. A. Harmonisation in rabbit meat research muscle and meat criteria. Proceeding of the 6th World Rabbit Congress, Toulouse, France. 1996. P. 217–224.
26. Ouyed A., Brum J. Heterosis direct and maternal additive effects on rabbit growth and carcass characteristics. Proceeding of the 9th World Rabbit Congress, June10-13, Verona, Italy. 2008. P.195-199.
27. Palazzo M., Vizzarri F., Arvay J., D'Alessandro G., Martemucci G., Casamassima D., Ratti S, Corino C., Rossi R. Dietary effect of dried bay leaves (Laurus nobilis) meal on selected productive performances and on quality meat traits in growing rabbits. Livestock Science. 2020. T. 242. DOI: 10.1016/j.livsci.2020.104301.
28. Pozahariskaja L., Kogan M., Ringina V., Frejidilin E. Physicochemical and bacteriological control in the meat industry. Moscow. Food industry. 1964. 285 p.
29. Ribarski S., Penchev G., Ivanova Zh., Georgiev I., Mircheva T., Bochukov A. Effect of castration on some physicochemical characteristics and histostructure of rabbit muscle. Jivotnovadni nauki, 2013. T. 50(1). P. 62-69.
30. Szendro Z. New perspectives of hoising reproducting and growing rabbits. Abstract of 10th World Rabbit Congres. Sharm El-sheikh, Egypt. 2012. P. 5-6.
31. Vachkova, E., Studies and some endocrine and metabolic parameters, morphometric characteristics and absorptive area of the gut in rabbits depending on weaning age. PhD Thesis, Stara Zagora, Bulgaria, 2008. 158 p
32. Vashin I., Pavlov A., Rusev V., Daskalov H., Dinkov D. Manual of veterinary sanitary control and expertise of foods of animal origin, Stara Zagora, Kota Publishing House, 1999. P. 76-83.
33. Xiccato G. Feeding and meat quality in rabbits: a review. World Rabbit Science. 1999. T. 7. P. 75–86.
34. Zhong B., Jiang D., Hong Y., Li L., Qiu L., Yang R., Jin X., Song Y., Chen C L, B. Glucose-6-phosphate dehydrogenase neutralises stresses by supporting reductive glutamine metabolism and AMPK activation. Signal transduction and targeted therapy. 2021. T. 6(1). P. 1-4.

Received 28 June 2021
Accepted 12 November 2021

Relationship between Duration from Proestrus until Predicted Ovulation and Changes of Progesterone Concentration during this Time on Canine Litter Size

Indrė Mečionytė[1], Sigita Kerzienė[1], Lina Anskienė[1], Renata Japertienė[1], Giedrius Palubinskas[1]
[1]*Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

Keywords: *canine, estrus, progesterone, litter size, POD.*

Abstract. *The aim of this research is to predict the number of puppies that may be born by duration of period from proestrus until POD and the change of P4 concentration during this time of medium size canines. The study was conducted when the oestrus cycles of clinically healthy medium size primiparous and multiparous canine females (n = 47) at 2–7 years old were observed. Canine females were observed from first proestrus signs (FPS) till litter was born. Blood samples were collected on day 5 from the onset of discharge from the vulva until the day when the progesterone (P4) concentration rate changed till 4 ng/mL. If P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until the predicted ovulation day (POD). Mating was performed 2 times with medium size breed clinically healthy 2–8–year–old canine males, which already had offspring. The number of alive and stillbirth puppies in a litter were counted at a time when they were born. All females were divided into 3 groups by the litter size: small, medium, and large. The mean POD for females in this study was 11.36 ± 2.17 day. It was found that the fastest increase of P4 concentrations was in the medium ($y = 2.1092x + 1.097$, $R^2 = 0.9833$) and in the large ($y = 1.9792x + 1.2844$, $R^2 = 0.9999$) litter groups and the largest litters were born when P4 reached 4–8 ng/mL on days 9–14 day from FPS ($P < 0.05$). It was also observed that there was no correlation between progesterone concentration during POD and the number of puppies born ($y = -0.0522x + 6.2322$, $R^2 = 0.0233$).*

Introduction

A canine female can bring limited numbers of litters. However, it is expected that the female of a high breeding value will produce as many offspring as possible who will continue to maintain the breed at a high level or even contribute to the improvement of the breed. Therefore, in order to improve these aspirations, it is required to get as many offspring of such a female as possible in its litters (Schrack et al., 2017).

There is a variety of research that determines how one or another trait (mating time, season of the year, female age, body weight, etc.) affects the size of a litter (Gaytán et al., 2019; Arlt, 2018; Schrack et al., 2017; Wigham et al., 2017). One of the factors determining the size of the litter is the phenomenon of ovulation (Lee at al., 2005). However, there are several ways to detect ovulation. The progesterone (P4) assay is the most commonly used method to predict the ovulation day (POD) for successful mating (Hollinshead, Hanlon, 2017). P4 concentration starts to rise (basal P4 concentration <1 ng/ml (Kustritz et al., 2012) in the phase of the oestrus cycle called proestrus before LH surge (P4 concentration is 1.5–2 ng/mL during the LH peak (Kustritz et al., 2012)), which is associated with preovulatory follicular luteinization. POD occurs about 2–3 days after LH peak when P4 is from 4 ng/mL till 8 ng/mL (Hollinshead and Hanlon, 2019; Kustritz et al., 2012; Lee et al., 2005). There is a lack of information about how a rise speed of P4 from the first proestrus signs (FPS) until POD can affect a litter size according to an average rise of P4 of each day until POD (Hollinshead and Hanlon, 2019) and according to when POD occurs. A P4 rise is faster when there are more ovulating follicles (Knox et al., 2003) and some authors believe that the same may be true with canine females (Stornelli et al., 2020). Consequently, it is possible to link this trait with a canine litter size.

The size of the breed may affect the reproductive characteristics of females. One of the factors on which the number of puppies to be born depends is the size of the breed (Borgea et al., 2011). For example, according to researchers (Hollinshead and Hanlon, 2017; Borgea et al., 2011), an average litter size is 5.74 puppies per litter for medium size breeds. P4 levels may also vary slightly depending on the size of the breed. For example, in Kutzler et al.'s (2003) research, P4 was 1.79–5.00 at LH0–LH3 for medium size breeds.

The aim of this research is to predict the number of puppies that may be born by duration of period from proestrus until POD and the change of P4 concentration during this time of medium size canines.

Correspondence to Indrė Mečionytė, Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania. E mail: indre.mecionyte@lsmuni.lt

Materials and methods

The research was conducted in compliance with the Law of Veterinary Medicine of the Republic of Lithuania (New wording from 1 July 2011: No. XI–1189, 30 November 2010 No. 148–7563 (20.10.2012), the Law on Animal Welfare and Protection of the Republic of Lithuania (No. XI–2271, 03.10.2012, Official Gazette 2012), and Keeping, care and usage requirements for animals used for scientific and educational purposes, approved by The State Food and Veterinary Service by official letter (No. B1–866, 31.10.2012).

Oestrus cycles of clinically healthy medium size primiparous and *multiparous* canine females (n = 47) at 2–7 years old were observed. Canine females were observed from FPS (vulva swelling and discharge from it) until a litter was born. Blood samples were collected on day 5 from FPS (onset of discharge from vulva) until the day when the P4 concentration rate changed up to 4 ng/mL. If the P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until POD. Blood samples were taken from the cephalic vein in the morning and P4 concentration was determined by a electrochemiluminescence (ECL) Cobas e 411 analyzer (Hitachi High–Technologies Corporation, JAPAN).

The oestrus cycles of clinically healthy medium size primiparous and *multiparous purebred (vizslas, setter, Labradors)* canine females (n = 47) at 2–7 years old were observed. Canine females were observed from FPS (vulva swelling and discharge from it) until a litter was born in dog kennels in Lithuania and Poland. Blood samples were collected on day 5 from the cephalic vein from FPS (onset of discharge from vulva) until the day when the P4 concentration rate changed up to 4 ng/mL. Blood samples of females were collected to 5–mL tubes without additional reagents in the mornings and P4 concentration was determined by a electrochemiluminescence (ECL) Cobas e 411 analyzer (Hitachi High–Technologies Corporation, JAPAN). If P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until POD.

Mating was performed 2 times with medium size breed clinically healthy 2–8–year–old canine males, which already had offspring in an environment familiar to the male (in its home). For the first time, it was done on day 2, and for the second time, it was done on day 3 after P4 reached 4–8 ng/mL.

The number of alive and stillbirth puppies in a litter were counted at a time when they were born. Thus, all females were divided into 3 groups by the litter size:

- small group – ⩽ 4 puppies in a litter (⩽ 25% of an average litter size) (n = 13);
- medium group – 5–7 puppies (n = 19);
- large group – ⩾ 8 puppies in a litter (⩾ 25% of an average litter size) (n = 15).

All data were collected into Microsoft Excel program and were processed using the statistical package SPSS 25.0 (SPSSInc., Chicago, IL, USA). A comparison between variables was calculated by the chi–square test (χ^2) (between POD days' groups: 5–8, 9–14 and from 15); one–way ANOVA test was used to define the differences among the means of investigated groups; the statistical significance of differences between groups was assessed by the post–hoc test criterion, LSD (between POD days' groups: 5–8, 9–14 and from 15; also between puppies' number in different POD groups). The results were considered statistically significant under $P \leqslant 0.05$. Linear regression was used to model the relationship between two variables (time in the peri–ovulatory period and litter size; P4 at POD and litter size) by fitting a linear equation to observed data.

Results

The increase of P4 concentration from FPS until POD varied depending on a litter size. The fastest increase of P4 concentrations was observed in a medium litter group and in a large litter group. The range between P4 rise was 32.32%–34.1% comparing the small litter group with the medium and the large group (Fig. 1).

The comparison of the concentration of P4 during POD and the number of puppies in the litter showed that the number of puppies in the litter did not depend on the concentration of P4 during POD. The most common P4 concentrations during POD were 4–5 ng/mL and 6–7 ng/mL, less frequently 5–6 ng/mL, 7–8 ng/mL, (Fig. 2).

POD of canine females was 11.36 ± 2.17 days from FPS used in this study. The highest number of females reached P4 concentration (specific for POD) on days 9–14. A smaller part (19.15%) reached POD on days 5–8 days and from day 15 from FPS ($\chi^2 = 45.957$, df = 2, $P < 0.001$). It was found that POD was related to a litter size. Canine females that ovulated from day 15 from FPS produced 40.22% less puppies than females that ovulated on days 9–14 ($P < 0.05$) and 33.8% less than females that ovulated on day 5–8 from FPS ($P > 0.05$), (Fig. 3).

Discussion

The rise of P4 can vary in each oestrus cycle of a canine female. According to our data, P4 rises from FPS until POD more slowly for females which produce small litters. Hollinshead and Hanlon (2019) found that slow rise of P4 concentration on this period had a negative effect on the litter size but this was statistically significant only when insemination was made with frozen semen. Their first theory for these results was that there are less ovulating follicles when the P4 curve rises slowly in oestrus. Their

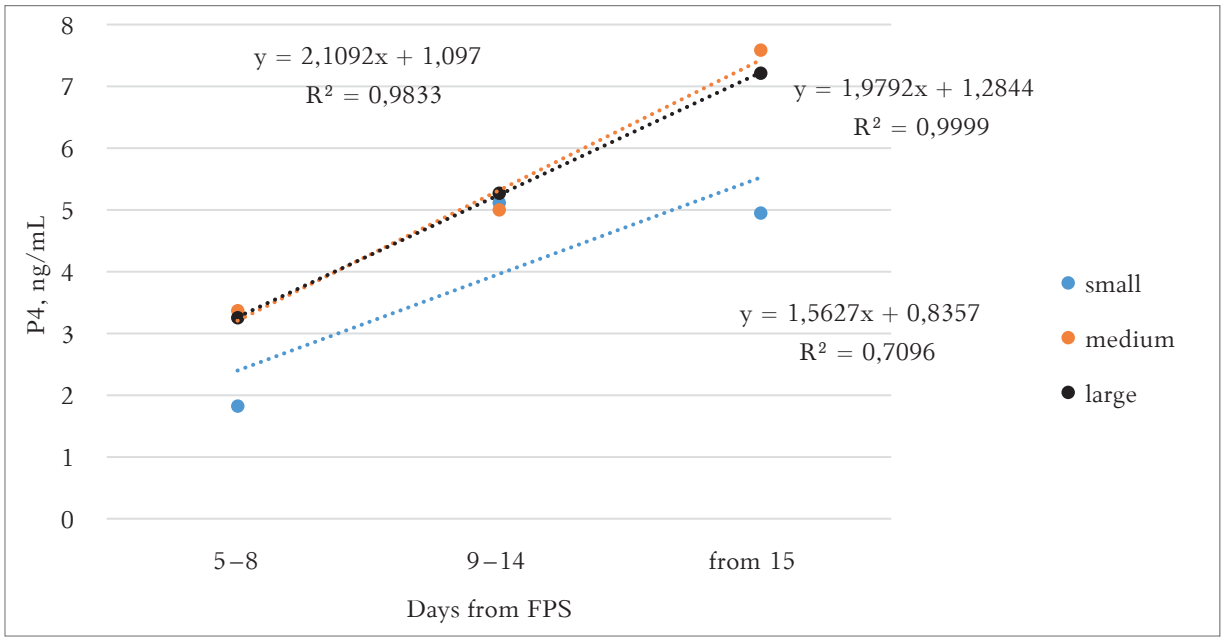

Fig. 1. Dependence of the litter size on progesterone level changes from the first proestrus signs until the predicted ovulation day

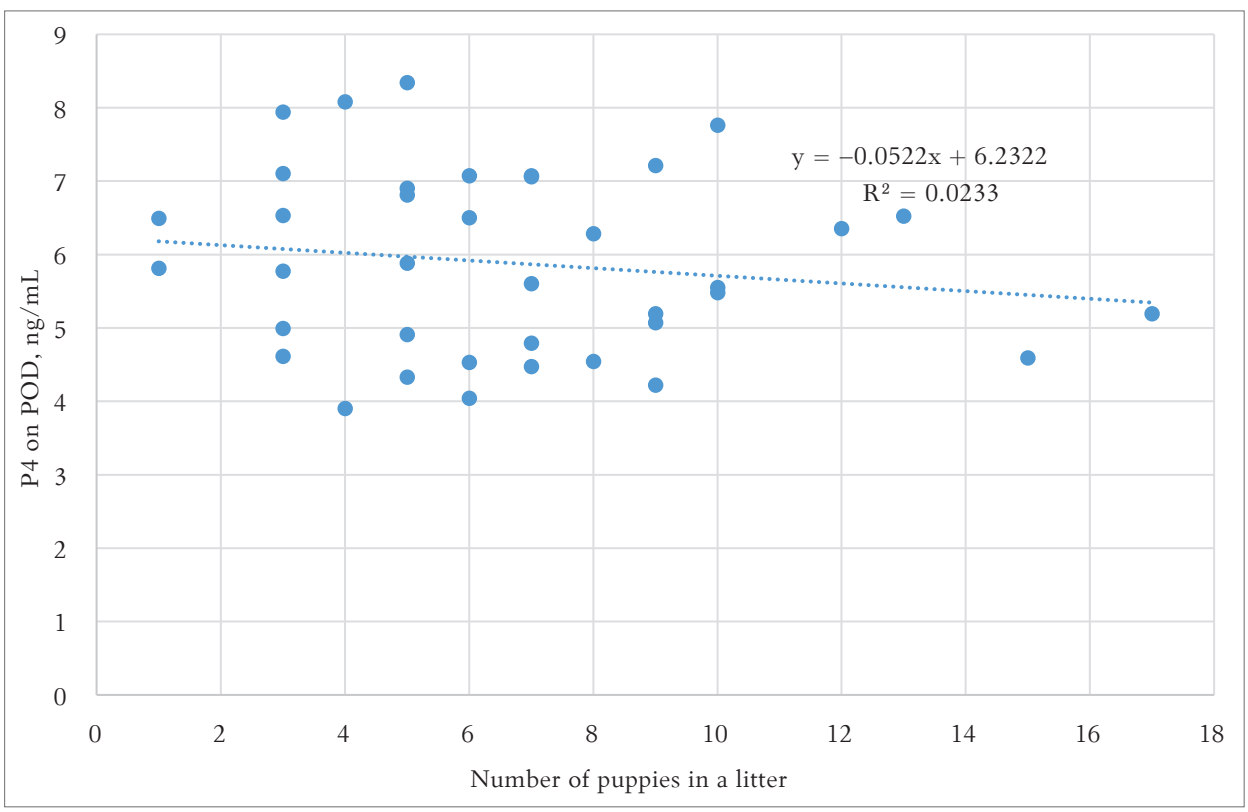

Fig. 2. Relationship between progesterone concentration during the predicted ovulation day and the number of puppies in a canine litter

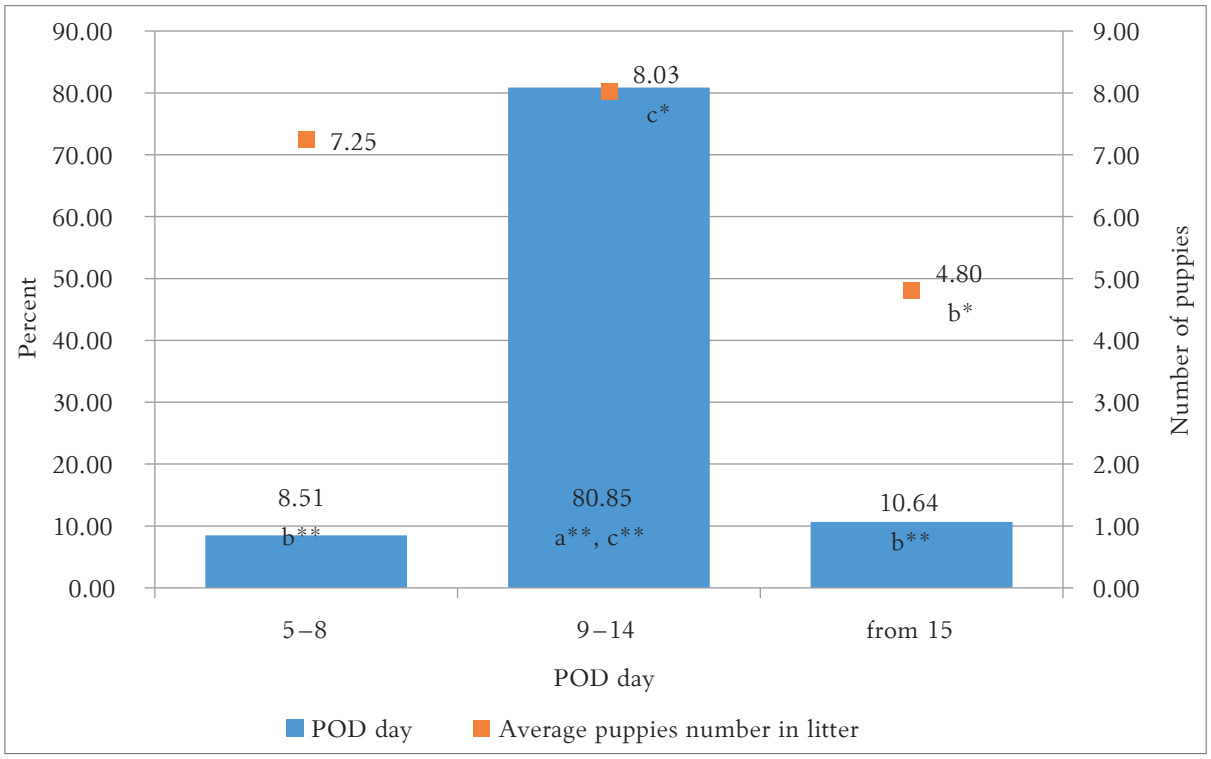

Fig. 3. The distribution of days from the first proestrus sign until the predicted ovulation day by progesterone concentration (4–8 ng/mL) and average litter size depending on POD.
Different letters a, b, and c indicate statistically significant differences between the groups (– $P < 0.05$; ** – $P < 0.01$).*

second theory was that follicles are ovulating later. This research was one of the first report about the dependence of the P4 concentration rate on the litter size. Our research suggests coming back to the first idea, and further research should focus on these ideas in order to get more accurate knowledge. It would be comprehensive to make P4 concentration and follicle count tests at the same time.

Some research studies have shown that ovulation could be predicted with a P4 test. P4 concentration at the POD is 4–8 ng/mL (Hollinshead and Hanlon, 2019; Rota et al., 2016; Lee et al., 2005). Other researchers have determined the effect of the breed size on P4 concentration at POD (Borgea et al., 2011), but there is no research available about the effect of P4 concentration at POD on the litter size. Thus, one of the objectives of our research was to find out how P4 concentration at POD affects the litter size. No statistically significant impact of this factor on the size of the litter in medium size canines was estimated ($P > 0.05$).

A range of P4 rise from FPS until POD on the same POD day can be different. This time depends on proestrus and preovulatory duration. These two periods can vary (proestrus may be 9–10 days, and preovulatory may be 1–7 days) (Concannon, 2010). The average POD day is day 9 (Domoslawska et al., 2014). According to our data, it occurs on days 9–14. The size of the litter depends on when the POD occurs. The largest litters are born on days 9–14.

Conclusion

The litter size was affected by an increasing mode of P4 concentration from proestrus until POD and the day of POD ($P < 0.05$), but P4 concentration at POD did not affect the litter size ($P > 0.05$).

References:

1. Arlt S. Canine ovulation timing: A survey on methodology and an assessment on reliability of vaginal cytology. Reproduction in domestic animals. 2018, 53(3):53-62
2. Borgea K.S., Tønnessena R., Nødtvedta A., Indrebøa A. Litter size at birth in purebred dogs—A retrospective study of 224 breed. Theriogenology. 2011, 75:911-919.
3. Concannon P.W. Reproductive cycles of the domestic bitch. Animal Reproduction Science. 2010, 124(3-4):200-10.
4. Domoslawska A., Jurczak A., Janowski T. Progesterone level does not distinguish the different course of canine ovulation determined by ultrasonography. Polish Journal of Veterinary Sciences. 2014, 17 (2): 293-297.
5. Gaytán L., Rascón C.R., Angel-García O., Véliz F.G., Contreras V., Mellado M. Factors influencing English Bulldog bitch fertility after surgical uterine deposition of fresh semen. Theriogenology. 2019, 142:315-319.
6. Hollinshead F.K., Hanlon DW. Normal progesterone profiles during estrus in the bitch: A prospectiveanalysis of 1420 estrous cycles. Theriogenology. 2019, 125:37-42
7. Hollinshead F.K., Hanlon DW. Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen. Theriogenology. 2017, 101:62-72.
8. Knox R.V., Vatzias G., Naber C.H., Zimmerman D.R. Plasma gonadotropins and ovarian hormones during the estrous cycle in high compared to low ovulation rate gilts. *Journal of Animal Science.* 2003, 81 (1): 249–260.

9. Kutzler M.A., Mohammed H.O., Lamb S.V., Meyers-Wallen V.N. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. Theriogenology. 2003, 60 (6): 1187-1196.
10. Lee B.C., Kim M.K., Jang G., Oh H.J., Yuda F., Kim H.J. Dogs cloned from adult somatic cells. Nature. 2005, 436:641.
11. Kustritz M.V. R. Managing the reproductive cycle in the bitch. Vet Clin North Am Small Anim Pract. 2012, 42:423–37
12. Rota A., Vannozzi I., Marianelli S., Gavazza A., Lubas G. Laboratory and Clinical Evaluation of a Feia Method for Canine Serum Progesterone Assay. Reproduction in Domestic animals. 2016, 51(1):69-74.
13. Schrack J., Dolf G., Reichler I.M., Schelling C. Factors influencing litter size and puppy losses in the Entlebucher Mountain dog. Theriogenology. 2017, 95: 163-17.
14. Stornelli M.C., Praderio R.G., García M.F., Favre R.N., Mitacek M.C.G., Sota R.L., Stornelli M.A. Serum progesterone concentration, volume, and apoptosis of corpora lutea in early, middle and late diestrus in the bitch. Animal Reproduction Science. 2020, 221: 106651
15. Wigham E.E., Moxon R.S., England G.C.W, Wood J.L.N, Morters M.K. Seasonality in oestrus and litter size in an assistance dog breeding colony in the United Kingdom. Veterinary record. 2017, 181(14):371.

Received 9 June 2021
Accepted 8 July 2021

Essential Oils as a Treatment Possibility Alternative in Dogs with Skin Infections

Arina Terentjeva[1], Modestas Ružauskas[2]
[1]*Lithuanian University of Health Sciences, Veterinary Academy, Kaunas, Lithuania,*
[2]*Lithuanian University of Health Sciences, Veterinary Academy, Microbiology and Virology Institute, Kaunas, Lithuania*

Keywords: *essential oils, antibacterial synergy, dog pyoderma, antibacterial resistance.*

Abstract*. The aim of this study was to investigate the susceptibility of bacteria to essential oils and to evaluate the possibility of using essential oils in treatment of skin infections in dogs. For the study, samples were taken from 6 dogs with skin infection, from which 11 bacterial species were identified and their susceptibility to antibiotics was determined. It was found that 70% of the isolates were multi-resistant. Bacterial susceptibility to essential oils was studied by the serial dilution method. Thyme, oregano and cinnamon leaf oils had broader-spectrum antibacterial activity (inhibited 90.9% of bacterial species) than other essential oils studied. Predominantly essential oils were effective in a concentration < 2.0%. An qual part mixture of 4 best acting essential oils – thyme, cinnamon leaf, oregano and geranium – showed synergistic properties and was effective even at a concentration of 0.1%. The mixture, however, did not have a bactericidal effect on Pseudomonas aeruginosa even at higher concentrations. Consequently, essential oils are effective against a wide range of bacterial species at low concentrations, well below the safe concentration recommendations, making them an effective alternative to antibiotics for the treatment of canine skin infections.*

Introduction

Skin diseases in dogs are among the most common reasons why owners consult a veterinarian (Soedarmanto et al., 2011; Tresch et al., 2019). When skin defence mechanisms are weakened, a transient or persistent skin microbiota can become pathogenic and cause a bacterial infection that often requires treatment with antibiotics (Miller et al., 2012). Carriers of multidrug-resistant bacteria are at particular risk because of a very limited choice of antibiotics (Nazarali et al., 2015). Due to the rapid antibacterial resistance development and multidrug-resistant bacteria spread, alternative antibacterial therapies are being actively investigated and the use of antibiotics in clinical practice is promoted to be minimized (Beever et al., 2015; Tresch et al., 2019).

There is currently a growing scientific interest in essential oils because of their great antibacterial properties found in a big number of *in vitro* studies, which suggest that essential oils could replace traditional therapies with antibiotics and antiseptics (Ruzauskas et al., 2020; Tresch et al., 2019). Essential oils are very complex mixtures of terpenoid and non-terpenoid substances, the amount and species of which depend on the plant species and growing conditions (Wynn & Fougère, 2007). It is terpenoids that have a broad spectrum of antibacterial properties – they act by breaking down bacterial membrane structures, causing lysis and intracellular fluid leakage (Khalil et al., 2017; Mann et al., 2000). The synergistic antibacterial effect of combining essential oils is often mentioned (Al-Bayati, 2008; Wynn & Fougère, 2007).

Multiple researches have not shown any risk of spontaneous bacterial resistance to essential oils development. The multicomponent nature of essential oils suggests that the likelihood of bacterial adaptation to multiple substances at a time is very low (Davis et al., 2005; Hammer et al., 2008). It is also thought that the antibacterial mechanisms of action of essential oils by rapid membrane damage limit the resistance development (Yap et al., 2014).

Pure essential oil is toxic to cells reaching 50% mortality in 24 hours even at low concentrations (0.1%), so it is necessary to dilute it with base oils which reduce the cytotoxicity by at least twice (Orchard et al., 2019). When not diluted, essential oils cause side effects such as contact dermatitis, sensitization, exacerbation of inflammation and pain (Tisserand, 2014), so it is necessary to follow the precautions of use: storage, dosing and dilution recommendations (Wynn & Fougère, 2007). Dilution recommendations in the literature are for humans only and have wide limits (Kerr, 2002; Tisserand, 2014; Wynn & Fougère, 2007). Few veterinarian clinical trials indicate that better treatment outcomes are achieved with lower concentrations of essential oils (up to 10%) by inhibition of inflammation, reduction in healing time, and reduction or absence of side effects (Costa et al., 2019; Dursun et al., 2003; Gunal et al., 2014; Kerr, 2002).

Most essential oils have an LD50 1–20 mL/kg, so dosing and dilution have to be especially careful for smaller animals (Wynn & Fougère, 2007) and those with concomitant diseases or conditions (Poppenga,

Correspondence to Arina Terentjeva Lithuanian University of Health Sciences, Veterinary Academy, Kaunas, Lithuania.
Email: terentjevaarina9@gmai.com

2002; Vanhaelen et al., 2002). It is important to thoroughly examine other effects of an essential oil intended to use to ensure safety in patients with contraindications, pregnancy, drug interactions possibility or coagulation issues (Khalil et al., 2017; Poppenga, 2002).

The aim of this study was to investigate the antibacterial effect of essential oils by determining their minimum inhibitory concentrations and to evaluate whether safe concentrations of essential oils can be sufficiently effective in the treatment of dog skin bacterial infections based on cytotoxicity studies and dilution recommendations provided in the scientific literature.

Materials and methods

Isolation and identification of bacteria

For the studies, samples were collected from the skin of 6 dogs with infection in Amies transport media with a swab (Transwab, MWE, UK). The clinical material was inoculated on universal soy-tryptone agar media with 7% sheep blood (Liofilchem, Italy) and selective media: Cetrimide Agar (Liofilchem, Italy), Slanetz-Bartley Agar (Liofilchem, Italy), Mannitol-Salt Agar (Liofilchem, Italy) and Endo Agar (Biolife, Italy). The media were incubated under aerobic conditions at +35°C and the blood agar under anaerobic conditions for up to 5 days, with daily media review and collection of grown colonies to identify the species.

Pure cultures were determined by morphological properties, growth pattern in universal (colony size, haemolysis, pigments) and selective media. Some bacteria were identified by oxidase, catalase, urease enzyme production, Gram-staining, agglutination reaction with specific sera, bacterial motility and gas (indole and hydrogen sulfide) production. In addition, biochemical studies were performed using Microgen (United Kingdom) identification systems (Staph-ID, Strep-ID, Bacillus-ID system, GN-A + B-ID system) according to the manufacturer's instructions. In cases when it was not possible to identify bacterial isolates by classical biochemical assays, the analysis of 16S rRNA sequences using primers 27F and 515R was performed as described previously (Ruzauskas et al., 2018).

Determination of bacterial susceptibility to antibiotics

After identification of the bacteria, their susceptibility to antibiotics was investigated by the disk diffusion method according to Kirby-Bauer. Susceptibility was assessed and interpretation of results was performed according to EUCAST recommendations (EUCAST, 2021) with updated clinical breakpoints. Antibiotics were cascaded by distinguishing the antibiotics of first, second, and third choice (Beco et al., 2013).

Selection of essential oils

Essential oils were selected according to dilution recommendations for humans, which are classified according to the skin irritation properties of the main substance (Table 1) (Tisserand, 2014). The selected substances belonged to moderately and mildly irritating substances, and the maximum concentration of 2.0% was chosen for the research.

Table 1. Classification of essential oils according to the degree of skin irritation and recommendations for their dilution for skin application

Skin irritation degree	Essential oil and its main substance		Maximum concentration recommendation
Severely irritating essential oils	Horseradish – sinigrin		Use on the skin is not recommended
	Mustard – allyl isothiocyanate		
	Garlic leaves – diallyl trisulfide		
Very irritating essential oils	Over 50%	Cinnamon bark, cassia – cinnamaldehyde	Dilute to 0.1%
	Sandalwood – santol		
	Saffron – safranal		
Moderately irritating essential oils	About 50%	Essential oils with cinnamaldehyde	Dilute to 1%
		Essential oils with eugenol	
		Essential oils with citral	
		Essential oils with carvacrol (> 50%)	
Mildly irritating essential oils	Essential oils with carvacrol (< 50%)		Dilute to 20%
	Essential oils with benzoic acid		
	Essential oils with citral (< 50%)		
	Essential oils with citronellol		
	Essential oils with thymol		
	Essential oils with geraniol		
	Essential oils with linalool		

Spain thyme essential oil (*Thymus zygis* thymol) with thymol (35.06%), sage essential oil (*Salvia sclarea*) with linalyl acetate (68.24%), geranium essential oil (*Pelargonium graveolens*) with citronellol (23.86%), radiant eucalyptus essential oil (*Eucalyptus radiata*) with eucalyptol (72.93%), ginger lemongrass essential oil (*Cymbopogon martinii* var. motia) with geraniol (73.08%), oregano essential oil (*Origanum vulgare*) with carvacrol (57.36 %), and cinnamon leaf essential oil (*Cinnamomum verum*) with eugenol (67.94%) were used in this study. The percentage of the main substances for each essential oil was indicated in GS/MS analysis provided by the manufacturer.

Determination of bacterial susceptibility to essential oils

The study was performed by the serial dilution method. Pure bacterial culture, cultured for 24 hours at 35°C under aerobic conditions taken from solid medium was diluted to 1.0 McFarland density with sterile saline. Then, 10.0 µL of culture solution was inoculated into 8 tubes with Mueller Hinton Broth (Liofilchem, Italy). The needed amount of essential oils was added to each tube. One tube was used for negative control without any oil. Then, the mixture was mixed for 20 seconds by an automatic shaker and incubated for 24 hours at +35°C. After incubation, 100 µL of the suspension was inoculated onto Tryptic soy agar to see the vitality of the bacteria. For this purpose, the plates were cultured for 24 hours at +35°C under aerobic conditions and bacteria growth was observed. In the absence of growth, the plates were incubated for up to 3 days. The process was performed with each of the 11 bacterial cultures tested with different concentrations of an essential oil – 2.0%, 1.5%, 1.0%, 0.5% and 0.2%.

Determination of synergistic antibacterial effects of essential oils

After evaluating four best acting essential oils – thyme, geranium, oregano and cinnamon leaf – the equal part mixture was made. All essential oils used for the mixture contained different main substances. The antibacterial effect of the mixture was tested by the serial dilution method, using the same technique as for the individual essential oils described in the section "Determination of bacterial susceptibility to essential oils". The antibacterial effect of the mixture at 0.1% concentration on isolated bacteria was studied. The susceptibility of *Pseudomonas aeruginosa* was further investigated for the mixture up to a concentration of 2.0%.

Statistical data analysis

The analysis of the results was performed using "Microsoft Office Excel 2017" and "SPSS/20" statistical programs. Crosstabulations were developed to examine the antibacterial activity of each essential oil, and the differences in the effect of the oils were determined according to the Pearson chi-square, the Kruskal–Wallis null hypothesis and the Fisher exact tests. The results of the calculations were considered statistically reliable if they reached more than 95% or $P < 0.05$.

Results

Pure bacterial cultures were isolated and identified from 6 dogs with skin infections (Table 2). Two different *S. pseudintermedius* isolates were isolated from the patient No. 1. All isolated bacteria species belonged to opportunistic pathogens and to transient or persistent microbial species.

The results of bacterial susceptibility to antibiotics showed that all bacteria were resistant to at least 2 antibacterial agents (Table 2). For most of the isolates, multidrug resistance was identified, especially for first- and second-line antibiotics. The resistance of *Citrobacter spp.*, *S. aureus*, *E. coli* and *P. aeruginosa* to the third-line antibiotics was found (erythromycin, imipenem, chloramphenicol and meropenem). *P. aeruginosa* was susceptible to only 2 of 7 antibiotics tested.

After studying the effect of individual essential oils, it was found that the effect of essential oils on bacteria differed depending on the type of an essential oil $P < 0.01$. Oregano, thyme and cinnamon leaf oils were found to inhibit 90.9% of bacterial species by a concentration of 1.0% (Table 3). Thus, these essential oils are established to have a broad-spectrum antibacterial effect. Other essential oils had a narrower bacterial species inhibition spectrum. All essential oils differed from each other in a statistically significant manner $P < 0.01$.

It was found that different bacterial species had different susceptibility depending on the type of an essential oil (Fig. 1). The figure shows the difference of an antibacterial effect on *E. coli* bacteria between sage and oregano essential oils: sage essential oil did not affect the bacterial growth even at 2.0% concentration, when oregano was effective at 0.5%. Data in the Table 3 show that the most sensitive bacteria to all essential oils were *P. multocida, Citrobacter spp., S. pseudintermedius-1, S. pseudintermedius-2* and *Streptococcus canis*, i.e., no specific essential oil was required to inhibit them. Other bacterial species required a specific essential oil for inhibition. *P. aeruginosa* bacteria was not suppressed by any of the 7 essential oils at a 1.0% concentration. This bacterium was affected only by thyme and oregano oil at 1.0% and 1.5%, respectively (Table 3).

Statistical analysis of the results to determine the relationship between bacterial Gram-staining (cell wall structure) and susceptibility to essential oils yielded statistically unreliable results ($P > 0.05$) meaning that essential oils may act equally on both gram-positive and gram-negative microorganisms.

The study of the made mixture of essential oils (thyme, geranium, oregano, cinnamon leaves) by serial dilutions resulted in a minimum inhibitory concentration of $\leqslant 0.1\%$ for all bacterial species (except

Table 2. Identified bacterial species and their susceptibility to antibiotics.

Patient number	Isolated bacteria species	Susceptibility to antibiotics														
		Penicillin	Gentamicin	Trimethoprim	Tetracycline	Cefalexin	Amoxicillin +CA	Cefoxitin	Enrofloxacin	Ciprofloxacin	Cefovecin	Erythromycin	Piperacine	Imipenem	Chloramphenicol	Meropenem
1	*Streptococcus canis*	R			R							S			S	
	Staphylococcus pseudintermedius-1	R	R	I	R			S	S			S				
	Staphylococcus pseudintermedius-2	R	R	S	R			S	I			S				
2	*Enterococcus faecalis*									R				I		
	Citrobacter spp.						R	R		S				S	R	S
	Staphylococcus chromogenes	R	R	S	S			R	S	R		S				
3	*Acinetobacter schindleri*		R							R				S		S
	Staphylococcus aureus	R	R	S	I			S	S			R				
4	*Escherichia coli*		R			S	R	R	S		R		S	R	R	S
5	*Pasteurella multocida*	R			R		R			S						
6	*Pseudomonas aeruginosa*		R						R		R		S	S	R	R

Note: R = resistant, I = intermediate, S = susceptible.

Table 3. Susceptibility of isolated bacteria to essential oils and their mixture.

Type of the essential oil	Main substance	Minimum inhibitory concentration of bacteria species (essential oil concentration, %)											Inhibition of bacteria species, n = 11, %
		E. coli	*S aureus*	*P. aeruginosa*	*Pasteurella multocida*	*Citrobacter spp.*	*Acinetobacter spp.*	*S. pseudintermedius-1*	*S. pseudintermedius-2*	*S. chromogenes*	*Enterococcus faecalis*	*Streptococcus canis*	
Oregano	Carvacrol 57.26%	0.5	0.2	1.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	90.9
Thyme	Thymol 35.06%	0.2	0.5	1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	90.9
Geranium	Citronellol 23.86%	> 2	0.2	**> 2**	0.2	0.5	1	0.5	0.2	> 2	1	0.2	54.6
Ginger lemongrass	Geraniol 73.08%	0.5	1	**> 2**	0.2	0.2	0.2	0.2	0.2	1	2	0.2	63.6
Eucalyptus	Eucalyptol 72.93%	0.2	0.5	**> 2**	0.5	0.5	0.2	0.2	0.2	> 2	0.5	0.2	71.9
Cinnamon leaf	Eugenol 67.94%	0.2	0.2	**> 2**	0.2	0.2	0.2	0.5	0.2	0.2	0.5	0.2	90.9
Sage	Linalyl acetate 68.24%	> 2	0.5	**> 2**	0.5	0.5	0.5	0.5	0.5	> 2	> 2	0.5	63.6
Mixture T,G,O,C 1:1:1:1	Carvacrol thymol, eugenol, citronellol	⩽ 0.1	⩽ 0.1	**> 2**	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	90.9
Bacteria species susceptibility to essential oils, n = 7, %		71.4	85.7	–	100	100	85.7	100	100	42.8	57.1	100	–

Note: T – thyme, G – geranium, O – oregano, C – cinnamon leaf essential oils

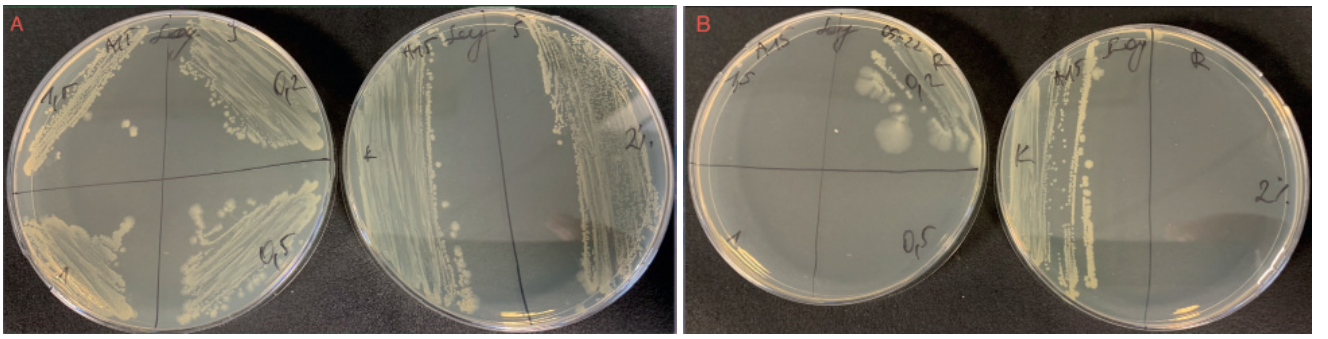

Fig. 1. Susceptibility comparison of *E. coli* (A15) to essential oils between two essential oils: A – sage essential oil had no effect (MIC > 2%), B – oregano essential oil – had a moderate effect (MIC = 0.5%)

P. aeruginosa). Synergy was found by lower MICs and a wider range of antibacterial effects. Statistical analysis of the results comparing the antibacterial effects of the individual oils and the mixture gave statistically significant results in all cases ($P < 0.01$).

Discussion

Bacterial multidrug resistance results confirm the problem of antibiotic resistance developing. The resistance found for the first, second and even third line antibiotics according to some authors could be greatly influenced by irresponsible use of antibacterial agents in clinical practice, not finishing the full course of antibiotics and contact transmission of multidrug resistant bacteria (Beever et al., 2015).

The studies have shown that the essential oils have a good effect on bacteria as mentioned in the literature. The antibacterial activity of essential oils has also been found to vary depending on the type of an essential oil (terpenoid type) (Oussalah et al., 2007; Tresch et al., 2019). The dependence of bacterial susceptibility to essential oils on the type of bacterium has been established as mentioned by other authors (Can Başer & Buchbauer, 2015; Orchard et al., 2019; Schnaubelt, 2012), but lower susceptibility of gram-negative bacteria to oils mentioned by Al-Bayati (2008) and Mann et al. (2000) was not statistically confirmed in this study.

The most resistant to essential oils was *Pseudomonas aeruginosa*, which was also mentioned by other authors as less sensitive than other bacteria. It requires higher concentrations of essential oils to inhibit. The resistance of this bacterium to essential oils is thought to be based on its inherent mechanisms of resistance to antibacterial agents (Arais et al., 2016; Chevalier et al., 2017; Smeriglio et al., 2017) and natural resistance to some natural antibiotics (Wynn & Fougère, 2007). In this study, only 2 essential oils were found to be effective against *Pseudomonas aeruginosa* – thyme and oregano with MIC 1.0% and 1.5%, respectively, although studies by different authors found significantly lower inhibitory concentrations of the same oils, such as 0.1% for thyme MIC. Such results may have been influenced by the reasons mentioned above. Although the literature mentions that *Pseudomonas aeruginosa* is more sensitive to a mixture of essential oils than to individual essential oils (Al-Bayati, 2008), the present study found the opposite: the mixture did not affect this bacterium even at higher concentrations. Data demonstrated that *P. aeruginosa* susceptibility may depend on the isolate.

The mixing of the 4 essential oils with different main substances achieved the synergistic effect mentioned in the literature, when the minimum inhibitory concentration for all bacterial species (except *P. aeruginosa*) was reduced at least twice (Al-Bayati, 2008; Wynn & Fougère, 2007). The synergy has also been identified for a wider range of bacteria species inhibition, making it possible not to look for a specific essential oil for the treatment. Research has also shown that mixing essential oils together increases their antibacterial activity and leaves cytotoxicity unchanged (Orchard et al., 2019; Ruzauskas et al., 2020). Thus, the blend was made from oils belonging to moderately and mildly irritating ones with dilution recommendations of up to 1% and up to 20%, and an effective concentration of 0.1% is more than 10 times lower than the safe one for dermal use (Beco et al., 2013; Tisserand, 2014). If such a mixture was prepared with *A. vera* or *S. chiensis* base oil, it is likely that the cytotoxicity would be reduced at least twice without attenuation of the antibacterial effect and it would have additional positive properties for wound healing (Edraki et al., 2014; Orchard et al., 2019).

Individual essential oils could be used to treat bacterial infections in dogs, but because bacteria species susceptibility to essential oils differs, the treatment should be started only after determining an effective essential oil type and its minimum inhibitory concentration to the infectious agent. A wider antibacterial spectrum and a lower effective concentration of the essential oil mixture would make it more convenient in clinical practice to use, thus achieving a positive effect and a minimal risk of side effects (Costa et al., 2019; Dursun et al., 2003; Gunal et al., 2014; Kerr, 2002). The treatment with essential oils is very promising, but until clinical trials of safety and effectiveness of their use in dogs are

made, such treatment can be used as an adjunct or as an alternative treatment when traditional treatment does not help.

Conclusions

In this study, 70% of dog skin isolates demonstrated multi-resistance to different classes of antibiotics.

The susceptibility of bacteria to essential oils was found to vary depending on the type of an essential oil and the species of bacterium: 6 of the 11 bacterial species required a specific essential oil to be inhibited. The most resistant to essential oils was *P. aeruginosa*, which was sensitive only to essential oils of thyme and oregano at concentrations $\geqslant$ 1.0%.

The essential oils of oregano, thyme and cinnamon leaf showed a broad antibacterial spectrum with 90.9% inhibition of bacterial species growth. It was found that the antibacterial potency of the essential oils did not depend on the Gram-staining of the bacteria ($P < 0.05$).

The essential oil mixture – thyme, geranium, oregano and cinnamon leaves – showed a synergistic effect by reduced minimum inhibitory concentration at least twice (to 0.1%) and by a broader spectrum of antibacterial effects than individual essential oils. The mixture did not affect *Pseudomonas aeruginosa*, so it may not be effective for treatment of the infection caused by *P. aeruginosa*.

The blend of essential oils was observed to be effective at very low concentrations against a wide range of bacteria and may therefore become an alternative to antibacterial therapy for the treatment of skin infections in dogs after further *in vivo* safety and efficacy clinical trials.

References

1. Al-Bayati F. A. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts. Journal of Ethnopharmacology. 2008. T. 116(3). P. 403–406.
2. Arais L. R., Barbosa A. V., Carvalho C. A., Cerqueira A. M. F. Antimicrobial resistance, integron carriage, and gyrA and gyrB mutations in Pseudomonas aeruginosa isolated from dogs with otitis externa and pyoderma in Brazil. Veterinary Dermatology. 2016. T. 27(2). P. 113-7e31.
3. Beco L., Guaguère E., Lorente Méndez C., Noli C., Nuttall T., Vroom M. Suggested guidelines for using systemic antimicrobials in bacterial skin infections: Antimicrobial choice, treatment regimens and compliance. Veterinary Record. 2013. T. 172(6). P. 156–160.
4. Beever L., Bond R., Graham P. A., Jackson B., Lloyd D. H., Loeffler A. Increasing antimicrobial resistance in clinical isolates of Staphylococcus intermedius group bacteria and emergence of MRSP in the UK. Veterinary Record. 2015. T. 176(7). P. 172.
5. Can Başer K. H., Buchbauer G. Handbook of essential oils: Science, technology, and applications, second edition. CRC Press, 2016. P. 433-619.
6. Chevalier S., Bouffartigues E., Bodilis J., Maillot O., Lesouhaitier O., Feuilloley M. G. J., Orange N., Dufour A., Cornelis P. Structure, function and regulation of Pseudomonas aeruginosa porins. FEMS Microbiology Reviews. 2017. T. 41(5). P. 698–722.
7. Costa M. F., Durço A. O., Rabelo T. K., Barreto R. de S. S., Guimarães A. G. Effects of Carvacrol, Thymol and essential oils containing such monoterpenes on wound healing: a systematic review. Journal of Pharmacy and Pharmacology. 2019. T. 71(2). P. 141–155.
8. Davis A. O., O'Leary J. O., Muthaiyan A., Langevin M. J., Delgado A., Abalos A. T., Fajardo A. R., Marek J., Wilkinson B. J., Gustafson J. E. Characterization of Staphylococcus aureus mutants expressing reduced susceptibility to common house-cleaners. Journal of Applied Microbiology. 2005. T. 98(2). P. 364-372.
9. Dursun N., Liman N., Özyazgan I., Güneş I., Saraymen R. Role of Thymus Oil in Burn Wound Healing. Journal of Burn Care and Rehabilitation. 2003. T. 24(6). P. 395–399.
10. Edraki M., Akbarzadeh A., Hosseinzadeh M., Tanideh N., Salehi A., Koohi-Hosseinabadi O. Healing effect of sea buckthorn, olive oil, and their mixture on full-thickness burn wounds. Advances in Skin and Wound Care. 2014. T. 27(7). P. 317–323.
11. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0. 2021. http://www.eucast.org
12. Gunal M. Y., Heper A. O., Zaloglu N. The effects of topical carvacrol application on wound healing process in male rats. Pharmacognosy Journal. 2014. T. 6(3). P. 10–14.
13. Hammer K. A., Carson C. F., Riley T. V. Frequencies of resistance to Melaleuca alternifolia (tea tree) oil and rifampicin in Staphylococcus aureus, Staphylococcus epidermidis and Enterococcus faecalis. International Journal of Antimicrobial Agents. 2008 . T. 32(2). P. 170-173.
14. Kerr J. The use of essential oils in healing wounds. International Journal of Aromatherapy. 2002. T. 12(4), P. 202–206.
15. Khalil A. A., Rahman U. U., Khan M. R., Sahar A., Mehmood T., Khan M. Essential oil eugenol: Sources, extraction techniques and nutraceutical perspectives. RSC Advances. 2017. T. 7(52), P. 32442–33060.
16. Mann C. M., Cox S. D., Markham J. L. The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). Applied Microbiology. 2000. T. 30(4), P. 2.
17. Miller W. H., Vmd J., Griffin C. E. Muller and Kirk ' s Small Animal Dermatology. 7th ed. Elsevier Health Sciences, 2012. P. 354-421.
18. Nazarali A., Singh A., Moens N. M. M., Gatineau M., Sereda C., Fowler D., Kim S. E., Kisiel A., Reynolds D., Ringwood B. R., Bruce C. W., Gibson T. W. G., Rousseau J., Weese J. S. Association between methicillin-resistant Staphylococcus pseudintermedius carriage and the development of surgical site infections following tibial plateau leveling osteotomy in dogs. Journal of the American Veterinary Medical Association. 2015. T. 247(8). P. 909–916.
19. Orchard A., Kamatou G., Viljoen A. M., Patel N., Mawela P., Vuuren S. F. V. The influence of carrier oils on the antimicrobial activity and cytotoxicity of essential oils. Evidence-Based Complementary and Alternative Medicine. 2019. T. 1. P. 1-24.
20. Oussalah M., Caillet S., Saucier L., Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food Control. 2007. T. 18(5). P. 414–420.
21. Poppenga R. H. Herbal medicine: potential for intoxication and interactions with conventional drugs. Clinical Techniques in Small Animal Practice. 2002. T. 17(1). P. 6–18.
22. Ruzauskas M., Bartkiene E., Stankevicius A., Bernatoniene J., Zadeike D., Lele V., Starkute V., Zavistanaviciute P., Grigas J., Zokaityte E., Pautienius A., Juodeikiene G., Jakstas V. The influence of essential oils on gut microbial profiles in pigs. Animals. 2020. T. 10. P. 1734.
23. Ruzauskas M., Klimiene I., Armalyte J., Bartkiene E., Siugzdiniene R., Skerniskyte J., Krasauskas R., Suziedeliene E. Composition and antimicrobial resistance profile of Gram-

negative microbiota prevalent in aquacultured fish. Journal of Food Safety. 2018. T. 38(3). P. e12447.
24. Schnaubelt K. Naujoji aromaterapija (Ed. Dalytė Mat). Kvapų namai, 2012. P. 48-52.
25. Smeriglio A., Denaro M., Barreca D., Calderaro A., Bisignano C., Ginestra G., Bellocco E., Trombetta D. In vitro evaluation of the antioxidant, cytoprotective, and antimicrobial properties of essential oil from Pistacia vera L. Variety Bronte Hull. International Journal of Molecular Sciences. 2017. T. 18(6), P. 1212.
26. Soedarmanto I., Kanbar T., Ülbegi-Mohyla H., Hijazin M., Alber J., Lämmler C., Akineden Ö., Weiss R., Moritz A., Zschöck M. Genetic relatedness of methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolated from a dog and the dog owner. Research in Veterinary Science. 2011. T. 91(3), P. e25-7.
27. Tisserand R. Essential Oil Safety (2nd ed.), Elsevier Health Sciences, 2014. P. 23-95.
28. Tresch, M., Mevissen M., Ayrle H., Melzig M., Roosje P., Walkenhorst M. Medicinal plants as therapeutic options for topical treatment in canine dermatology? A systematic review. BMC Veterinary Research. 2019. T. 15(1), P. 174.
29. Vanhaelen M., Pereira F. D. O., Mendes J. M., Lima I. O., Mota K. S. D. L., Oliveira W. A. De, Lima E. D. O., Habulin M., Šabeder S., Sampedro M. A., Knez Ž., Nerio L. S., Olivero-Verbel J., Stashenko E., Lawless J. The Encyclopedia of Essential Oils: The Complete Guide to the Use of Aromatic Oils in Aromatherapy, Herbalism, Health & Well-Being. Conary Press, 2002. P. 37-48, 60-76.
30. Wynn S. G., Fougère B. J. Veterinary Herbal Medicine. Mosby Elsevier, 2007.P. 314-329, 508.
31. Yap P. S. X., Yiap B. C., Ping H. C., Lim S. H. E. Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance. The Open Microbiology Journal. 2014. T. 8. P. 6-14.

Received 22 March 2021
Accepted 6 November 2021

Macro-Anatomical Investigations on the Course and Branches of the Celiac Artery in the Sparrow Hawk (*Accipiter nisus*)

Mehmet Can[1], Zekeriya Özüdoğru[2], Derviş Özdemir[3], Hülya Balkaya[3] , Şükrü Hakan Atalgın[1]
[1]Department of Anatomy, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey.
[2]Department of Anatomy, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey.
[3]Department of Anatomy, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey.

Keywords: *anatomy, aorta, celiac artery, sparrow hawk and splenic artery.*

Abstract. *The aim of this study was to investigate the course and distribution of the celiac artery in the sparrow hawk. The present work was carried out with 7 sparrow hawks of different ages and sexes. Non-curable birds were injected with 5–10 mg/kg doses of xylazine for premedication and 20–40 mg/kg doses of ketamine for anaesthesia intramuscularly. Under deep anaesthesia, their blood was drained by cutting off the apex of the heart and the vessels were cleaned out by administering 0.9% of normal saline water into the vessels. Latex coloured with dye was injected into the ventriculus sinister of the heart through the aorta at 7 materials. Then the specimens were subjected to fine dissection to demonstrate the origin, course and distribution of the celiac artery. The celiac artery originated from the aorta at the beginning of the synsacrum and provided the arterial nutrition of the last part of the oesophagus, cecum, small intestines, pancreas, lien, liver, ventriculus and proventriculus. Along its course, it was found to terminate splitting into the right branch of the celiac artery and the left branch of the celiac artery after giving off the common root of the oesophageal artery with the superior proventricular artery and splenic artery. The left branch of the celiac artery was determined to extend cranioventrally in the proximity of isthmus gaster. After it gave off the right hepatic artery to nourish the lobus hepatis dexter of the hepar, it was passed to the left side of the hepar. In this part, this blood vessel was found to terminate splitting into the inferior proventricular artery, left inferior gastric artery and left gastric artery after giving off the left hepatic artery to the lobus hepatis sinister of the liver with three to four branches. The right branch of the celiac artery was determined to run along under the lien in a caudoventral direction. It was observed to lie on the junction of the gaster with duodenum. It was found to give off the right superior gastric artery before reaching the indicated junction. At the level of the indicated junction, the right branch of the celiac artery was determined to split into its terminal branches which extended in different directions, namely ileocecal artery, gastroduodenal artery, pancreaticoduodenal artery, duodenojejunal artery and right inferior gastric artery.*

Introduction

Sparrow hawk is a bird of prey in the order of daytime predators (*Falconiformes*), family *Accipitridae* and subfamily *Accipitrina*e. Sparrow hawks have short, rounded wings, a long tail, a hooked beak, and curved claws. Unlike raptors belonging to the *Falconidae* family, it kills its prey not with its beak, but with its feet and claws. Therefore, the feet are relatively well developed compared with the beak (Sustaita, 2008). The most important route of raptors, especially sparrow hawks, is Turkey, where they come from Europe and Asia and pass to the Black Sea Region (Greenberg and Marra, 2004).

The celiac artery originating from the aorta nourishes proventriculus, gizzard, ventriculus, liver, lien, pancreas and small intestines (Doğuer and Erençin, 1964; Malinovsky and Novotna, 1977; Baumel et al., 1993; Dursun, 2002). During its course, this artery bifurcates into the right branch of the celiac artery and the left branch of the celiac artery after giving off oesophageal artery and superior proventricular artery (McLeod et al., 1964; Malinovsky, 1965; Gadhoke et al., 1975; Getty, 1975; Baumel et al., 1993; Pinto et al., 1998; Cardoso et al., 2000). The superior proventricular artery gives off the oesophageal branches and the dorsal gastric artery (Baumel et al., 1993; Dursun, 2002).

The inferior proventricular artery, left gastric artery, rami sacci, inferior gastric artery, left hepatic artery and gastroduodenal artery arise from the left branch of the celiac artery; meanwhile, splenic artery, right hepatic artery, ileocecal artery, pancreaticoduodenal artery, doudenojejunal artery, right superior gastric artery, right inferior gastric artery and rami sacci have their origin from the right branch of the celiac artery (Baumel et al., 1993).

This study aimed to increase the knowledge of the gross morphology of the celiac artery, especially in sparrow hawks, and to compare it with other birds, and also to contribute to veterinary comparative anatomy.

Correspondence to Mehmet Can, Balıkesir University, Faculty of Veterinary Medicine, Department of Anatomy, 10463, Balıkesir, Turkey. E-mail: mehmetcan@balikesir.edu.tr

Materials and Methods

In this study, 7 sparrow hawks were used. Their weights ranged from 150 to 250 g and sex differences were not considered. These hawks were birds that were brought to the surgical clinic of Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey, for treatment purposes because they had broken wings or legs and were euthanized after it was confirmed that treatment was not possible. After injecting 5–10 mg/kg xylazine for premedication and 20–40 mg/kg ketamine for anaesthesia intramuscularly (Flecknell, 1987; Belge and Bakır, 1999), surgical clinic staff performed euthanasia by cutting the apex of the heart under deep anaesthesia. Then, the vessels were cleaned by giving 0.9% physiological saline to the vessels. Latex coloured with dye was injected into the ventriculus sinister of the heart through the aorta at 7 materials (Hassa, 1967). After keeping in tap water at room temperature for 24 hours, photographs of the cadavers were taken, dissecting of the celiac artery and its branches.

For angiography, 45 kW doses of barium sulphate solution were injected into the aorta in 3 materials. Angiographies were taken at a ventrodorsal position, and 100 kW 30 mAS Poskom brand x-ray was used for angiography.

In the presented study, mathematical-statistical evaluation was not performed because the number of animals was not high and there were gender and age differences.

For the terminology, Nomina Anatomica Avium (NAA) was used (Baumel et al., 1993).

Results

The celiac artery originated from the ventral wall of the aorta at the beginning of the synsacrum, at the junction where the glandular stomach joins the oesophagus. This artery was determined to provide the nutrition of the last part of the oesophagus, proventriculus, ventriculus, liver, lien, pancreas, small intestines and cecum. The celiac artery was found to run along in between the lobus hepatis dexter et sinister of the liver on the right side of the proventriculus in caudoventral direction and, along its course, it terminated by splitting into the right branch of the celiac artery and the left branch of the celiac artery in-between the medial surface of the lien after first giving off the common root of the oesophageal artery and superior proventricular artery and then splenic artery (Fig. 1.1 and 2.2).

The first vessel originating from the celiac artery was found to be the common root of the superior

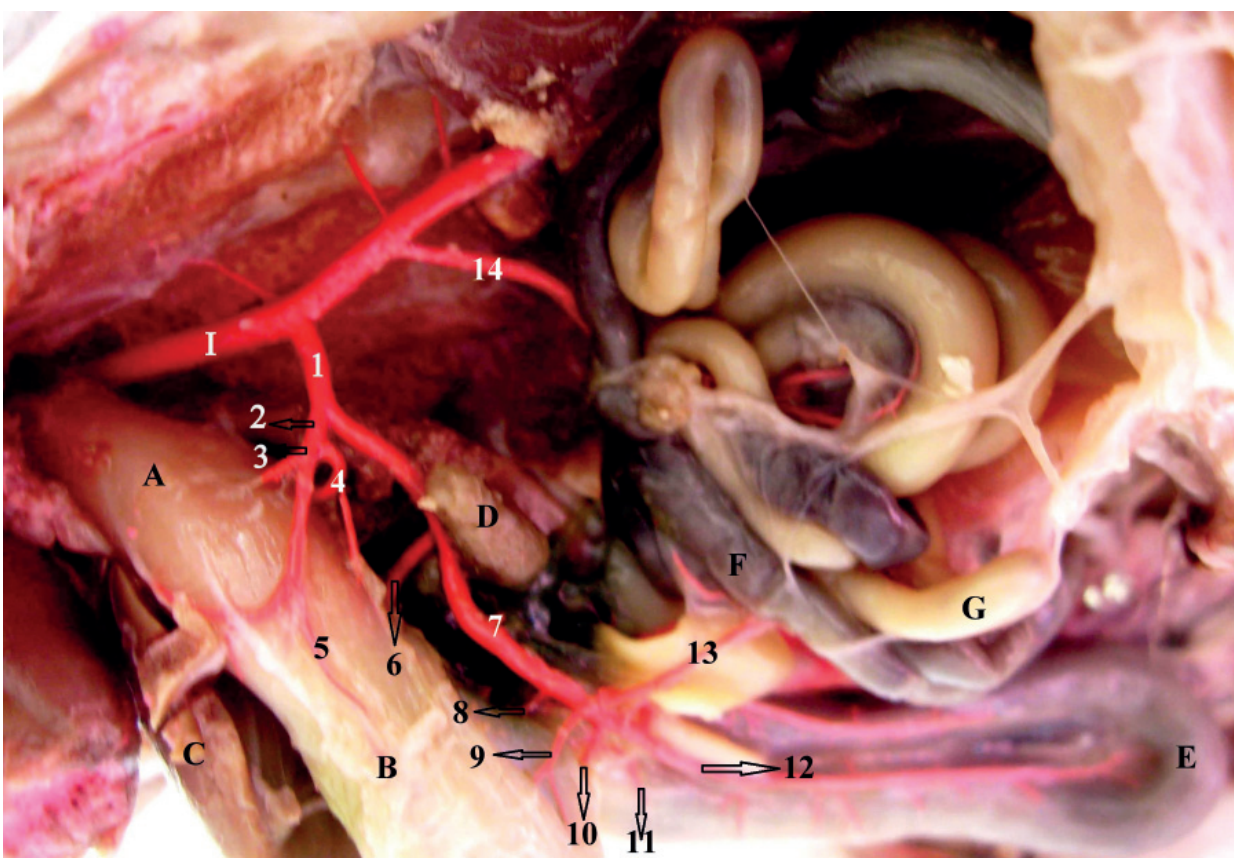

Fig. 1. Lateral view of the celiac artery. I: Aorta, 1: Celiac artery, 2: Common trunk of the esophageal artery and superior proventricular artery, 3: Esophageal artery, 4: Superior proventricular artery, 5: Superior gastric artery, 6: Left branch of the celiac artery, 7: Right branch of the celiac artery, 8: Right superior gastric artery, 9: Right inferior gastric artery, 10: Gastroduodenal artery, 11: Pancreaticoduodenal artery, 12: Duodenojejunal artery, 13: Ileocecal artery, 14: Cranial mesenteric artery, A: Esophageus, B: Proventriculus, C: Lobus hepatis sinister, D: Lobus hepatis dexter, E: Duodenum, F: Ileum, G: Cecum.

proventricular artery and the esophageal artery. Immediately after its origin, the oesophageal artery was terminated by giving off the branches, namely, rami oesophagi, which nourish the oesophagus, extending in craniodorsal direction (Fig. 1.3 and 3c).

The superior proventricular artery was found to distribute to the dorsal surface of the proventriculus as 3 to 4 branches (Fig. 1.4; 1 A–C and 3d). The superior gastric artery emanated from the superior proventricular artery and was observed to provide the vascularisation of the left and dorsal surface of the ventriculus (Fig. 1.5).

The splenic artery was the last branch given by the celiac artery before splitting into the right branch of the celiac artery and the left branch of the celiac artery. These vessels were determined to vary from 3 to 4 in number and to nourish the medial and distal part of the lien (Fig. 2.4).

The left branch of the celiac artery, one of the last two branches of the celiac artery, was determined to extend cranioventrally in the proximity of isthmus gaster. After it gave off the right hepatic artery to nourish the lobus hepatis dexter of the hepar, it passed to the left side of the hepar. In this part, this blood vessel was found to terminate splitting into the inferior proventricular artery, the left inferior gastric artery and the left gastric artery after giving off the left hepatic artery to the lobus hepatis sinister of the liver with 3 to 4 branches (Fig. 1.6 and 3f).

The first branch given off the left branch of the celiac artery prior to reaching the portae hepatis, the right hepatic artery, was established to terminate distributing to the lobus hepatis dexter of the hepar. In addition, the right hepatic artery was determined to give off the artery of the gall bladder which provides the arterial nourishment of the vesicae fellae before terminating (Fig. 2.7).

Arising from the left branch of the celiac artery at the cranial region of the vesicae fellae, the left hepatic artery was observed to provide the vascularisation of the lobus hepatis sinister of the hepar (Fig. 2.9–9a–b). In addition, the left hepatic artery was determined to be thicker than right hepatic artery in all materials.

Having its origin at the left branch of the celiac artery at the caudal of the proventriculus as 2 to 3 branches, the inferior proventricular artery was observed to nourish the ventral region of the proventriculus.

Arising from the left branch of the celiac artery at the cranial region of the ventriculus as 2 to 3 branches, the left inferior gastric artery was observed to nourish of the ventral region of the ventriculus (Fig. 1.9).

The left gastric artery was determined to be the prolongation of the left branch of the celiac artery and terminating to distribute at the left surface of the ventriculus.

After stemming from the celiac artery, the right branch of the celiac artery was determined to run

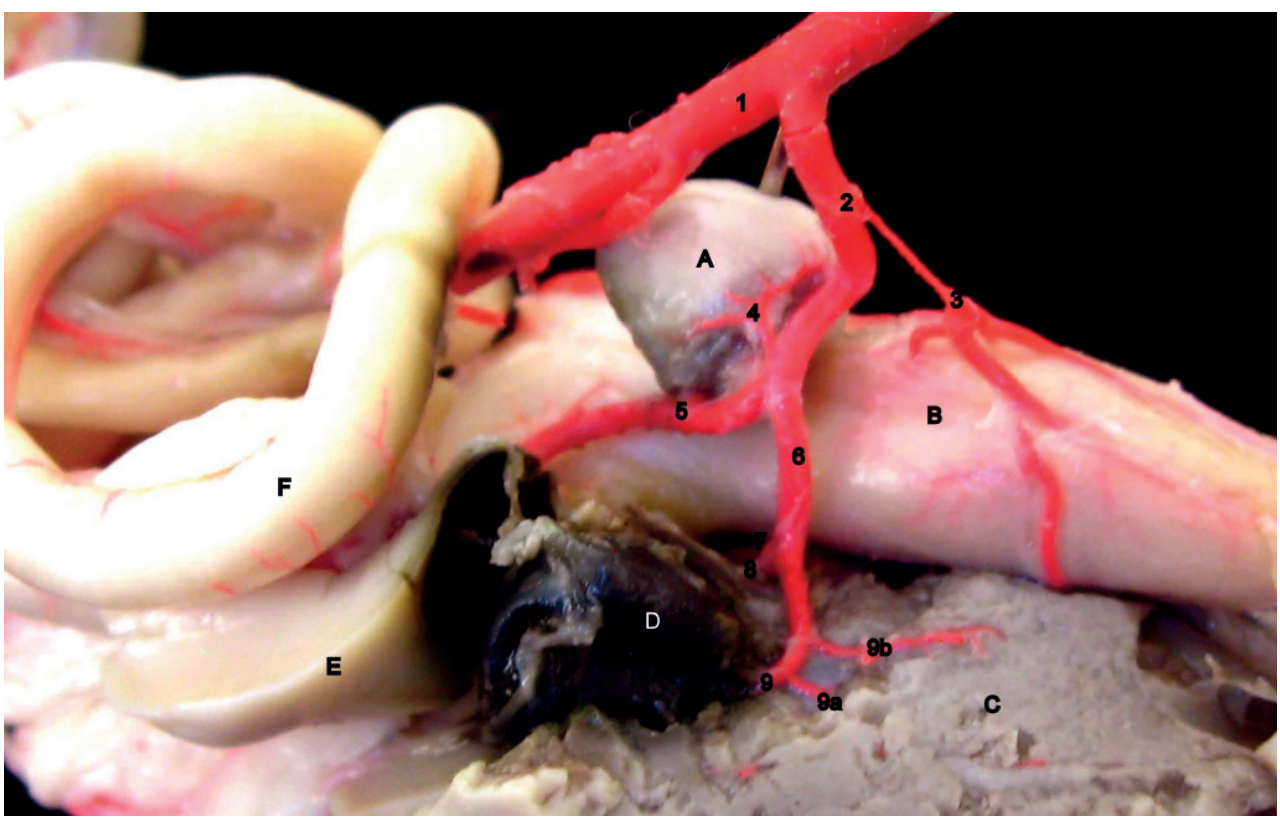

Fig. 2. Dorsal view of the celiac artery. 1: Aorta, 2: Celiac artery, 3: Common trunk of esophageal artery and superior proventricular artery, 4: Splenic artery, 5: Right branch of celiac artery, 6: Left branch of celiac artery, 7: Right hepatic artery, 8: Artery of gall bladder, 9-9a-b: Left hepatic artery, A: Lien, B: Proventriculus, C: Hepar, D: Vesicae fellae, E: Pancreas, F: Jejunum.

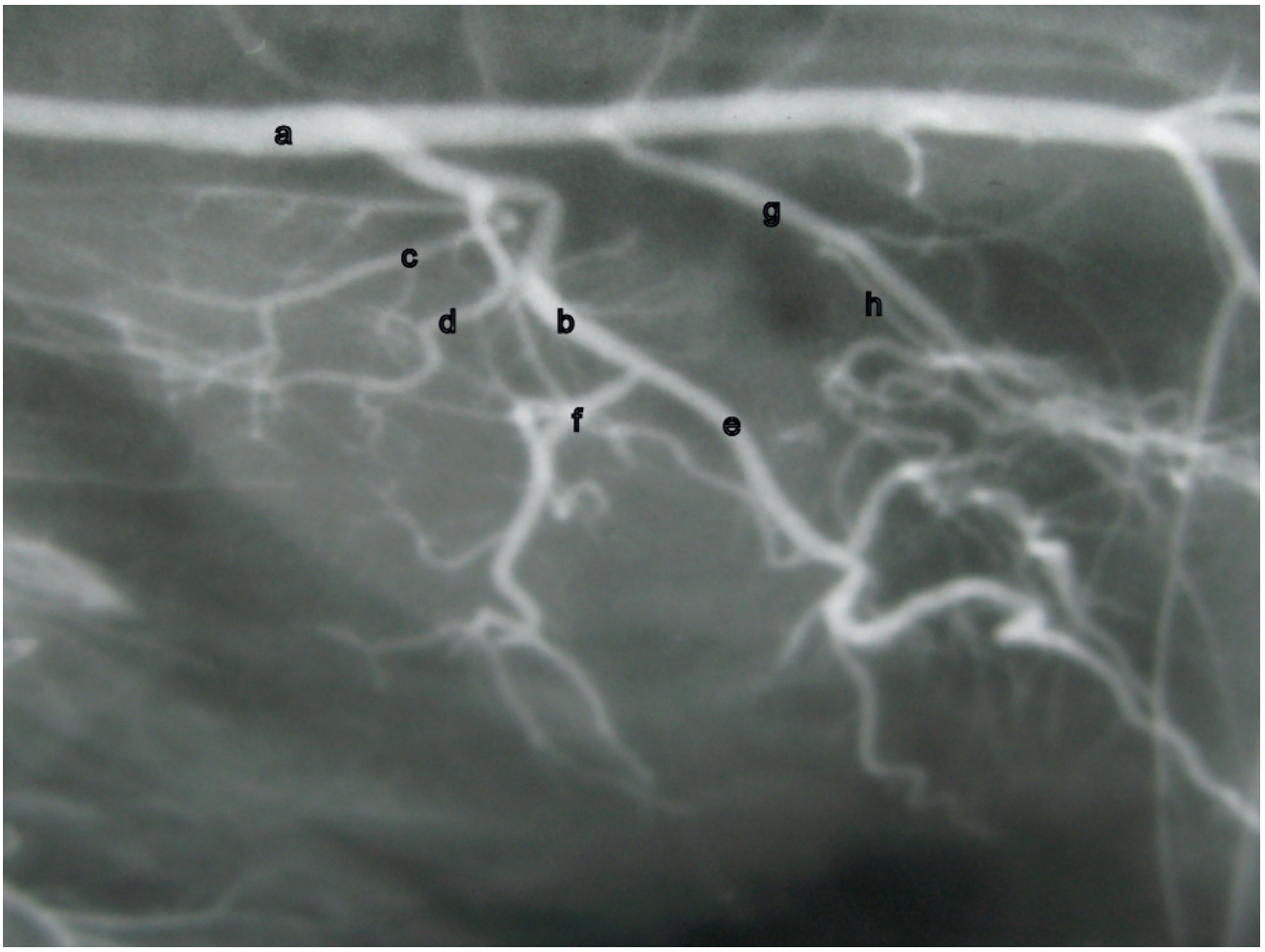

Fig. 3. Radiograph of the celiac artery. a: Aorta, b: Celiac artery, c: Esophageal artery, d: Superior proventricular artery, e: Right branch of celiac artery, f: Left branch of celiac artery, g: Cranial mesenteric artery, h: Ileocecal artery.

along under the lien in a caudoventral direction. This blood vessel was observed to lie on the junction of the gaster with the duodenum. It was determined to give off the right superior gastric artery before reaching the indicated junction. At the level of the indicated junction, the right branch of the celiac artery was determined to split into its terminal branches which extended in different directions, namely the ileocecal artery, the gastroduodenal artery, the pancreaticoduodenal artery, the duodenojejunal artery and the right inferior gastric artery (Fig. 1.7 and 3e).

Having its origin at the caudal region of the gaster, the right superior gastric artery was determined to nourish the right surface of the ventriculus, running along in the dorsocaudal direction (Fig. 1.5).

The ileocecal artery was observed to spring from the right branch of the celiac artery at the craniodorsal of the right surface of the ventriculus and gave off branch only for ileum (Fig. 1.13).

The gastroduodenal artery was detected to separate from the right branch of the celiac artery at the caudal region of the ventriculus as one branch. This vessel was terminated to give off 2 to 3 branches which nourish the pars descendens region of the duodenum (Fig. 1.10).

One of the last branches separating from the right branch of the celiac artery, the pancreaticoduodenal artery, was established to bifurcate into 2 branches at the beginning part of the duodenum by extending to the caudal direction. The first branch was determined to terminate distributing to the duodenal fold and pars descendens duodeni and the second to the pars ascendens duodeni. The two bifurcated branches were determined to anastomose with each other at the ansa duodenalis. One small vessel ramifying from the second branch was determined to nourish the lobus pancreaticus dorsalis et ventralis of the pancreas (Fig. 1.11).

Originating from the right branch of the celiac artery at the level of the junction of the duodenum and ventriculus, the duodenojejunal artery was determined to give off 3 to 5 branches to the duodenum along its course to the jejunum (Fig. 1.12).

The right inferior gastric artery was separated from the right branch of the celiac artery. This blood vessel was observed to distribute to the ventral surface of the gaster as 2 to 4 branches extending to the ventral direction (Fig. 1.9).

Discussion

The results of this study showed that the celiac artery was established to have its origin from the aorta at the beginning of synsacrum at the junction where the proventriculus joins the oesophagus. This result is similar in falcons, buzzards, chickens, roosters, ducks, pigeons, eagle owls, white turkeys, red falcons, domestic geese, cattle egrets and hooded crows (Malinovsky, 1965; McLeod et al., 1965; Malinovsky et al., 1973; Malinovsky et al., 1975; Kuru, 1996; Aycan and Duzler, 2000; Dursun, 2002; Kurtul and Haziroglu, 2002; Kurtul, 2002; Arı et al., 2010; Halıgur and Duzler, 2010; Ragap et al., 2013; Khalifa, 2014; Hassan and El-Sayed, 2018).

In various reports, the celiac artery was reported to split into the right branch of the celiac artery and the left branch of the celiac artery. Similarly, in 7 sparrow hawks examined in the present study, the celiac artery was also determined to split into right branch of the celiac artery and left branch of the celiac artery (Getty, 1975; Malinovsky and Novotna, 1977; Schummer and Seifirle, 1977; Baumel et al., 1993; Nickel et al., 2014; Alan et al., 2016; Hassan and El-Sayed, 2018). Contrary to the above reports, Aycan and Duzler (2000) and Chiasson (1964) reported that this vessel was not split into the above-mentioned branches.

In 7 sparrow hawks examined, the superior gastric artery was found to spring from the superior proventricular artery. This is in agreement with the findings of previously conducted studies that have reported the superior gastric artery to originate from the superior proventricular artery (Getty, 1975; Baumel et al., 1993; Dursun, 2002; Kuru, 2010; Rezk and El-Bably, 2014). In contrast to this, in the literature, Aycan and Duzler (2000), Ari et al. (2010), and Halıgur and Duzler (2010) have indicated that the superior gastric artery was determined to spring from the celiac artery.

Kuru (1996), Aycan and Duzler (2000), Arı et al. (2010), Nickel et al. (1977) and Rezk and El-Bably (2014) have reported the oesophageal artery to be the first branch originating from the celiac artery. Kurtul and Haziroglu (2002) in roosters, ducks, and pigeons and Hassan and El-Sayed (2018) in hooded crows have reported that the first branch of the celiac artery was the dorsal proventricular artery. Alan et al. (2016) have observed that the oesophageal artery stemmed from the superior proventricular artery. However, Halıgur and Duzler (2010) and Ragap et al. (2013) have determined the oesophageal artery to have its origin from the celiac artery as a common root with the superior proventricular artery. Similarly, in sparrow hawks examined in the present study, the oesophageal artery originated from the celiac artery as a common root with the superior proventricular artery.

Chiasson (1964), Kuru (1996), Aslan and Takçı (1998), Aycan and Duzler (2000), and Rezk and El-Bably (2014) have reported that the splenic artery stemmed from the right branch of the celiac artery. In contrast to these studies, Pinto et al. (1998), Cardoso et al. (2000), and Dursun (2002) have reported that the splenic artery stemmed from the left branch of the celiac artery. Contrary to the above reports, Malinovsky et al. (1973), Baumel et al. (1993), Kuru (1996), Ari et al. (2010) and Khalifa (2014) have reported that the splenic artery originated from the celiac artery. In the present study, the splenic artery was determined to spring from the celiac artery and not to the right branch or left branch of the celiac artery.

Alan et al. (2016) have determined that right hepatic artery arose from the right branch of the celiac artery in 2 flamingos, in other 2 birds it stemmed from the left branch of the celiac artery, and in the fifth flamingo it was observed that 2 separate right hepatic arteries originated as one vessel from the right and left branch of the celiac artery. Ari et al. (2010) have reported the right hepatic artery to spring from the right branch of the celiac artery and the left hepatic artery from the left branch of the celiac artery. In contrast to this study, in the present study, which was carried out in the sparrow hawks, the right hepatic artery was determined to spring from the left branch of the celiac artery, which is in agreement with a report of Halıgur and Duzler (2010).

Reports exist that the left gastric artery arises from the left branch of the celiac artery (Baumel et al., 1993; Dursun, 2002; Halıgur and Duzler, 2010; Khalifa, 2014). Similarly, in the present study, the left gastric artery was also determined to arise from the left branch of the celiac artery stem from truncus celiacus.

In the sparrow hawks examined, contrary to the above reports, Nishida et al. (1969) describes that the left gastric artery originating from the artery of the gall bladder was detected to spring from the right hepatic artery. This is in agreement with the findings of previously conducted studies (Malinovsky, 1965; Malinovsky 1973; Malinovsky and Visnanska, 1975; Malinovsky, 1977; Baumel et al., 1993; Aslan and Takçı, 1998; Aycan and Duzler, 2000; Dursun, 2002; Kurtul, 2002; Arı, 2010; Kuru, 2010; Alan et al., 2016). In contrast to this, Halıgur and Duzler (2010) have indicated that the artery of the gall bladder was determined to originate from the left branch of the celiac artery.

In conclusion, in the present study, origin and branches of the celiac artery, which were specific to the sparrow hawk, were determined and compared those reported in the literature available for other avian species.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1. Alan A., Duzler A., Orhan I. Ramification of the celiac artery in the greater flamingo (*Phoenicopterus roseus*). Veterinární Medicina. 2016. T. 61(2). P. 97-101.
2. Arı HH., Karadağ H., Çınaroğlu S., İstanbullugil FT., Sefergil S. Beyaz hindide a. celiaca ve dallarının makroanatomik ve subgros incelenmesi. Van Veterinary Journal. 2010. T. 21(3). P. 147-152.
3. Aslan K., Takçı I. The arterial vascularisation of the organs (stomach, intestinum, spleen, kidneys, testes and ovarium) in the abdominal region of the geese obtained from Kars surrounding. Journal of the faculty of Veterınary Medıcıne, Kafkas Unıversıty. 1988. T. 4(1-2). P. 49–53.
4. Aycan K., Duzler A. The anatomy of celiac artery in the eagle owl (*Bubo bubo*). Veterinary Journal of Ankara University. 2000. T. 47. P. 319–323.
5. Baumel J.J., King S.A., Breasile J.E., Evans H.E., Berge J.C.V. Handbook of avian anatomy (nomina anatomica avium). Cambridge, Massachusetts: Publications of the Nuttal Ornithological Club, 1993. P. 407-436.
6. Belge A., Bakır B. Veteriner anesteziyoloji ve reanimasyon. Van Veterinary Journal. 1999. T. 2.
7. Cardoso JR., Drummond S.S., Martins A.K., Carnerio e Silva FO., Severino K.S., Mota FCD. Anatomical aspects of the coeliac artery in gallus gallus of the Peterson. Arquivos deCiências Veterinárias e Zoologia da Unipar. 2000. T. 3(2). P. 151-157.
8. Chiasson BR. Laboratory anatomy of the pigeon. Dubuque, Iowa, W.M.C. Brown Company Publishers, 1964. P. 34-41.
9. Doğuer S., Erençin Z. Evcil kuşların komparatif anatomisi. Ankara, Ankara University Press, 1964. P. 68-77.
10. Dursun N. Anatomy of domestic birds. Ankara, Medisan Publishing, 2002. P. 140-141.
11. Flecknell PA. Laboratory animal anaesthesia. an introduction for research workers and technicians. London, Academic Press, 1987.
12. Gadhoke JS., Lindsay R.T., Desmand RK. Comparative study of the major arteriel branches of the descending aorta and their supply to the abdominal viscera in the domestic Turkey (*Meleagris gallopova*). Anatomischer Anzeiger. 1975. T. 138(5). P. 438-443.
13. Getty R. Sisson and grossman's the anatomy of the domestic animals. 5th ed, Philadelphia, W.B. Saunders Company, 1975. P. 1990-1991.
14. Greenberg R., Marra P.P. Birds of two worlds: the ecology and evolution of migration. Johns Hopkins University Press, 2004.
15. Halıgur A., Duzler A. Course and branch of the celiac artery in the red falcon (*Buteo rufinus*). Veterinarni Medicina. 2010. T. 55(2). P. 79-86.
16. Hassa O. Koroner damarların plastic demostrasyonu için pratik enjeksiyon metodu. Veterinary Journal of Ankara Univercity. 1967. T.14. P. 444-453.
17. Hassan S., El-Sayed AK. Gross anatomy of the celiac, cranial mesenteric and caudal mesenteric arteries in Hooded Crow (*Corvus cornix*). Journal of Veterinary Anatomy. 2018. T. 11(2). P. 41-55.
18. Khalifa E.F. Gross anatomical studies on the celiac artery in Cattle Egret (*Bubulcus ibis*) with special reference to the arterial supply of the stomach. Journal of Veterinary Anatomy. 2014. T. 7(1). P. 1-13.
19. Kurtul I. Comparative macroanatomical investigations on the pattern and branches of the aorta descendens among the rooster, drake, and pigeon (in Turkish). Faculty of Veterinary Medicine, Ankara University, 2002. P. 24–37.
20. Kurtul I., Haziroglu R.M. Comparative macroanatomical investigations on the pattern and branches of the descending aorta among the rooster, drake, and pigeon. Veterinary Journal of Ankara Univercity. 2002. T. 51. P. 1-6.
21. Kuru N. Macroanatomical investigation of course and branching of aorta in domestic chick and new zealand rabbit (in Turkish). (PhD thesis). Faculty of Biology, Selcuk University, 1996. P. 30–37.
22. Kuru N. Macroanatomic investigations on the course and distribution of the celiac artery in domestic fowl (*Gallus gallus domesticus*). Scientific Research and Essays. 2010. T. 5(23). P. 3585-3591.
23. Malinovsky L. Blood supply to stomachs and adjacent organs in Buzzard. Folia Morphologica (Praha). 1965. T. 13. P. 191-201.
24. Malinovsky L., Novotna M. Branching of the celiac artery in some domestic birds, III. A comparison of the pattern of the celiac artery in three breeds of the domestic fowl. Anatomischer Anzeiger. 1977. T. 141(2). P. 137-146.
25. Malinovsky L., Visnanska M. Branching of the celiac artery in some domestic birds, II. The domestic goose. Folia Morphologica. 1975. T. 23(2). P. 128-135.
26. Malinovsky L., Visnanska M., Roubal P. Branching of the celiac artery in some domestic birds. I. Domestic Duck. Scripta-Medica. 1973. T. 46. P. 325-336.
27. McLeod W.M., Trotter D.M., Lumb J.M. Avian anatomy. Minneapolis, Burgess Publishing Company, 1964. P. 133-134.
28. Nickel R., Schummer A., Seifirle E. Anatomy of the domestic birds. Berlin, Verlag Paul Parey, 1977. P. 87-102.
29. Nishida T.T., Paik Y.Y., Yasuda M.M. Comparative and topographical anatomy of the fowl. 58. Blood vascular supply of the glandular stomach (ventriculus glandularis) and the muscular stomach (ventriculus muscularis). Nihon Yakugaku Zasshi. 1969. T. 31(2). P. 51-70.
30. Pinto M.R.A., Riberiro A.A.C.M. Souza, W.M. The arrangements configured by the cranial and caudal mesenteric arteries in the domestic duck (*Cairinamoshata*). Brazilian Journal of Veterinary Research Animal Science. 1998. T. 35. P. 107-109.
31. Ragap S.A., Farag F.M.M., Tolba A.R., Saleh A.A., El-Karmoty A.F. Anatomical study of the celiac artery in the domestic goose (*Anser anser domesticus*) with special reference to the arterial supply of the stomach. Journal of Veterinary Anatomy. 2013. T. 6(2). P. 23-40.
32. Rezk H.M., El-Bably S.H. Gross anatomical studies on the celiac artery in the domestic fowl (*Gallus gallus domesticus*). Journal of Veterinary Anatomy. 2014. T. 7(1). P. 127-141.
33. Sustaita D. Musculoskeletal underpinnings to differences in killing behavior betweenNorth American accipiters (*Falconiformes: Accipitridae*) and falcons (*Falconidae*). Journal of Morphology. 2008. T. 269. P. 283-301.

Received 9 September 2021
Accepted 25 November 2021

The Experimental Infection of Vaccine-like Isolates of Infectious Laryngotracheitis Virus Isolated in Ukraine in 2010–2012

Andriy Veretsun[1], Borys Stegniy[1], Larysa Usova[1], Oleksandr Rula[1], Denys Muzyka[1]
[1]Department of Avian Diseases, National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Ukraine

Keywords: *infectious laryngotracheitis virus, vaccine-like isolates, biological properties, experimental infection, pathological changes.*

Abstract. *Infectious laryngotracheitis (ILT) is an important respiratory infection of chickens that can pose a serious threat to poultry. Despite the fact that the infection has been known for a long time and a large number of vaccines have been developed for specific prevention, the disease still occurs quite often worldwide. This is due to the pathogen characteristics and its ability to mutate, resulting in new isolates. Despite the extensive ILT vaccination program used in commercial poultry farms, the circulation of 2 types of virus isolates is registered among poultry. The first type is the field isolates of the ILT virus (ITLV), which are significantly different from the vaccine strains of the ILTV. The second type is vaccine-like isolates, which have differences compared with vaccine strains and can cause clinical disease in birds.*

The paper presents the data on the study of the biological properties of 2 ILTV isolates: A 4-12 and B 2-10. These isolates were isolated from sick hens from commercial and backyard poultry of Ukraine in 2010–2012. Both isolates were identified as vaccine-like viruses by PCR. All isolates were pathogenic for chicken embryos (CE) and caused death and typical postmortem changes. During the experimental infection of susceptible chickens (at age of 60 days) at the laboratory conditions, it was found that the isolates caused typical clinical signs and pathological macroscopic and microscopic changes in internal organs. ILTV isolate B 2-10 caused the death of 80% of the experimental chicken, and isolate A 4-12 caused only clinical manifestations of the disease in chickens, but not death of chickens.

Postmortem studies showed that vaccine-like isolates cause a threat to cells and changes in the tissue of the internal organs (trachea, lungs, spleen, intestines) of infected chickens, which are typical for other ILTV strains. At the same time, the reaction of the immune system of chickens against the virus was observed at the cell level.

Introduction

Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory tract disease of chickens and hens caused by a Gallid herpesvirus 1 (GaHV-1) belonging to the genus *Iltovirus* and subfamily *Alphaherpesvirinae* within *Herpesviridae* family. The disease is characterized by sinusitis, conjunctivitis, oculo-nasal discharge, respiratory distress, bloody mucus, swollen orbital sinuses, high morbidity, considerable mortality and decreased egg production. Co-infections with other respiratory pathogens and environmental factors adversely affect the respiratory system and prolong the course of the disease. Latently infected chickens are the primary source of ILT virus (ILTV) outbreaks irrespective of vaccination (Gowthaman et al., 2020).

The disease is reported in most countries with developed industry poultry farming, but outbreaks occur mostly in small-scale poultry farms (Bagust et al., 2000). Chickens are considered like the primary host of the virus (Bagust, 1986), but natural disease has been reported in peafowls and pheasants (Crawshaw et al., 1982). ILT causes production losses due to increased morbidity, moderate mortality, decreased weight gain, reduced egg production and expenses spent on vaccination, biosecurity measures and therapy to counteract secondary infection by other avian pathogens (Jones, 2010; Saif et al., 2008).

Ukrainian poultry industry is an important sector of the national economy. There are two segments of the poultry farming: developed industrial poultry farming as well as significant backyard poultry farming. Therefore, ILT as a respiratory disease can pose a threat to the poultry industry. Considering the circulation of the ILTV in Ukraine, the significant economic losses from the disease and the need to choose the right vaccination strategy, the constant monitoring of the ILT, isolation and study of new isolates of the virus are important to ensure the epizootic well-being of the poultry industry. The National Scientific Center Institute of Experimental and Clinical Veterinary Medicine (Kharkiv) has been a center for scientific research on poultry infectious diseases, including infectious laryngotracheitis, for almost 100 years. Scientists constantly conduct

Correspondence to Denys Muzyka Department of Avian Diseases, National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, 83 Pushkinska st., Kharkiv, 61023, Ukraine. E-mail: dmuzyka77@gmail.com

epizootological monitoring of ILTV circulation among industrial and backyard poultry farms in different regions of Ukraine, virus isolation and in-depth studies of their biological properties, as well as their impact on poultry.

Over the last 10 years, several cases of ILT have been recorded in Ukraine: in the Kharkiv region in 2010, 2011, and 2012; in the Luhansk region in 2010; in the Autonomous Republic of Crimea in 2012 (Воротилова, 2014); in Donetsk region in 2012 (Музика et al., 2021; Veretsun et al., 2021); and in the Sumy region in 2019 (Veretsun et al., 2021). In addition, due to the results of serological monitoring, 7.1%–42.9% of unvaccinated chickens in industrial poultry farms reveal specific antibodies to the ILTV (in press). Thus, the ILTV is an important pathogen for poultry in Ukraine. It is also important to estimate the pathotype of the isolates which are circulating in the country. Particular attention is paid to the study of the features of vaccine-like ILTV isolates, their ability to cause clinical disease and postmortem changes. The aim of the research was to study the biological properties (infectious activity, ability to cause death of chicken embryos, ability to cause clinical disease in experimental infection) and the postmortem changes in the tissues of experimentally infected chickens of 2 vaccine-like isolates of the ILTV isolated in 2010–2012.

Materials and Methods

Sampling. Samples (trachea, lungs, and spleen) were obtained from sick and dead hens in 2 poultry farms, where the disease cases were registered during 2010–2012.

Farm 1 is located in Kharkiv region. The flock was vaccinated against ILT. Birds were at the age of 180 days. Clinical signs were general oppression, feed refusal, cough. Postmortem changes were tracheitis, conjunctivitis, sinusitis, and catarrhal rhinitis.

Farm 2 is located in Donetsk region. The flock was not vaccinated against ILT. Birds were at the age of 138 days. Clinical signs were a sudden death of birds without significant visible clinical signs. Postmortem changes were hyperemia of the mucous membrane of the lower eyelid, tracheitis, blood shears in the lumen of trachea, pneumonia, aerosacculitus, pericarditis, hydropericarditis, hemorrhages on the heart, enlarged spleen and kidneys, and catarrhal-hemorrhagic enteritis.

Virological studies. The studies were conducted at the Department of Poultry Diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". Virological studies were performed by conventional methods (inoculation of chicken embryos, determination of infectious and lethal titers) (A laboratory manual, 2008). For this purpose, a 10%–20% suspension of pathological material on phosphate buffer (pH 7.2–7.4) was prepared. The suspension was centrifuged at 3000 rpm for 30 min, and then a mixture of antibiotics was added and kept for 30 min at room temperature. The 10–12-day-old CE were infected (inoculation dose was 0.2 mL) on the chorio-allantoic membrane. Infected embryos were incubated for 7 days (A laboratory manual, 2008; OIE, 2008).

Statistical analysis. The definition and evaluation of biological activity (infectious and lethal titer) was carried out according to the common method on chicken embryos by titration. Infectious titer was calculated by the method of Reed and Muench (A laboratory manual, 2008).

Experimental infections. Two experiments on sensitive chickens were conducted. All studies were conducted from observing all necessary requirements of biosafety and biosecurity in conditions that exclude the release of the pathogen. Experimental infection was carried out taking into account the principles of bioethics (the scheme of the experiment was considered and approved at the meeting of the Bioethics Commission of the NSC IECVM).

In the experiments, 60-day-old specific pathogen-free non-vaccinated chickens were used. The absence of specific antibodies to ILT virus was confirmed by ELISA (Infectious Laryngotracheitis Antibody test kit, BioChek, UK)(OIE, 2008).

The first experiment was to determine the pathogenicity of the isolate B 2-10. Experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (spleen, trachea, and lungs), which contained isolate B 2-10 (dose 0.5 mL per bird). The infected birds were monitored for 15 days. In the first experiment, 15 chicks were used.

The second experiment was to determine the pathogenicity of the isolate A 04-12. Experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (spleen, trachea, and lungs), which contained isolate A 04-12, using a dose of 0.5 mL per bird (group 1), and extraembryonic fluid of infected CE passage I, using a dose of 0.5 mL (2.15 lg EID_{50}) per bird (group 2). The infected birds were monitored for 15 days. In the second experiment, 2 chickens in each group were used.

Molecular genetic studies. Nucleic acid extraction was performed using the AmpliSens® DNA-sorb-B kit (Russian Federation). Detection of the ILTV DNA was conducted using conventional PCR according to the typical PCR protocol (Kirkpatrick et al., 2006). In brief, a pair of forward (5'-CTGGGC-TAA-ATC-ATC-CAA-GAC-ATC-A-3') and reverse (5'-GCT-CTC-TCG-AGT-AAGAAT-GAG-TAC-A-3') primers was used for the amplification of 2.24 kbp region of the ILTV thymidine kinase gene following the amplicons separation by electrophoresis through 0.8% agarose gels, stained with an appropriate nucleic acid stain and exposed to UV light for visualisation.

Field type or vaccine isolates of the ILTV were determined in a commercial laboratory Royal GD (Deventer, The Netherlends) by PCR in a combination of the restriction analysis method in accordance with the OIE recommendations (OIE, 2008) and local SOPs.

Histopathological studies. Internal organs of chickens were routinely processed, embedded in paraffin wax, and histological sections were stained by hematoxylin-eosin. Organ samples were washed under running water for 12 hours to remove the formalin solution. Dehydration of organ samples was carried out in alcohols of increasing concentration: 60, 70, 80, 90, 96 for 12 hours. Further organ slices were treated with a solution of alcohol-chloroform for 30 min, chloroform for 1 hour, chloroform-paraffin for 1 hour and alternately moved into paraffin 1, paraffin 2, and paraffin 3 for 60 min. The sections were prepared using rotary microtome, 3–5 microns thick. Sections were stained with hematoxylin-eosin according to the accepted procedure (Luna, 1968).

Results

Isolate B 2-10 was isolated from pathological material of affected and dead hens with clinical signs typical for ILT from farm 1 (Kharkiv region). Hens from this farm were routinely vaccinated against ILT. Isolate A 04-12 was isolated from pathological material of infected chickens without clinical signs, but with pathological changes of respiratory organs from farm 2 (Donetsk region), where vaccination against ILT was not carried out.

As a result of molecular biological studies (PCR), the samples contained the genetic material of the ILTV. Restriction analysis and PCR studies conducted in Royal GD showed that both isolates (B 2-10 and A 04-12) belonged to a vaccine-like ILTV.

Also, we tested the biological activity (infection and lethal titer) of the isolates B 2-10 and A 04-12. It was found that the infection titer of ILTV isolate B 2-10 was 6.5 lg EID_{50}/1 mL, and that of isolate A 04-12 was 5.3 lg EID_{50}/1 mL.

The next stage of our research was to study the pathogenicity of these isolates in the laboratory conditions. In order to study the pathogenicity of the isolates, infection of 60-day-old chickens, free from antibodies to ILT virus, was performed. The infected birds were monitored for 15 days. The results of experimental inoculation of chickens with isolate B 2-10 (number of chickens with clinical signs and the number of fatalities) are shown in Table 1.

The results in Table 1 show that the clinical signs of the disease (general depression, diarrhea, respiratory noises, labored breathing) were observed from day 10. The incidence was 100% of the experimentally infected population. Specific deaths of chicken were observed from day 13 (for isolate B 2-10). All infected chickens died at the end of the experiment as a result of ChP 96-10 isolate. Dead bird autopsy showed pathological changes typical for ILT.

For the second isolate A 04-12, experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (group 1) and intratracheal application of extraembryonic fluid from infected CE of passage I (group 2). The number of affected and dead chickens was recorded in Table 2.

Obtained results showed that in both groups

Table 1. Dynamics of the clinical signs and death of the experimentally infected chickens with ILTV isolate B 2-10 (application of 10% suspension of native pathological material).

Clinical state of poultry	Day after virus inoculation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Amount of chickens														
Healthy	15	15	15	15	15	15	15	15	15	–	–	–	–	–	–
Affected	–	–	–	–	–	–	–	–	–	15	15	15	9	3	–
Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	6	6	3

Table 2. Dynamics of clinical signs and death of chickens experimentally infected with ILTV isolate A 04-12.

Clinical state of poultry		Day after virus inoculation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Amount of chickens														
Group 1	Healthy	2	2	2	2	2	2	2	–	–	–	–	–	–	–	–
	Affected	–	–	–	–	–	–	–	2	2	2	2	2	2	2	2
	Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Group 2	Healthy	2	2	2	2	2	2	2	–	–	–	–	–	–	–	–
	Affected	–	–	–	–	–	–	–	2	2	2	2	2	2	2	2
	Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

after virus inoculation the clinical signs of the disease (general depression, diarrhea, respiratory noises, labored breathing) appeared from day 8 of the experiment. No dead chickens were registered throughout the observation period.

On day 15, a forced slaughter of the experimental poultry was conducted. At autopsy, pathological changes characteristic of the ILTV were revealed (catarrhal tracheitis, hyperplasia and hyperemia of the spleen).

For the estimation of tissue changes in the internal organs of experimentally infected chickens with a vaccine-like isolate of the ILTV, postmortem studies were performed. For this purpose, internal organs (trachea, lungs, spleen, intestinal) were obtained from infected chickens with A 04-12 isolate.

As a result of histological examination of the trachea, thickening of the own lamina of the mucous membrane due to infiltration by small and medium lymphocytes, macrophages, and eosinophiles, was determined (Fig. 1). Tracheal epithelial cells have signs of mucoid swelling. The submucosal base of the trachea has signs of edema.

The number of goblet cells was reduced. In some areas of the mucous membrane, there was a desquamation of epithelial cells with exposure of the submucosal base (Fig. 3). There was a small amount of exudate in the lumen.

In chickens of group 2, the changes were less pronounced, but the mucosa was also infiltrated with lymphoid cells, the glands had a larger number of goblet cells, and the capillaries were enlarged. Epithelial cells of the trachea have signs of mucoid swelling, and the lumen contains a small amount of exudate.

In the study of the lungs, it was found that the alveoli wall was formed by a single-layer flat epithelium, a thin layer of connective tissue and blood vessels, mainly capillaries, and lined endothelium, which formed a plexus around each alveolus. Most of the surface of the alveoli was lined by highly flat epithelial cells – respiratory epitheliocytes. Alveolar macrophagocytes were found in the walls of the alveoli. The walls of the bronchioles were lined with a single-layer prismatic ciliated epithelium. The lamina propria was formed by a thin layer of the loose fibrous connective tissue. The muscle plate consisted of smooth muscle cells. In the study of lungs of chickens of group 1, the thickening of the wall of the bronchioles was established (Fig. 4). Lymphoid cells accumulated around them. Capillaries were enlarged, overflowing with blood cells. Actually, the mucous membrane was infiltrated by lymphoid cells. In group 2 chickens, histomorphological changes in the lungs were less pronounced. Infiltration of lymphoid cells, pseudo-eosinophils, and macrophages around the blood vessels and in the lamina propria of some bronchioles was observed (Fig. 5).

As a result of histomorphological examination, it was established that the spleen was surrounded by a connective tissue capsule with elastic fibers and smooth muscle cells. The connective tissue stroma of the organ was poorly developed. In the course of large vessels, there was a small amount of connective tissue. The basis of the parenchyma of the spleen was

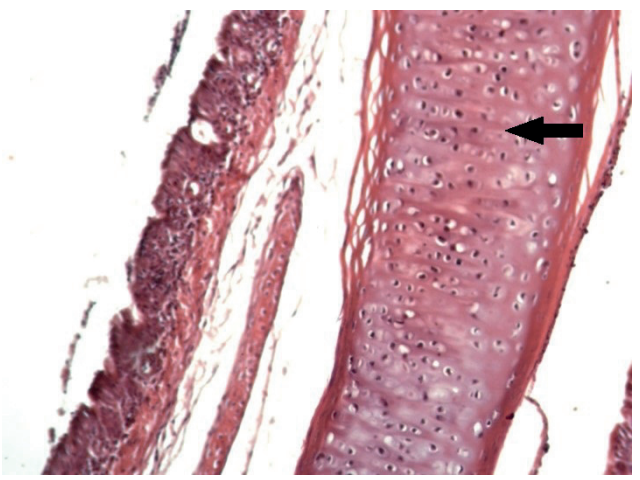

Fig. 1. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). The lamina of the mucous membrane infiltrated with lymphoid-histiocytic cells. × 100, H+E

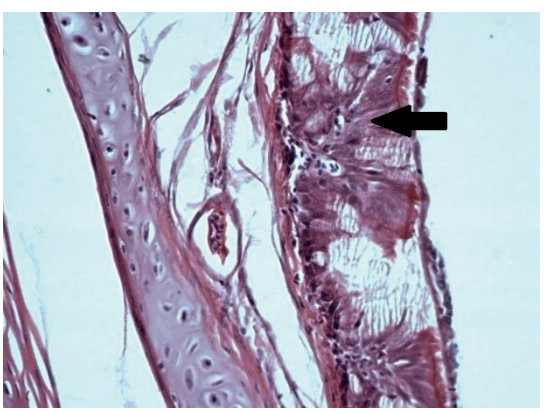

Fig. 2. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). Epithelial cells with signs of mucoid swelling. × 200, H+E

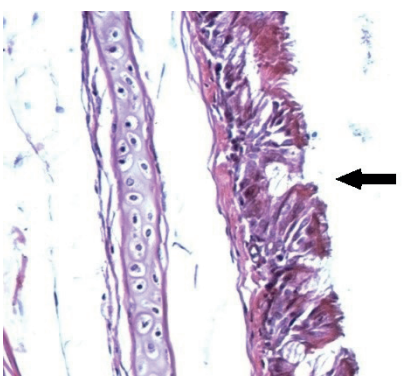

Fig. 3. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). Desquamation of epithelial cells. × 100, H+E

the reticular tissue. Numerous reticulo-endothelial clutches were seen in the slice plane, having the shape of elongated, elliptical clusters of reticuloendothelial cells around the terminal arterial vessels. Around the blood vessels, lymphoid clutches were visible that were not clearly delimited and had the appearance of homogeneous clusters of lymphoid cells. It was also possible to distinguish germinal follicles that were clearly delimited by the membrane and consisted of blasts, lymphocytes and, to a lesser extent, plasma cells. Germination follicles were bound with small and medium sized arteries and veins. The arterial germinal follicles adjoined the surface of the vessel. And the venous germinative follicles were immersed deeply in the lumen of the veins, lay inside them and changed the shape of the lumen. Histological examination of the spleen of chickens in groups 1 and 2 revealed that the capsule of the organ was unchanged. The organ was hematopoietic, and the white pulp occupied a large portion of the slice area (Fig. 6). The germinal follicles were single, and the periarterial lymphoid clutches were wide. Also, we detected an increasing number of pseudo-eosinophils. Reticuloendothelial clutches were not enlarged in size.

Histological examination of the large intestine and cecal tonsils revealed that the *mucous lamina propria* contained lymphoid tissue with numerous lymphoid follicles and diffuse lymphoid tissue, represented by small and medium lymphocytes, macrophages, plasma cells, and pseudo-eosinophils. The muscular membrane of the mucosa was represented by smooth muscle cells. The mucous membrane was lined by a single-layer prismatic epithelium containing many goblet cells. The muscular membrane consisted of two layers. In chickens of group 2, cecal tonsils contained a small number of lymph nodes that were not densely filled with lymphoid cells (Fig. 7). Signs of catarrhal inflammation were observed. The epithelial layer was thickened, and some of the cells were desquamated. In chickens of group 1, hemorrhages located mainly on the tips of the villi were observed.

The villi were thickened and deformed. In some areas, there was a desquamation of cells and, in others thickening of the epithelial layer was observed.

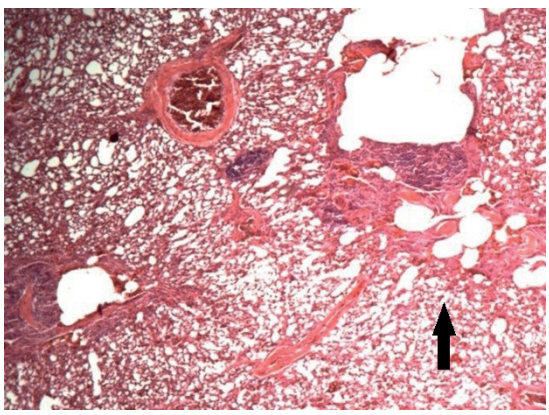

Fig. 4. Lungs of a chicken infected with the ILTV (group 1, isolate A4-12). Thickness of the walls of the bronchioles. × 100, H+E

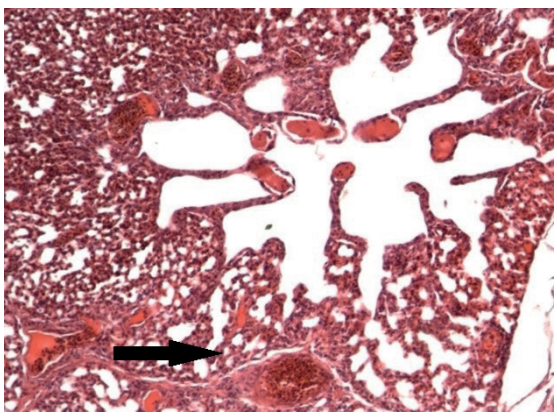

Fig. 5. Lungs of a chicken infected with the ILTV (group 2, isolate A4-12). Lymphoid-histiocytic cell cluster. × 100, H+E

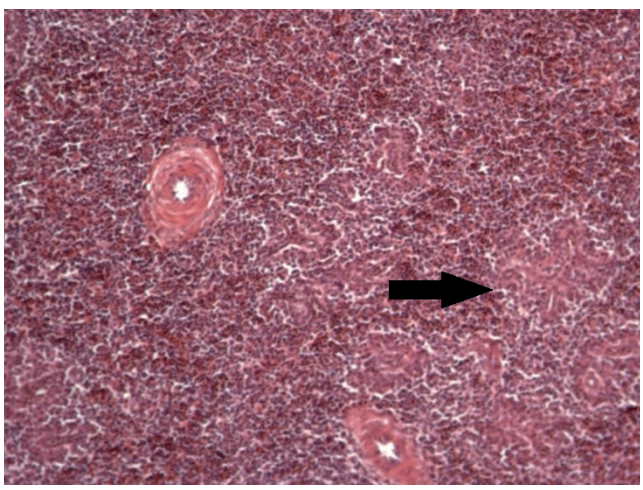

Fig. 6. Spleen of a chicken infected with the ILTV (group 1, isolate A4-12). The white pulp occupies a large portion of the slice area. × 50, H+E

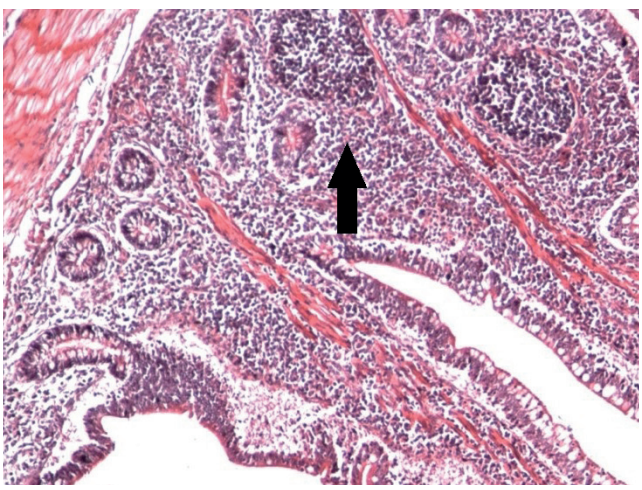

Fig. 7. The cecum of a chicken infected with the ILTV (group 2, isolate A4-12). The lymph nodes are few, loosely filled with lymphoid cells. × 100, H + E

Discussion and Conclusions

Nowadays, one of the biggest problems of industrial poultry farming in the world is infectious diseases. Despite much research in the study of the biological properties of pathogens, the development of modern tools for early diagnosis and specific prevention, outbreaks of infectious viral and bacterial diseases are registered almost constantly in different countries. Respiratory viral diseases such as infectious laryngotracheitis remain an urgent problem and are often complicated by bacterial infections, which are significantly aggravating the course of the disease.

Ukraine has a developed industrial poultry farming (Асоціація «Союз птахівників України», 2014) and the problem of infectious laryngotracheitis has been known since the 70s of the last century. At that time, this disease was registered quite often and led to significant economic losses (Бабкин, 1975; Бабкин, 1986; Бабкин et al., 1997). Nowadays, ILT remains an important viral disease for poultry. Active international trade between Ukraine and other countries, export and import of poultry products, genetic material, veterinary drugs and veterinary technology contribute to the circulation of the pathogen. In addition, the properties of the ILTV and the ability of latent infection lead to the emergence of new isolates. In the last 10 years, the circulation of ILTV isolates in both industrial and backyard poultry farms has been detected in Ukraine [Музика et al., 2021; Veretsun et al., 2021]. Several ILTV strains have been isolated from infected birds in different regions of Ukraine, but at the same time their pathotype and affiliation to field or vaccine-like isolates remains unknown.

The results presented in this work show the first data regarding the vaccine-like strain circulating in Ukraine. For the first time in Ukraine, we found that the ILTV isolates from industrial poultry farms were characterized as vaccine strains. It should also be noted that isolate B2-10 was obtained from sick vaccinated birds, while isolate A 04-12 was isolated from sick non-vaccinated chickens. This information suggests that vaccine strains are able to spread between poultry farms and more detailed investigation is needed.

In our studies, vaccine-like isolates of the ILTV (A 04-12 and B2-10), which were isolated in different regions of Ukraine in 2010 and 2012, showed high reproducibility in CE and were pathogenic for them. They caused typical changes in the chorioallantoic membrane of CE. Similar changes were caused by other isolates of the ILTV (Saif, 2008). During experimental infection, both isolates were pathogenic for 60-day-old chickens. According to our data, the incubation period of vaccine-like ILTV infection in chickens varied from 7 to 9 days, which is consistent with other authors (Saif, 2008). Ukrainian isolates ILTV (A4-12 and B2-10) caused not only typical clinical signs in chickens, but also death of chickens (B2-10) within 12 days after infection. These results confirm the ability of ILTV vaccine-like isolates to cause disease in susceptible chickens (Hughes et al., 1987; Hughes et al., 1989; Saif et al., 2008).

Concerning postmortem studies, the changes we found in the tissues of internal organs, especially the respiratory system, indicate that vaccine-like isolates of the ILTV can cause significant damage to organs at the cellular level. At the same time, we also found the reaction of the immune system of chickens to infection (Fletcher et al., 2008).

Based on the obtained data, we can conclude that vaccine-like isolates of the ILTV can be pathogenic to birds, cause them clinical manifestations of the disease and death of infected chicken. Thus, there is no effective method regarding differentiating between vaccine-like and field isolates of the virus without conducting in-depth molecular genetic studies and sequencing (A laboratory manual, 2008; Creelan et al., 2006, Saif et al., 2008). The pathogenicity level of the vaccine-like isolates can be different, and the key factors in the deterioration of the disease in poultry will be external factors (veterinary and sanitary conditions of poultry farms, feed quality, and other infections, especially bacterial). In conclusion, it is necessary to note the importance of the further research: regular epidemiological monitoring, isolation of pathogens and study of their biological and molecular genetic properties, which will allow timely control of the emergence of new variants of viruses, improvement of diagnostics and specific prevention. These studies are extremely important for poultry farming in Ukraine, because it remains unknown which field ILTV isolates circulate in Ukraine and their origin.

Acknowledgements. This study was funded within the State Scientific Program of NAAS of Ukraine (#26.3-01.01.1 and #38.01.02.02).

References

1. A laboratory manual for the isolation, identification and characterization of avian pathogens. American Association of Avian Pathologists, Athens, GA. 2008
2. Офіційний сайт Асоціації «Союз птахівників України». Союз птахівників України www.ptaha.kiev.ua. 22-1-2014.

Бабкин, В. Ф. 1986. Инфекционный ларинготрахеит, p. 143-150. *In*: Респираторные болезни сельскохозяйственных животных. Киев.

3. Бабкін, В. Ф. 1975. Інфекційний ларинготрахеїт птиці. Київ.
4. Бабкін, В. Ф., Макогон В, and Герман В. 1997. Вакцинопрофілактика інфекційного ларинготрехеїту та ньюкаслської хвороби птиці в умовах півдня України. Вет. медицина України. 12:20-22.
5. Воротилова Н.Г. 2014.Епізотологічний моніторинг високопатогенного грипу, інфекційного ларинготрахеїту птиці та біологічні властивості збудників, Харків.
6. Bagust, T. J. 1986. Laryngotracheitis (Gallid-1) herpesvirus infection in the chicken. 4. Latency establishment by wild and vaccine strains of ILT virus. Avian Pathol. 15:581-595.
7. Bagust, T. J., R. C. Jones, and J. S. Guy. 2000. Avian infectious laryngotracheitis. Rev.Sci Tech. 19:483-492.
8. Crawshaw, G. J. and B. R. Boycott. 1982. Infectious laryngotracheitis in peafowl and pheasants. Avian Dis. 26:397-401.

9. Creelan, J. L., V. M. Calvert, D. A. Graham, and S. J. McCullough. 2006. Rapid detection and characterization from field cases of infectious laryngotracheitis virus by real-time polymerase chain reaction and restriction fragment length polymorphism. Avian Pathol 35:173-179.
10. Gowthaman, V., S. Kumar, M. Koul, U. Dave, T. R. G. K. Murthy, P. Munuswamy, R. Tiwari, K. Karthik, K. Dhama, I. Michalak, and S. K. Joshi. 2020. Infectious laryngotracheitis: Etiology, epidemiology, pathobiology, and advances in diagnosis and control - a comprehensive review. Vet.Q. 40:140-161
11. Hughes, C. S., R. M. Gaskell, R. C. Jones, J. M. Bradbury, and F. T. Jordan. 1989. Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. Res Vet Sci 46:274-276.
12. Hughes, C. S., R. C. Jones, R. M. Gaskell, F. T. Jordan, and J. M. Bradbury. 1987. Demonstration in live chickens of the carrier state in infectious laryngotracheitis. Res Vet Sci 42:407-410.
13. International Office of Epizootics, Biological Standards Commission, International Office of Epizootics, and International Committee. 2008. Manual of diagnostic tests and vaccines for terrestrial animals mammals, birds and bees. Office international des epizooties, Paris.
14. Музика Д.В., Верецун А.Л., Стегній Б.Т., Рула О.М., and Усова Л.П. 2021. Виділення та ідентифікація епізоотичного ізоляту вірусу інфекційного ларинготрахеїту. Вет. медицина: міжвід.темат.наук.зб. 104:137-142.
15. Jones, R. C. 2010. Viral respiratory diseases (ILT, aMPV infections, IB): are they ever under control? Br.Poult.Sci 51: 1-11.
16. Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York: McGraw Hill Book Co..
17. Oscar Jasper Fletcher and C. Riddell. 2008. Avian histopathology. Jacksonville, Fla. : American Association of Avian Pathologists.
18. Saif, Y. M. and A. M. Fadly. 2008. Diseases of poultry. Blackwell Pub, Ames, Iowa.
19. Veretsun, A., Stegniy, B., Rula, O., Bolotin, V., Stegniy, A., Gerilovych, A., & Muzyka, D. (2021). Molecular and genetic characterization of avian laryngotracheitis virus isolates obtained in Ukraine. *Agricultural Science and Practice*, *8*(1), 37-46. https://doi.org/10.15407/agrisp8.01.032

Received 3 September 2020
Accepted 15 September 2021

Identification of Bacteria in Aceh Cattle with Repeat Breeding

Cut Nila Thasmi[1], Husnurrizal Husnurrizal[1], Sri Wahyuni[2], Hafizuddin Hafizuddin[1], Tongku Nizwan Siregar[1]

[1]*Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[2]*Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*

Keywords: *Aceh cattle; bacteria; repeat breeding.*

Abstract. *Initial studies report that the incidence of repeat breeding (RB) in Aceh cattle is associated with bacterial infection in the reproductive tract, particularly in the uterine cornual. Therefore, it is necessary to conduct research to identify the type of bacteria in the uterus of Aceh repeat breeding cows. The objective of this study was to identify the types of bacteria that infect the uterus of Aceh cows that experiencing RB. In this study, 16 Aceh cows were used: 7 fertile Aceh cows and 9 RB ones, all aged 3–8 years with a body condition score (BCS) of 3–4. Uterine swab samples were taken from all cows for examination and identification of bacteria. In the uterine samples of two groups of Aceh cows (RB and fertile), 41 bacterial isolates from 11 different types of bacteria were found. The results showed that the most prevalent bacteria in fertile cows was E. coli (26.7), while no Salmonella was found. In RB Aceh cows, the most common bacteria were Salmonella sp. (29.6%), isolated from 8 samples, and E. coli (22.7%) isolated from 6 samples. In conclusion, RB Aceh cows showed higher numbers of bacterial isolates than fertile Aceh cows, with the most dominant bacterial isolate being Salmonella sp. (29.6%).*

Introduction

Repeat breeding (RB) is a clinical reproductive disorder which can cause infertility. It can decrease reproductive efficiency and livestock productivity, especially in cattle (Prihatno et al., 2013; Thasmi et al., 2020). Early diagnosis of RB disorders minimizes economic losses among farmers (Bonneville-Hébert et al., 2011; Carneiro et al., 2016). Subclinical endometritis in cattle is considered an important etiological factor for RB disorders (Bedewy and Rahaway, 2019; Jeremejeva et al., 2016).

During the reproductive period in cows, the uterus can be exposed to infectious agents, especially during mating and after birth (Yilmaz et al., 2012). Uterine infection can be caused by microorganisms originating from the posterior genital organs (vulva, vagina, and cervix) or from the uterine tract (Bhat et al., 2014; Yilmaz et al., 2012). Of cows with RB, 71.2% were reported to have bacterial infection (El-Khadrawy et al., 2011). Uterine infection causes 20% of RB cases in cattle (Azawi, 2010).

Isolates from the early postpartum uterus include anaerobic bacteria and both Gram-positive and Gram-negative aerobic bacteria (Nath et al., 2014). The bacteria commonly found in cows with RB are *Staphylococcus* spp. (21.0%), *E. coli* (18.4%), *Bacillus* spp. (13.1%), *Corynebacterium* spp. (13.1%), *Pseudomonas* spp. (10.5%), *Proteus* spp. (10.5%), *Klebsiella* spp. (7.9%) and *Streptococcus* spp. (5.3%) (El-Khadrawy et al., 2011). Bacteria that infect the uterus are divided into 3 categories, namely pathogenic bacteria, potential pathogens, and contaminant bacteria. Pathogenic bacteria include *E. coli*, *Trueperella pyogenes*, *Prevotella* spp., *Fusobacterium necrophorum*, and *Fusobacterium nucleatum*. Other potentially pathogenic bacteria include *Acinetobacter spp., Bacillus licheniformis, Enterococcus faecalis, Haemophilus somnus, Mannheimiahaemolytica, Pasteurella multocida, Peptostreptococcus spp., Staphylococcus aureus* (coagulase +), *Streptococcus uberis*, and *Fusobacterium* sp. Contaminant bacteria include *Aerococcus viridans, Clostridium butyricum, Clostridium perfringens, Corynebacterium spp., Enterobacter aerogenes, Klebsiella pneumoniae, Micrococcus spp., Providencia rettgeri, Providencia stuartii, Proteus spp., Propionibacterium granulosum,* and *Streptococcus acidominimus* (Carneiro et al., 2016).

Pathogenic microorganisms isolated from the infected uterus are commonly found in livestock environments. They are capable of infecting other tissues and organs (Moreno et al., 2016). These microorganisms can cause changes in the pH of the cervical, vaginal, and uterine mucus (Bedewy and Rahaway, 2019; El-Khadrawy et al., 2011). They can inflame and denude the uterine mucosa, interfering with the implantation process (Siregar et al., 2017). Generally, uterine infection can lead to endometritis (Carneiro et al., 2016; Melia et al., 2014), failure of pregnancy, and infertility in cattle (Yilmaz et al., 2012). It is suspected that abnormal uterine conditions can also cause failure of pregnancy and endometritis in Aceh cattle.

Several causes of reproductive disorders in Aceh cows have been reported. Low progesterone levels at the onset of the luteal phase do not appear to cause RB among Aceh cows (Jeon et al., 2015; Thasmi et al., 2017). Rather, initial studies report that the incidence of RB in Aceh cattle is associated with

Correspondence to Hafizuddin Hafizuddin, Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. E - mail: hafizuddin_umar@unsyiah.ac.id

bacterial infection of the reproductive tract, especially in the uterine cornual (Dolezel et al., 2010; Rafika et al., 2020). The objective of this study was to identify the types of bacteria that infect the uterus of Aceh cows experiencing RB.

Materials and methods

Experimental animals

In this study, 16 Aceh cows were used: 7 fertile Aceh cows and 9 RB Aceh cows, all aged 3–8 years with a body condition score (BCS) of 3–4. Uterine swab samples were taken from all cows for examination and identification of bacteria.

Isolation and identification of bacteria from the uterine mucosa

Bacterial samples were collected from the uterine tract using a sterile cotton swab attached to the tip of an AI gun (Hasan et al., 2015). The swab sample was steeped in nutrient broth (NB) media and incubated at 37°C for 24 hours. Bacteria were cultured in nutrient agar (NA), blood agar, Mac Conkey agar (MCA), and mannitol salt agar (MSA) (Oxoid Ltd.).

It was incubated at 37°C for 24–48 hours; following the procedure of Yilmaz et al. (2012) and Hasan et al. (2015), bacteria were identified by morphology with Gram staining, catalase test, haemolysis, and biochemical tests including motility, indole and urease test (MIU test), triple sugar iron agar (TSI-A), and Voges Proskauer Test (VP) (Oxoid Ltd.) (Hasan et al., 2015; Joy and Faruk, 2011).

Data analysis

The observations resulting from identification of morphology, types of bacteria and intensity of the isolates in this study were analysed descriptively.

Results

The results showed that all Aceh cows sampled showed bacterial isolates in the uterine mucosa (100%). The intensity of bacterial isolates found in Aceh cattle is presented in Table 1. The results showed that the most common bacteria found in uterine samples of fertile Aceh cows was *E. coli* (26.7%). In RB Aceh cows, the most common bacteria found were *Salmonella* sp. (29.3%), isolated from 8 samples, and *E. coli* (22.7%), isolated from 6 samples. No *Salmonella* sp. was found in fertile Aceh cows.

The results of bacterial isolation showed that all Aceh cows had bacterial isolates (100%) in the uterine mucosa. All 9 uterine samples from RB cows contained 3 types of bacterial isolates, including 6 isolates of *E. coli* (22.2%); 8 isolates of *Salmonella* sp. (29.6%); 4 isolates of *Corynebacterium* sp. (14.8 %); *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Shigella* sp., each 2 isolates (7.4%); and 1 isolate of *Citrobacter freundii* (3.7%) as presented in Table 2.

Discussion

In the RB and fertile Aceh cows, 42 bacterial isolates were found from 11 different types of bacteria, as presented in Table 1. Based on Table 1, the greatest mean of bacteria is isolated from the uterus of RB Aceh cows. Previous reports stated that the highest average number of microorganisms, mainly pathogenic, was found in cows with uterine infections. About 60% of uterine bacteria were found in RB cows and sick cattle, a much higher frequency of isolates than in fertile cows (Gani et al., 2008; Kather et al., 2012; Sumiarto, 2013).

In fertile Aceh cows (n = 7), only 2 samples (28.6%) contained single isolates, while 3 samples (42.9%) contained 2 types of bacterial isolates. Two samples

Table 1. Frequency distribution of bacterial isolation from uterus of fertile and RB Aceh cows

No	Bacterial type	Number of bacterial isolates	
		Fertile, n = 7 (%)	RB, n = 9 (%)
1	*Escherichia coli*	4 (26.7)	6 (22.2)
2	*Salmonella* sp.	0	8 (29.6)
3	*Corynebacterium* sp.	3 (20.0)	4 (14.8)
4	*Enterobacter* sp.	2 (13.3)	2 (7.4)
5	*Pseudomonas* sp.	2 (13.3)	2 (7.4)
6	*Staphylococcus* sp.	1 (6.7)	2 (7.4)
7	*Shigella* sp.	0	2 (7.4)
8	*Bacillus* spp.	1 (6.7)	0
9	*Klebsiella* sp.	1 (6.7)	0
10	*Citrobacter freundii*	0	1 (3.7)
11	*Streptococcus* sp.	1 (6.7)	0
		15	27
	Total	42	

Table 2. Intensity of bacterial isolates found in endometrial mucosa of fertile Aceh cows and RB Aceh cows

Parameter	Fertile Aceh cows		RB Aceh cows	
	n	(%)	n	(%)
Number of samples with a single bacterial isolate	2	28.6	0	0
Number of samples with 2 bacterial isolates	3	42.9	0	0
Number of samples with 3 bacterial isolates	2	28.6	9	100
Number of samples with bacterial isolates	7	100	9	100

contained 3 bacterial isolates (28.6%). The uterine bacterial isolates from fertile Aceh cows included *E. coli* with 4 isolates (26.7%), *Corynebacterium* sp. with 3 isolates (20%), *Enterobacter* sp. and *Pseudomonas* sp., each with 2 isolates (13.3%). Other bacteria observed were *Staphylococcus* sp., *Bacillus* spp., *Klebsiella* sp., and *Streptococcus* sp., each with 1 isolate (6.7%). Most of the bacterial isolates found in this study greatly resembled those in the report mentioned above. The identified bacterial isolates included *Staphylococcus* spp. (37.8%), *Bacillus* spp. (35.1%), *E. coli* (29.7%), *Pseudomonas* spp. (18.9%), and Gram-negative rod-shaped bacteria (24.3%) (20). Another report found that the most common bacterial isolates from the bovine uterus were *E. coli* (38.3%), followed by *S. aureus* (20.0%), *Proteus* spp. (10.0%), and *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., 5.0%, 5.0% and 6.7%, respectively (Kusumastuti, 2014).

RB cows had more bacterial isolates than fertile cows (62.5% vs. 28.6%). Bacterial isolates found include *Bacillus* sp. (50%), *Staphylococcus* sp. (40%), *E. coli* sp. (20%), and *Streptococcus* sp. (10%) (Kusumastuti, 2014). Several types of bacteria isolated from the uterus of dairy cows were *Bacillus* sp., *Staphylococcus* sp., *E. coli,* and *Streptococcus* sp. (Singh et al., 2000), *E. coli*, *Streptococcus* spp., *Arcanobacterium pyogenes, Bacillus licheniformis*, *Prevotella* spp., and *Fusobacterium necrophorum* (Azawi, 2008; Földi et al., 2006; Petit et al., 2009; Singh et al., 2000; Yavari et al., 2007). This shows that uterine bacteria are closely related to the incidence of endometritis. Uterine infection and endometritis in Aceh cattle are probably caused by bacterial infection.

The number of bacterial isolates in RB Aceh cows (2.45 ± 2.58) was higher than in fertile Aceh cows (1.36 ± 1.28), but it was not statistically significant ($p > 0.05$). However, the number of bacteria isolated from the uterus of Aceh RB cows was higher (64.3%) than that of fertile Aceh cows (35.7%). The most common bacteria found in fertile Aceh cows included *E. coli* (26.6%) and *Corynebacterium* sp. (20%), and in RB Aceh cows, it was *Salmonella* sp. (29.6%), *E. coli* (22.2%) and *Corynebacterium* sp. (14.8%). The discovery of E. coli bacteria in the uterus of Aceh cows in this study was probably due to faecal contamination containing these bacteria. Bacterial contamination can also occur during the implementation of artificial insemination, embryo transfer, uterine biopsy, and intrauterine drug infusion that does not pay attention to aspects of hygiene (Casarin et al., 2018).

Bacterial contamination causes abnormalities or infections of the uterus (Jun et al., 2008). Uterine infections are always associated with the presence of *Arcanobacterium pyogenes*, *E. coli, Fusobacterium necrophorum,* and *Prevotella melaninogenica* (Frazier et al., 2002; Gani et al., 2008; Jun et al., 2008; Källerö, 2010; Petit et al., 2009; Yavari et al., 2007), and *Corynebacterium pyogenes* (Abere and Belete, 2016).

In the genital organs of domestic animals, there are several bacteria that are harmful and pathogenic to susceptible animals. Bacteria can travel from the vagina to the uterus. In the cervix and uterus, it can multiply, leading to contamination and infection of the uterus (Chapwanya et al., 2012). When the uterus is infected, inflammatory cells infiltrate the endometrium and cause acute and chronic inflammation, followed by necrosis, hyperaemia, increased numbers of neutrophils, lymphocytes, and macrophages, cystic dilation or atrophy of the uterine endometrium (Bajaj et al., 2016; Chethan et al., 2015; Thasmi et al., 2018), endometrial hyperplasia, endometrial atrophy, and pyometra (Källerö, 2010). A cow's uterus with endometritis shows exudates in the endometrial lumen that vary, such as serum, mucus, and purulence that fill the inside of the uterus (Frazier et al., 2002; Källerö, 2010).

Conclusion

The RB Aceh cows showed higher numbers of bacterial isolates than fertile Aceh cows, with the most dominant bacterial isolate being Salmonella sp. (29.6%). The RB Aceh cows tend to be infected by more than 3 bacterial isolates.

Acknowledgments

The author would like to thank the assistants at the Reproductive Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, for assistance in uterine swab collection, and the Health Laboratory of Aceh Province, Banda Aceh, for their assistance in the identification of bacteria from uterine mucus.

Conflict of interests

The authors declare that they have no conflict of interests.

Author's contribution

Cut Nila Thasmi and Husnurrizal Husnurrizal participated in performing uterine swabs, selecting samples, and writing the initial manuscript. Sri Wahyuni performed manuscript revision and assisted bacterial identification. Tongku Nizwan Siregar performed practical experiments and sample collection. Hafizuddin Hafizuddin developed the original idea and protocol and revised the final manuscript.

References

1. Abere, T., Belete, H. Infections of the uterus on postpartum cows. J. Reprod. Infertil. 2016. Vol. 7(2). P. 34–40.
2. Azawi, O. Postpartum uterine infection in cattle. Anim. Reprod. Sci. 2008. Vol. 105(3–4). P. 187–208.
3. Azawi, O. Uterine infection in buffalo cows: a review. Buffalo Bull. 2010. Vol. 29(3). P. 154–171.
4. Bajaj, N.K., Shukla, S.P., Agrawal, R.G., Agrawal, S., Honparkhe, M. Subclinical endometritis in postpartum buffaloes: an emerging threat. Journal of Animal Research. 2016. Vol. 6(5). P. 819.
5. Bedewy, R., Rahaway, M. Comparative study for detection of subclinical endometritis in local cows. Adv. Anim. Vet. Sci. 2019. Vol. 7(4). P. 289–294.
6. Bhat, F.A., Bhattacharyya, H.K., Hussain, S.A.White side test: A simple and rapid test for evaluation of nonspecific bacterial genital infections of repeat breeding cattle. *In*: Veterinary research forum: an international quarterly journal. 2014. pp. 177.
7. Bonneville-Hébert, A., Bouchard, E., Tremblay, D.D., Lefebvre, R. Effect of reproductive disorders and parity on repeat breeder status and culling of dairy cows in Quebec. Can. J. Vet. Res. 2011. Vol. 75(2). P. 147–151.
8. Carneiro, L.C., Cronin, J.G., Sheldon, I.M. Mechanisms linking bacterial infections of the bovine endometrium to disease and infertility. Reprod.Biol. 2016. Vol. 16(1). P. 1–7.
9. Casarin, J., Martini, A.P., Trentin, J.M., Fiorenza, M.F., Pessoa, G.A., Barros, S.S., Rubin, M.I. Bacteriological, cytological and histopathological evaluation of the reproductive tract of slaughtered cows. Pesqui. Vet. Bras. 2018. Vol. 38(1). P. 53–58.
10. Chapwanya, A., Meade, K.G., Foley, C., Narciandi, F., Evans, A.C., Doherty, M.L., Callanan, J.J., O'Farrelly, C. The postpartum endometrial inflammatory response: a normal physiological event with potential implications for bovine fertility. Reprod. Fertil. Dev. 2012. Vol. 24(8). P. 1028–1039.
11. Chethan, S., Singh, S., Karikalan, M., Kharayat, N., Behera, B., Narayaman, K., Kumar, H., Anjaneya, A. Histopathological evaluation of important uterine pathological affections in riverine buffalo (Bubalus bubalis): an abattoir study. Asian. J. Anim. Vet. Adv. 2015. Vol. 10(8). P. 406–415.
12. Dolezel, R., Palenik, T., Cech, S., Kohoutova, L., Vyskocil, M. Bacterial contamination of the uterus in cows with various clinical types of metritis and endometritis and use of hydrogen peroxide for intrauterine treatment. Vet. Med. 2010. Vol. 55(10). P. 504–511.
13. El-Khadrawy, H., Ahmed, W., Hanafi, M. Observations on repeat breeding in farm animals with emphasis on its control. J. Reprod. Infertil. 2011. Vol. 2(1). P. 01–07.
14. Földi, J., Kulcsar, M., Pecsi, A., Huyghe, B., De Sa, C., Lohuis, J., Cox, P., Huszenicza, G. Bacterial complications of postpartum uterine involution in cattle. Anim. Reprod. Sci. 2006. Vol. 96(3–4). P. 265–281.
15. Frazier, K.S., Baldwin, C.A., Pence, M., West, J., Bernard, J., Liggett, A., Miller, D., Hines, M.E. Seroprevalence and comparison of isolates of endometriotropic bovine herpesvirus-4. J. Vet. Diagn. Invest. 2002. Vol. 14(6). P. 457–462.
16. Gani, M., Amin, M., Alam, M., Kayesh, M., Karim, M., Samad, M., Islam, M. Bacterial flora associated with repeat breeding and uterine infections in dairy cows. Bangladesh. J. Vet. Med. 2008. Vol. 6(1). P. 79–86.
17. Hasan, M.A., Al Mamun, A., Uddin, A.A., Hassan, M.Z., Hasan, M., Rahman, M.H. Investigation into gyneco-pathological disorders and identification of associated bacteria from the genital organs of cows in Dinajpur, Bangladesh. J. Adv. Vet. Anim. Res. 2015. Vol. 2(2). P. 165–169.
18. Jeon, S.J., Vieira-Neto, A., Gobikrushanth, M., Daetz, R., Mingoti, R.D., Parize, A.C.B., de Freitas, S.L., da Costa, A.N.L., Bicalho, R.C., Lima, S. Uterine microbiota progression from calving until establishment of metritis in dairy cows. Appl. Environ. Microbiol. 2015. Vol. 81(18). P. 6324–6332.
19. Jeremejeva, J., Orro, T., Waldmann, A., Aunapuu, M., Blank, I., Arend, A., Kask, K. Early versus late postpartum treatment of clinical endometritis in dairy cows using a combination of systemic administrations of ceftiofur with prostaglandin F2A. Vet. Med. Zoot. 2016. Vol. 74(96). P. 9–13.
20. Joy, S., Faruk, M. Isolation and identification of uterine microorganisms in postpartum dairy cows. Bangladesh Journal of Microbiology. 2011. Vol. 28(1). P. 19–23.
21. Jun, W., Meiyan, C., Cai, J. Separated and identified bacteria from the womb of infertility milk cow of endometritis being not ovariopathy. J. China. Agric. Univ. 2008. Vol. 13(6). P. 77-80.
22. Källerö, E.E. Uterine Pathology and Physiology in the Post Partum Period in Ethiopian Cattle. Examensarbete Inom Veterinärprogrammet, Uppsala, SE. 2010..
23. Kather, N.Y., Hasan, A.S., Dawood, W.S., Mohammed, S.N. Bacterial flora isolated from genital tract of cows submitted for artificial insemination in Balad district. Kufa. J. Vet. Sci.. 2012. Vol. 3(1). P. 91-97.
24. Kusumastuti, N. Identifikasi Jenis Bakteri pada Uterus Sapi Perah Normal. Doctoral Dissertation, Universitas Gadjah Mada, Yogyakarta, ID. 2014.
25. Melia, J., Amrozi, A., Tumbelaka, L.I. Ovarian dinamics of endometritis cows treated with a combination of intrauterine infusion of gentamicine, flumequine, and PGF2α analog. J. Kedokt. Hewan. 2014. Vol. 8(2). P. 111–115.
26. Moreno, I., Codoñer, F.M., Vilella, F., Valbuena, D., Martinez-Blanch, J.F., Jimenez-Almazán, J., Alonso, R., Alamá, P., Remohí, J., Pellicer, A. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am. J. Obstet. Gynecol. 2016. Vol. 215(6). P. 684–703.
27. Nath, B., Das, B., Bari, M., Rahman, M. Prevalence and risk factors of repeat breeding in commercial dairy farms of Chittagong district of Bangladesh. Int. J. Sci. Nat. 2014. Vol. P. 21–27.
28. Petit, T., Spergser, J., Rosengarten, R., Aurich, J. Prevalence of potentially pathogenic bacteria as genital pathogens in dairy cattle. Reprod. Domest. Anim. 2009. Vol. 44(1). P. 88–91.
29. Prihatno, S.A., Kusumawati, A., Karja, N.W.K., Sumiarto, B. Prevalensi dan faktor resiko kawin berulang pada sapi perah pada tingkat peternak. J. Vet. 2013. Vol. 14(4). P. 152–461.
30. Rafika, I., Thasmi, C.N., Herrialfian, H., Rosmaidar, R., Hafizuddin, H. Isolation and identification of gram negative bacteria in uterine Aceh cow with repeat breeding. J. Agripet. 2020. Vol. 20(2). P. 187–192.
31. Singh, J., Sidhu, S., Dhaliwal, G., Pangaonkar, G., Nanda, A., Grewal, A. Effectiveness of lipopolysaccharide as an intrauterine immunomodulator in curing bacterial endometritis in repeat breeding cross-bred cows. Anim. Reprod. Sci. 2000. Vol. 59(3-4). P. 159–166.
32. Siregar, T.N., Agustina, I., Masyitah, D., Al-Azhar, D., Thasmi, C., Sulaiman, R., Daud, R. Physical properties of cervical mucus of repeat breeder Aceh cattle. J. Vet. 2017. Vol. 3(18). P. 378–382.
33. Sumiarto, D.B. Kajian Epidemiologi Kawin Berulang Pada Sapi Perah di Daerah Istimewa Yogyakarta. Universitas Gadjah Mada, Yogyakarta, ID. 2013.
34. Thasmi, C.N., Husnurrizal, H., Wahyuni, S., Siregar, T.N. Detection of serum macro minerals concentration in repeat breeding Aceh cattle. J. Kedokt. Hewan. 2020. Vol. 14(2). P. 25–28.

35. Thasmi, C.N., Siregar, T.N., Wahyuni, S., Aliza, D., Hamdan, H., Panjaitan, B., Asmilia, N., Husnurrijal, H. Estrus performance and steroid level of repeat breeding Aceh cattle synchronized with PGF2α. Veterinaria. 2017. Vol. 66(1). P. 36–41.
36. Thasmi, C.N., Siregar, T.N., Wahyuni, S., Aliza, D., Panjaitan, B., Nazaruddin, N., Sabila, F.N., Fallatanza, M. Anatomical and histological changes of uterine horn of Aceh cattle with repeat breeding. J. Adv. Vet. Anim. Res. 2018. Vol. 5(4). P. 445.
37. Yavari, M., Haghkhah, M., Ahmadi, M.R. Bacterial study of clinical postpartum endometritis in Holstein dairy cows. Online. J. Vet. Res. 2007. Vol. 11. P. 14–23.
38. Yilmaz, O., Kuyucuoğlu, Y., Sevimli, A. Uterine microbiology and histopathology in repeat breeder Anatolian Water buffaloes: An Abattoir study. Kafkas. Univ. Vet. Fak. Derg. 2012. Vol. 18(5). P. 791–798.

Received 7 July 2021
Accepted 29 November 2021

Comparative Analysis of Motility Characteristics and Kinematic Parameters of Fresh, Chilled and Sexed Ram Semen – Preliminary study

Stanimir Yotov[1], Elena Kistanova[2], Desislava Abadjieva[2], Manol Karadaev[1], Boyana Ivanova[1], George Bonev[1], Dobri Yarkov[1], Branimir Sinapov[1]

[1]Department Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

[2]Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Keywords: *ram, semen motility, kinematics, chilling, sexing.*

Abstract. *The application of the bovine serum albumin (BSA) column method for sexing of spermatozoa is cost effective and appropriate for small ruminants. The current study compares motility characteristics and kinematic parameters of fresh, chilled and sexed ram semen with the aim to select the high-quality ejaculates for semen sexing. Fresh, chilled and sexed semen from 4 East Friesian rams was analysed by CASA, and immotile sperm cells, motile sperm cells, progressive motility, non-progressive motility, VCL, VAP, VSL, STR, LIN and WOB were determined. Semen sexing was carried out in bovine serum albumin columns and incubation for 45 min at a temperature of 25°C. The average values of all semen parameters and the change of the most important indices in individual rams were estimated. Significant differences ($P < 0.05$) were detected between immotile and motile sperm cells, progressive and non-progressive motility, VCL and WOB of fresh and chilled semen. All investigated parameters between fresh and isolated upper and bottom layer spermatozoa, excluding STR and WOB, differed significantly ($P < 0.05$). The same dependency ($P < 0.05$) was detected for motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed spermatozoa. In conclusion, the average values for motile sperm, progressive motility, VCL, STR and LIN of chilled and sexed ram spermatozoa are significantly ($P < 0.05$) affected by chilling and sexing in BSA columns at 25°C for 45 min. CASA analysis of motility and kinematic parameters of chilled semen can provide a correct choice of ejaculates with high quality for sexing. The individual features of rams have influence on the semen characteristics and should be take into consideration in selection of the donors for a production of the sexed semen.*

Introduction

During different procedures as dilution, chilling or sexing alters the quality of the sperm cells, which can affect their fertilizing ability (Urry et al., 1983; Rodríguez-Martínez and Pena Vega, 2013; Acharya et al., 2020; Steele et al., 2020). The accurate determination of the different changes has a crucial role for a selection of the ejaculates for future handling. Motility characteristics and kinematic parameters determined by computer-assisted semen analysis (CASA) are used by many authors with an increasing trend worldwide in the last years, for exclusion of subjectivity in evaluation of the semen quality and for prediction of fertility of different type of semen (Robayo et al., 2007; Buchelly Imbachi et al., 2018). Additionally, CASA systems provide clear digital images of each spermatozoa track that allows for individual motion analysis and accurate assessment of important kinetic parameters (Verstegen et al., 2002, Wilson-Leedy and Ingermann, 2011; Amann and Waberski, 2014).

The introduction of sexed sperm is a new tool for improvement of the reproductive efficiency of small ruminants, allowing effective use of high producing animals and an optimal production of males and females in production systems (Hollinshead et al., 2002; Hamano, 2007; Ferreira-Silva et al., 2017; Gonzalez-Marín et al., 2021). The ram semen can be sexed by different sorting systems such as using an albumin gradient (Maxwell et al., 1984), flow-cytometry (Johnson, 2000) or a centrifugal counter with an aqueous two-phase system (Ollero et al., 2000). A few studies (Hadi and Al-Timimi, 2013; Solihati et al., 2019) report on the application of the bovine serum albumin (BSA) column for sexing of fresh ram semen. They describe this method as easier, cheaper and practically applicable, compared with others, but the obtained results are still debatable. Moreover, many experiments are conducted at different temperature and time for semen incubation with microscopic record of motility characteristics only, without determination of kinematic parameters. According to Solihati et al. (2019), the viability of X- and Y-sperm incubated from 45 min was better than obtained after incubation for 60 and 75 min, and the X-sperm has the highest viability. Agasi et al. (2020) separated bull sperm cells by the BSA column in a laminar cabinet at a room temperature of 27°C. They reported that motility, viability, and plasma membrane integrity of this semen could be maintained on the

Correspondence to Elena Kistanova, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. E-mail: kistanova@gmail.com

quality level after freezing and thawing. Sometimes after collection semen has to be preserved for an extended period before transporting it to the lab and submission of semen sexing procedure. It is associated with semen preservation at a low temperature (Maicas et al., 2020). There is a single report (Hollinshead et al., 2004) for sorting frozen semen in ruminants, but the information about CASA analysis of motility and kinematics of sexed spermatozoa of chilled ram semen was not available.

The aim of this study was to compare the motility characteristics and the kinematic parameters of fresh, chilled and sexed ram semen by CASA analysis in relation to the selection of high-quality ejaculates for semen sexing.

Material and methods

The study was carried out in 4 East Fresian rams at the age of 3.6 ± 0.4 years, body weight of 75 ± 8.5 kg, reared in a group box at a small ruminant farm, located at N 42.25 and E 25.38. The animals were housed in the uniform technology, and feeding included alfa-alfa and meadow straw, concentrate, vitamin and mineral premix and drinking of water *at libitum*. Investigation was performed during the breeding season after 20 days of sexual abstinence of all rams. The experiment was conducted according to the recommendations of the Local Animal Ethics Committee and regulations for human attitude and animal protection.

Semen collection, primary assessment, dilution and chilling

The semen was collected by the artificial vagina method in presence of a teaser sheep, between 8.00–9.00 am, transported to the laboratory and placed on a water bath at 37°C, and submitted to a primary assessment. The volume was measured on the graduated semen collection tube, the motility and sperm concentration ($\times 10^9$/mL) were evaluated by the CASA system. The percentage of abnormal sperms for fresh semen was recorded in stained slides by microscopic examination using of Motic Image Plus digital software system (Motic China Group Ltd, 2001–2004).

Only semen with normal color and transparency, volume > 1.5 mL, sperm concentration > 1.5×10^9/mL, motile sperm > 70% and abnormal sperms < 15% was used. After the primary assessment and on the basis of CASA results, each ejaculate was diluted with Tris-glucose-glycerol-based extender to a concentration of 400×10^6 sperm cells per mL and stored in a refrigerator at 5°C for 24 h.

Semen sexing method

Semen sexing was carried out by albumin gradient separation of the BSA column. Initially, BSA fraction V was placed in the Brackett and Oliphant (BO) medium in concentrations of 5% or 10% and stored in a refrigerator for dissolving. BSA columns were prepared in graduated glass tubes as the bottom layer contained 1 mL of 10% BSA and the upper layer contained 1 mL of 5% BSA. Chilled ram semen (1 mL) was added to each BSA column; it was preliminarily diluted with a BO medium until adjustment to a concentration of 200×10^6 cells per mL. The BSA columns were incubated at a temperature of 25°C for 45 min. After that, each layer was carefully separated in an individual tube and centrifuged at 1800 rpm for 10 min. The supernatant was discarded, and the semen was diluted with a semen extender and placed in a water bath at a temperature of 37°C until to a CASA analysis. The layers of the BSA column with a concentration of 5% (upper) and with a concentration of 10% (bottom) were accepted to contain spermatozoa bearing X and Y chromosome, respectively (Solihati et al., 2019).

Motility and kinematic parameters evaluation

The semen was evaluated immediately after collection (fresh), at 24 h after storage at 5°C and before dilution with the BO medium (chilled) and after processing by the BSA column method (sexed). Immediately before examination, the semen samples were gently mixed and a 5 μL drop was placed on a slide warmed at 37°C, and covered with a 20 mm × 20 mm cover slip. Computer-assisted semen analysis was carried out by qualified operator using the Sperm Class Analyzer software (SCA® 2002, Microptic, Barcelona, Spain). The measured motility characteristics and kinematic parameters included immotile sperm cells (%), motile sperm cells (%), progressive motility (%), non-progressive motility (%), curve linear velocity (VCL; μm/s), average path velocity (VAP; μm/s), straight-line velocity (VSL; μm/s), linearity (LIN; %), straightness (STR; %) and oscillation index (WOB; %). The software settings were adjusted to ram semen assessment according to manufacturer recommendations. A percentage of reduction for the different indices was calculated as the values before chilling and sexing were accepted as 100% and this information is presented in Figure 1.

Statistical analysis

The data were processed by statistical program Statistica version 7.0 (Stat-Soft., 1984–2000 Inc., Tulsa, OK, USA). The motility characteristics and kinematic parameters for each type of semen were given as mean ± standard deviation. Initially, the values were tested for normal distribution of variances by Kolmogorov-Smirnov and Lilliefors tests, then they were transformed logarithmically. The mean values of each index between fresh, chilled semen and spermatozoa originated from the upper and bottom layers were compared by non-parametric Mann-Whitney test. Statistical significance was considered at $P < 0.05$.

Results

During the primary assessment, considerable differences between the quality of ejaculates collected from different rams were not determined. The semen

was without impurity and with normal colour. The values for volume of ejaculate, sperm concentration, total motility, abnormal spermatozoa and pH varied between 1.5–2.4 mL, 2.5–3.2 x 10^9 per mL, 89.02%–98.5% and 8.4%–12.2%, 6.5–6.9, respectively. All parameters of fresh semen were in a normal range for small ruminants.

The average values for immotile and motile sperm cells, progressive and non-progressive motility, curve linear velocity and oscillation index (WOB) between fresh and chilled semen differed significantly ($P < 0.05$) while the rest parameters were identical (Table 1).

In rams 2 and 4, the progressive motility dropped with 46.4% and 61.4%, respectively, compared with the initial values (Fig. 1A). The same situation was recorded for curve linear velocity, but this process was strictly individual. The highest decrease (64.3%)

Table. 1 Motility characteristics and kinematics of fresh, chilled and sexed ram semen (Mean ± SD).

Parameter	Type of semen			
	Fresh (n = 4)	Chilled (n = 4)	Sexed (n = 4)	
			Upper layer (X)	Bottom layer (Y)
Immotile sperm (%)	6.23 ± 6.31^{a}	18.6 ± 6.97^{b}	32.4 ± 7.88^{bc}	44.27 ± 23.01^{bcd}
Motile sperm (%)	93.77 ± 6.31^{a}	85.15 ± 3.60^{b}	67.6 ± 7.88^{c}	63.23 ± 9.24^{cd}
Progressive motility (%)	70.27 ± 22.75^{a}	40.05 ± 5.25^{b}	15.05 ± 8.46^{c}	12.51 ± 7.70^{cd}
Non-progressive motility (%)	23.5 ± 16.48^{a}	45.1 ± 6.33^{b}	52.55 ± 0.49^{c}	50.72 ± 1.86^{bc}
VCL (µm/s)	149.44 ± 44.76^{a}	89.86 ± 22.46^{b}	51.53 ± 10.44^{c}	48.67 ± 10.22^{cd}
VAP (µm/s)	75.29 ± 22.25^{a}	47.94 ± 8.41^{ab}	35.34 ± 7.97^{bc}	32.64 ± 8.32^{bcd}
VSL (µm/s)	40.64 ± 12.00^{a}	27.39 ± 4.64^{ab}	25.29 ± 6.11^{abc}	23.16 ± 6.56^{abcd}
STR (%)	39.73 ± 16.06^{a}	55.7 ± 1.45^{ab}	63.34 ± 0.68^{c}	62.15 ± 2.30^{cd}
LIN (%)	38.68 ± 11.05^{a}	35.77 ± 2.28^{b}	47.01 ± 0.08^{ac}	45.01 ± 2.60^{cd}
WOB (%)	50.24 ± 1.18^{a}	59.21 ± 2.35^{b}	67.31 ± 0.55^{c}	65.18 ± 2.35^{abc}

Values with different superscript within a row differ each other at $P < 0.05$.

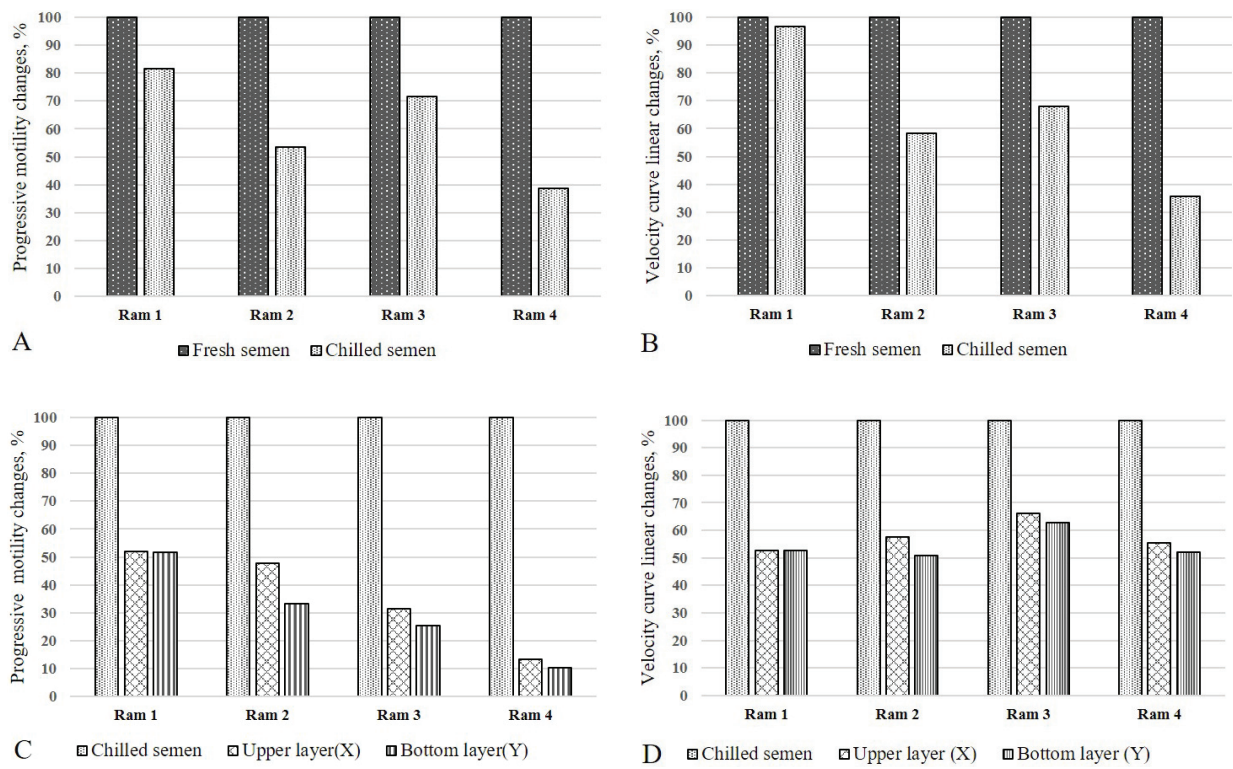

Figure 1. Changes of some motility characteristics and kinematic parameters of individual rams.
A – progressive motility of fresh semen vs. chilled semen; B – velocity curve linear of fresh semen vs. chilled semen; C – progressive motility of chilled semen vs. upper (X) and bottom (Y) layer; D – velocity curve linear of chilled semen vs. upper (X) and bottom (Y) layer.

was demonstrated in the semen of ram 4, followed by this of ram 2 (46.4%), while VCL in rams 1 and 3 was changed insignificantly (Fig. 1B).

After incubation of semen in the BSA column for 45 min, immotile sperm, non-progressive motility, STR, LIN and WOB were increased while motile sperm, progressive motility, VCL, VAP and VSL ($P < 0.05$) were decreased. All investigated parameters between fresh and isolated upper and bottom layer spermatozoa, excluding STR and WOB, differed significantly ($P < 0.05$). The same dependency ($P < 0.05$) was detected for motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed spermatozoa. However, the greatest reduction of progressive motility (over 65%) was observed in samples from rams 3 and 4 for both types of sperm cells X and Y (Fig. 1C), while in ram 2, the same effect was registered only for spermatozoa with Y chromosome. The comparative analysis between the average values of all parameters for spermatozoa isolated from the upper and the bottom layer showed no significant differences (Table 1; $P > 0.05$). Nevertheless, there was a clear tendency ($P < 0.067$) to more motile sperm and a lower decrease of progressive motility for the spermatozoa from the upper layer, mainly in rams 1 and 3. The motility and progressive motility of sexed spermatozoa in rams 1 and 2 were the highest (> 65% and > 22%), whereas in ram 4, they were the lowest (< 60 % and < 10%). The sexing process resulted in an additional drop in VCL of the sorted semen with some differences between the individuals (Fig. 1D). Even in ram 1 showing the best semen quality, VSL was reduced with 47% for both types of sperm cells.

Discussion

The kinematic parameters, especially sperm motility, are the commonly used indicators in measurement of male fertility. The good motility is very important for sperm migration through the female genital tract and for gamete interaction at fertilization (Robayo et al., 2007). The current study indicated that chilling process significantly ($P < 0.05$) affects the average values for immotile and motile sperm cells, progressive and non-progressive motility, curve linear velocity and oscillation index with a slow effect on the other kinematic parameters. A similar decline of CASA recorded motility, progressive motility and VCL in Katahdin rams at 24 h after chilling was determined by Acharya et al. (2020). Regardless of this result, the additional analysis of the data showed high individual sensitivity of the spermatozoa from different samples to chilling process. It was clearly demonstrated by a considerable drop of the progressive motility in rams 2 and 4, compared with the obtained values for fresh semen. The same strict individuality was registered for curve linear velocity, presented with the highest decrease of this parameter in the semen of ram 4, followed by this of ram 2, ram 3 and ram 1. Rickard et al. (2016) reported also different semen resistance to a low temperature. They related the variation in freezing resilience of ram spermatozoa with the source and composition of the seminal plasma. Spermatozoa produced from low-resilience rams frozen with high-resilience seminal plasma exhibited higher motility than those from low-resilience rams frozen with low-resilience seminal plasma.

Different studies have reported using the BSA column method in fresh semen sexing, but information on sexing of chilled ram semen is too limited. The incubation of chilled semen in the BSA column for 45 min affected semen motility characteristics and kinematic parameters of the spermatozoa, irrespective of presence of X or Y chromosome. It was conducted to increase the average values of immotile sperm, non-progressive motility, STR, LIN and WOB and decrease motile sperm, progressive motility, VCL, VAP and VSL, compared with the obtained values immediately before sexing ($P < 0.05$). Solihati et al. (2019) determined that sexing of ram semen by the same method had a significant effect on motility, intact plasma membrane and intact acrosome cup, but did not significantly affect abnormalities. The incubation time of 45 min resulted in the same motility as the incubation time of 60 min, but at the incubation of 45 min, the highest percentage of spermatozoa were with intact plasma membrane and intact acrosome cup.

The chilling and sexing changed the motility and kinematics of the sorted spermatozoa, compared with the values for fresh and chilled semen. Evidence for that was significant differences ($P < 0.05$) in almost all investigated parameters between fresh and isolated upper and bottom layer spermatozoa and differences in motile sperm, progressive motility, VCL, STR and LIN between chilled and sexed sperm cells.

According to Agasi et al. (2020), the motility decrease during the separation process might be the result of reduced nutrition. In regard to sperm morphokinetics, sex sorting resulted in increased numbers of immotile sperm and decreased numbers of progressive and hyperactivated sperm (Steele et al., 2020). The highest reduction of progressive motility for sperm cells from upper (X) and bootom layers (Y), observed in samples of rams 3 and 4, and the same effect for spermatozoa with Y chromosome in ram 2 can be explained by an individual response of both types of gametes in different rams to separating process. Related to this, Burroughs (2011) reveals that in some animals BSA can bind sperm plasma membrane and adsorb cholesterol. It is a reason for damage of the plasma membrane causing loss of motility and fertilization of the sperm.

In spite of insignificant differences between the average values of all parameters for spermatozoa isolated from the upper and the bottom layer, there was a clear tendency ($P < 0.067$) to more motile sperm and a lower decrease of progressive motility for the spermatozoa from the upper layer, especially

in samples for rams 1 and 3. In support of the abovementioned are results obtained by Solihati et al. (2019) who also used the BSA column for ram semen sorting. They registered longer longevity for X sperm, compared with Y sperm after incubation time of 45, 60 and 75 min. The higher percentage of sperm with mitochondrial membrane potential and the percentage of live sperm with a reacted acrosome for X than Y group from 0 h to 4 h of incubation were observed by Carvalho et al. (2018). This preliminary study indicated that the chilled ram semen incubated at a temperature of 25°C for a period of 45 min can produce sexed sperm of good quality, but not from each ram. This was confirmed with the recorded highest motility and progressive motility of sexed spermatozoa in rams 1 and 2 and very low values in ram 4. The reduction in VCL of sorted semen, even in rams with the best semen quality, before sexing can be accepted as additional evidence for individual features of ejaculates in different animals. From the practical point of view, only sexed semen from rams 1 and 2 could be recommended for artificial insemination, because it had enough motile sperm cells. In agreement with this, Hollinshead et al. (2002) reported pregnancy after laparoscopic insemination with low numbers of sex-sorted frozen-thawed motile sperm per dose (2–4 x 10^6).

In the current study, we can speculate that high quality of motility and kinematic parameters of fresh ram semen not always is a guaranty for good results after chilling and sexing. The individual characteristics of different rams can affect significantly semen indices such as immotile sperm cells, motile sperm cells, progressive motility, non-progressive motility, velocity curve linear and oscillation index during the chilling and sorting. Sexing of chilled ram semen by the BSA column at a temperature of 25°C and incubation for 45 min can be used for separation of spermatozoa, bearing X or Y chromosome, but preliminary CASA analysis of the chilled semen is recommended. The determination of the kinematic parameters will be beneficial in selection of ejaculates for future semen sorting. Robayo et al. (2007) showed VCL, VAP, STR and LIN as highly significantly ($P < 0.01$) related with migration of sperm through the cervical mucus and suggested that specific kinematic parameters confer the ability of spermatozoa to colonize and migrate through epithelial mucus with different rheological properties. Future investigations with ejaculates from a large number of animals and use of sorted semen for artificial insemination will clarify additionally the questions about sexing of chilled ram semen.

In conclusion, the average values for motile sperm, progressive motility, VCL, STR and LIN of chilled and sexed ram spermatozoa are significantly ($P < 0.05$) affected by chilling process and incubation in the BSA column at 25°C for 45 min. CASA of motility and kinematic parameters of chilled semen provides a correct choice of ejaculates with high quality for sexing. The individual features of rams have influence on the semen characteristics and should be taken into consideration for a selection of the donors for production of sexed semen. The obtained information can be beneficial for optimization of the sheep reproduction.

Acknowledgments

The research leading to these results has received funding from the Ministry of Education and Science under the National science program INTELLIGENT ANIMAL HUSBANDRY, grant agreement № Д01-62/18.03.2021.

References

1. Acharya, M., Burke, J. M., Rorie, R. W. Effect of Semen Extender and Storage Temperature on Motility of Ram Spermatozoa. Advances in Reproductive Sciences. 2020. T. 8. P. 14–30.
2. Agasi F. A., Yusuf M., Said S., Toleng A. L. The quality of Sumba Ongole sperms after sexing using bovine serum albumin column in Bracket Oliphant extender at different temperatures. 2020. IOP Conference Series: Earth and EnvironmentalScience. T. 492. 012066. doi:10.1088/1755-1315/492/1/012066
3. Amann RP, Waberski D. Computer-assisted sperm analysis (CASA): capabilities and potential developments. Theriogenology. 2014. T. 81. P. 5–17.
4. Buchelly Imbachí F, Zalazar L, Pastore JI, Greco MB, Iniesta-Cuerda M, Garde JJ, Soler AJ, Ballarin V, Cesari A. Objective evaluation of ram and buck sperm motility by using a novel sperm tracker software. Reproduction. 2018. T. 156. P. 11–21.
5. Burroughs, C. A. Removing Seminal Plasma Improves Sex-Sorting of Bovine Sperm.Thesis. 2011. Department of Biomedical Sciences. Colorado State University. Colorado. P. 17.
6. Carvalho J. O., Sartori R., Rodello L., Mourão G. B., Bicudo S. D., Dode M. A. N. Flow cytometry sex sorting affects bull sperm longevity and compromises their capacity to bind to oviductal cells. Livestock Science. 2018. T. 207. P. 30–37.
7. Ferreira-Silva J.C., Moura M.T., Basto S.R.L., Oliveira L.R.S., Caldas E.L.C., Filho M.L.S., Oliveira M.A.L. Use of Percoll Density Centrifugation for Sperm Sexing in Small Ruminants. Global Journal of Science Frontier Research: D Agriculture and Veterinary. 2017. T. 17. P. 55–59.
8. Gonzalez-Marín C., Gongora C.E., Moreno J.F., Vishwanath R. Small ruminant SexedULTRA™ sperm sex-sorting: Status report and recent developments. Theriogenology. 2021. T. 162. P. 67–73.
9. Hadi S., Al-Timimi I.H. Separation of Y-chromosome Bearing Ram's Sperms using an Albumin Gradient Technique and Identification of Embryos by PCR. AL-Qadisiyah Journal of Veterinary Medicine Sciences. 2013. T. 12. P. 144–151.
10. Hamano K. Sex preselection in bovine by separation of X- and Y-chromosome bearing spermatozoa. The Journal of reproduction and development. 2007. T. 53. P. 27–38.
11. Hollinshead F.K., O'Brien J.K.,Maxwell W.M.C., Evans G. Production of lambs of predetermined sex after the inseminatin of ewes with low numbers of frozen-thawed sorted X- or Y-chromosome-bearing spermatozoa. Reproduction, Fertility and Development. 2002. T. 14. P. 503–508.
12. Hollinshead FK, Evans G, Evans KM, Catt SL, Maxwell WM, O'Brien JK. Birth of lambs of a pre-determined sex after in vitro production of embryos using frozen-thawed sex-sorted and re-frozen-thawed ram spermatozoa. Reproduction. 2004. T. 127. P. 557–68.
13. Johnson LA. Sexing mammalian sperm for production of offspring: the state of- the-art. Animal Reproduction Science .2000. T. 61. P. 93–107.

14. Maicas C, Holden SA, Drake E, Cromie AR, Lonergan P, Butler ST. Fertility of frozen sex-sorted sperm at 4 × 106 sperm per dose in lactating dairy cows in seasonal-calving pasture-based herds. Journal of Dairy Science. 2020. T. 103. P. 929–939.
15. Maxwell WMC, Meddoa G, Shite IG. Post thawing survival of motile ram sperm after isolation by layering on protein columns. Theriogenol. 1984. T. 21. P. 601–606.
16. Ollero M, Perez-Pe R, Gargallo I, Morlanes S, Osada J, MuinoBlanco T, Cebrian-Perez J (2000). Separation of ram spermatozoa bearing X and Y chromosome by centrifugal countercurrent distribution in an aqueous two phase system. 2000. Journal of Andrology. T. 21. P. 921–928.
17. Rickard J. P., Schmidt R. E., Maddison J. W., Bathgate R., Lynch G. W., Druart X., de Graaf S. P. Variation in seminal plasma alters the ability of ram spermatozoa to survive cryopreservation. Reproduction, Fertility and Development. 2014. T. 28. P. 516–523.
18. Robayo I, Montenegro V, Valdés C, Cox JF. CASA assessment of kinematic parameters of ram spermatozoa and their relationship to migration efficiency in ruminant cervical mucus. Reproduction Domestic Animmals. 2008. T. 43. P. 393–399. doi: 10.1111/j.1439-0531.2007.00920.x.
19. Rodríguez-Martínez H., Vega F.P. Semen technologies in domestic animal species. Animals Frontiers. 2013. T. 3. P. 26–33.
20. Solihati N, Rasad SD, Yusrina A, Winangun K, Toha. Quality and longevity of local ram's sexed sperm with albumin column. Jurnal Ilmu Ternak. 2019. T. 19. P. 113–121. DOI: 10.24198/jit.v19i2.25632
21. Steele H, Makri D, Maalouf WE, Reese S, Kolle S. Bovine sperm sexing alters sperm morphokinetics and subsequent early embryonic development. Scientific Reports. 2020. T. 10. P. 6255.
22. Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology. 2002. T. 57. P. 149–179.
23. Wilson-Leedy J., Ingermann R. 2011. Computer assisted sperm analysis using ImageJ; description of necessary components and use of free software. (available at: https://imagej.nih.gov/ij/plugins/docs/CASAinstructions.pdf)
24. Urry RL, Middleton RG, McNamara L, Vikari CA. The effect of single-density bovine serum albumin columns on sperm concentration, motility, and morphology. Fertility and Sterility. 1983. T. 40. 666–669.

Received 8 July 2021
Accepted 15 November 2021

Effect of Hydroponic Green Forage Supplementation during Prepartum and Lactation on Sow and Litter Performances

Soledad Velásquez Amores[1], Elizabeth Breininger[1,2], Alejandra Lorenzo Smirnoff[1], Nicolás Caggiano[1], Emilio De Simone[1], Marcelo Acerbo[1], Verónica De Luca Sarobe[1]

[1]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias. Buenos Aires, Argentina

[2]CONICET - Universidad de Buenos Aires. Investigaciones en Producción Animal (INPA). Buenos Aires, Argentina

Keywords: *Swine, hydroponic green forage, lactation, nutrition, fibre.*

Abstract. *The incorporation of fibre in the diet of pregnant sows improves the performance during pregnancy and increases voluntary consumption during lactation. Hydroponic green forage (HGF) constitutes a method of cultivation without soil in controlled environmental conditions. The aim of this study was to evaluate the impact of the supplementation with HGF in lactating sows on productive performance and to investigate the metabolic state through the analysis of different biochemical parameters. Twelve sows of the Porcine Productive Unit of the Faculty of Veterinary Sciences were randomly assigned to 2 feeding groups: control (conventional diet) or HGF (hydroponic green forage diet, conventional diet supplemented with HGF) from 7 days prepartum until day 28 postpartum. Productive parameters in sows (weaning-to-oestrus interval and back fat thickness) and in the litter (number of piglets born and weaned, and piglet weight) were evaluated. The effect of the diet in the metabolism of the sows was evaluated by biochemical parameters (total plasma proteins, albumin, glucose, urea and creatinine). Supplementation with HGF did not significantly affect the litter size nor productive parameters but produced a higher weight of piglets at day 60. Although lactation affected some biochemical parameters, no substantial negative consequences of the HGF supplemented diet were observed. Our results suggest that the use of HGF could be an option to take into account in porcine production with economic and environmental benefits.*

Introduction

The transition from pregnancy to lactation is characterized by physiological changes in sows. It is accompanied by important changes in feeding. Nutritional restriction is a very common feed management performed in pregnant sows in order to avoid extra weight gain and problems with locomotion and farrowing. In contrast, ad libitum ingesting is encouraged during lactation to cover the nutritional requirements (Dourmad et al., 1996). Nevertheless, in prolific sows, the voluntary feed intake is generally insufficient to meet the demands (Boulot et al., 2008). The incorporation of fibre in the diet of pregnant sows, without altering the supplementation of daily energy, has shown a decrease in the stereotypic behaviour associated with the level of restricted feeding during pregnancy (Meunier-Salaün et al., 2001) and an increase in voluntary feeding during lactation (Courboulay & Gaudre, 2002). Worldwide, porcine diets are supplemented with a wide range of high fibre ingredients. These diets do not always maximize production parameters but they allow the use of locally grown food and thus contribute to a sustainable production (Jarrett & Anshworth, 2018). Although its use in non-ruminant animals has some limits, fibre can produce a large amount of benefits that requires further investigation, especially in peripartum sows (Oliviero et al., 2009).

Hydroponic green forage (HGF) constitutes a method of cultivation without soil in controlled environmental conditions, which allow obtaining a feed supplement in a few days with a higher crude protein content than conventional forage. The forage produced by this method is highly nutritious and with good palatability. Furthermore, it offers a sustainable production throughout the year, conserves water, requires minimal work for its production and is friendly to the environment because it does not use pesticides and does not present wasted nutrients (Pandey & Pathak, 1991). Animals fed with HGF have increased milk production with a higher content of fat and total solids (García-Carrillo et al., 2013). In this regard, HGF can be an easy and quick alternative to apply for the porcine producer. It is also a non-expensive option that would promote their animal feed production.

Many factors can affect the productive performance of pigs. Among them, nutrition plays an important role affecting both the metabolic state and productive parameters. However, little information is available about nutritional supplementation with HGF in pigs. The aim of this study was to evaluate the productive performance of sows and their litters after supplementing the sows with HGF during prepartum and lactation. In addition, the metabolic status was investigated by analyzing different biochemical sow parameters.

Correspondence to Elizabeth Breininger, INPA. Faculty of Veterinary Sciences. University of Buenos Aires. Chorroarín 280. C1427CWO, CABA, Argentina. E-mail: ebreininger@fvet.uba.ar

Materials and methods

This study was conducted in accordance with the guidelines of the Institutional Committee for Care and Use of Experimental Animals, Faculty of Veterinary Sciences, Buenos Aires University.

Animals and diet

The study was conducted with the animals of the Porcine Productive Unit of the Faculty of Veterinary Sciences (University of Buenos Aires). Twelve parity sows (Landrace x Yorkshire) of the first or the second gestation were used, and all of them mated with Landrace x Yorkshire boars. These sows were randomly assigned to 2 feeding groups: control (conventional diet) and HGF (hydroponic green forage diet, conventional diet supplemented with HGF) from 7 days prepartum until day 28 postpartum. The composition of the 2 diets is shown in Table 1. Sows started feeding the maintenance recommendation (6.8 Mcal metabolizable energy (ME) / day) since the day of birth, and the amount of food was increased until day 8 (1.7 ME / piglet / day). Then, the feed intake was maintained until weaning. The animals were fed twice a day, once in the morning and once in the afternoon. Water was available ad libitum and no considerable feed refusal was observed during the whole study.

Productive parameters evaluation

Oestrus detection was evaluated after weaning. A weaning-to-oestrus interval was recorded after oestrus confirmation by a female standing reflex in the presence of a boar. Back fat thickness (BF) was measured at partum (day 0) and weaning (day 28). The measurements of BF were performed at the P2 point (between the last and penultimate rib, at a distance of 5 cm from the vertebral column) with a Sonoscape A5 with a linear transducer of 5 to 12 Mhz.

Litter size, number of piglets born, number of piglets weaned and total kg of piglets weaned per litter were determined. Piglet weight was evaluated over 60 days (day 0, 28 and 60).

Metabolic parameters evaluation

Blood samples were taken to evaluate metabolic parameters during the whole study. The samples were obtained from the jugular vein from day 7 prepartum until day 28 postpartum. Samples were centrifuged at 400 g for 10 min and the serum obtained was immediately frozen at –20°C until analysis. Plasma concentrations of total plasma proteins, albumin, glucose, urea and creatinine were measured in a Shimadzu UV-VIS spectrophotometer, model UV-1900i. Total plasma protein and albumin levels were determined by the biuret colorimetric method. Glucose concentration was measured using a spectrophotometric assay based on the oxidation of the sugar by glucose oxidase and the subsequent determination of the hydrogen peroxide formed. Urea was measured with the assay of urease that decomposes urea, producing carbon dioxide and ammonia. The latter reacts with phenol and hypochlorite in the alkaline medium, producing indophenol blue, which is colourimetrically measured. Creatinine reacts with the alkaline picrate (Jaffe reaction) yielding a red chromogen that can be quantified by a photometric reading. All determinations were carried out with Wiener lab kits according to the manufacturer's instructions (Wiener lab, Rosario, Argentina).

Statistical analyses

Results are given as mean and standard error of the mean (SEM). The quantitative data collected were analyzed for normality assumption by the Shapiro-Wilk test and variances homogeneity using the Levene test. Productive parameter values were analysed by the Student t test except for BF, which was evaluated by the paired Student t test. Piglet weight and metabolic parameters were analysed by two-way ANOVA (treatment, days and their interactions) according to a repeated measure model. The Bonferroni test or post hoc general contrast was used for comparison among means. A value of $P < 0.05$ was considered as statistically significant. All statistical tests were performed with InfoStat (Córdoba University, Córdoba, Argentina, see http://www.infostat.com.ar/).

Results

Supplementation with hydroponic green forage did not significantly affect litter size nor sow productive parameters. However, this supplementation produced a higher weight in piglets at 60 days of life.

No significant effect of HGF supplementation

Table 1. Ingredients and dietary composition

	Control				HGF		
Ingredients	%	g	Mcal ME		%	g	Mcal ME
Ground corn	68	680	2.40		54.1	605	2.13
Soybean pellet	28.9	289	1.07		23	257	0.94
Vitamin and mineral premix	3.1	31	–		2.8	31	
HGF	–	–	–		20.1	225	0.35
Total	100	1000	3.47		100	1118	3.42

HGF: hydroponic green forage; ME: Metabolizable energy. Control diet: maintenance recommendation 2000 g per day; lactation recommendation 500 g per piglet per day. HGF diet: maintenance recommendation 2240 g per day; lactation recommendation 560 g per piglet per day.

was observed in the weaning-to-oestrus interval. Regarding back fat thickness, a similar significant decrease was observed during lactation days (23% in control and 17% in HGF treatment) without differences between treatments (Table 2).

Litter productive sizes were not significantly affected by HGF supplementation. Piglet weight increased during lactation time. At day 60, a significant difference was observed in the HGF group (Table 3).

Some metabolic parameters in sows were affected in the lactation period. Total plasma proteins increased up to day 7 postpartum and then returned to prepartum levels, but only for the control treatment; in HGF supplementation, no differences were observed during lactation days (Fig. 1). Albumin was not affected by supplementation, as its value decreased

Table 2. Effect of hydroponic green forage supplementation on sows' productive parameters

	Treatment	
Productive parameter	Control	HGF
Weaning-to-oestrus interval	5.25 ± 0.22^{a}	4.93 ± 0.16^{a}
BF (mm) at day 0	22.89 ± 0,83^{a}	23.40 ± 0.73^{a}
BF (mm) at day 28	18.58 ± 1.05$^{a\#}$	19.94 ± 0.88$^{a\#}$

Values (mean ± SEM) of control and HGF (hydroponic green forage) treatments. BF: back fat thickness. Columns with different letters indicate significant ($P < 0.05$) differences between treatments. $^{\#}$ indicates significant differences between day 0 and 28.

Table 3. Effect of hydroponic green forage supplementation on litter productive parameters

	Treatment	
Productive parameter	Control	HGF
Number of piglets born	13.15 ± 0.61^{a}	13.43 ± 0.79^{a}
Number of piglets weaned	10.54 ± 0.57^{a}	10.57 ± 0.69^{a}
Total kg of piglets weaned per litter	81.60 ± 4.72^{a}	85.46 ± 3.02^{a}
Piglet weight (kg) at day 0	1.24 ± 0.04$^{a\#}$	1.48 ± 0.04$^{a\#}$
Piglet weight (kg) at day 28	7.57 ± 0.27$^{a\#\#}$	7.94 ± 0.14$^{a\#\#}$
Piglet weight (kg) at day 60	13.61 ± 0.51$^{a\#\#\#}$	16.13 ± 0.53$^{b\#\#\#}$

Values (mean ± SEM) of control and HGF (hydroponic green forage) treatments. Columns with different letters indicate significant (< 0.05) differences between treatments. $^{\#, \#\#, \#\#\#}$ indicate significant differences between days.

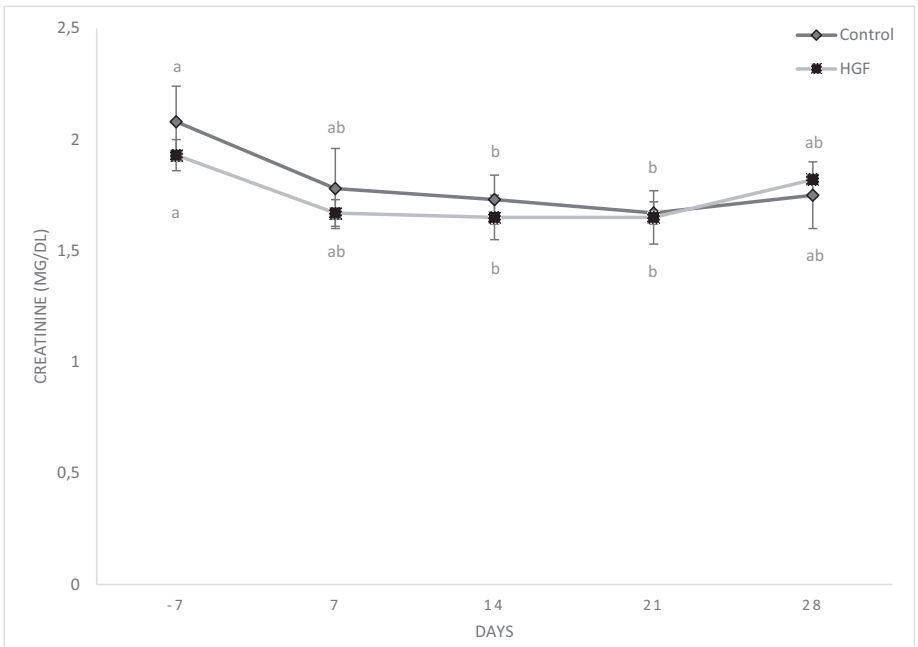

Fig. 1. Mean ± SEM of total plasma protein blood concentration in control and HGF supplemented diet. Values with different letters indicate significant ($P < 0.05$) differences between days.

at day 7 and then returned to prepartum levels in both treatments (Fig. 2). Glucose was not affected by supplementation treatment nor lactation (Fig. 3). Only in the control treatment, the urea increased up to day 21 and then returned to prepartum levels. In the case of HGF supplementation, a slight increase was observed with no significant differences with prepartum levels (Fig. 4). Creatinine was not affected by supplementation, decreased up to day 21, and then returned to prepartum levels (Fig. 5).

Discussion

The incorporation of HGF in diets of lactating sows did not affect productive parameters. Our results demonstrated no substantial negative consequences of the HGF supplemented diet on metabolic and productive parameters. In addition, a positive effect was observed on the weight of piglets, which was higher in HGF litters at 60 days.

Milk production in the sow has the highest priority during lactation and is positively affected by

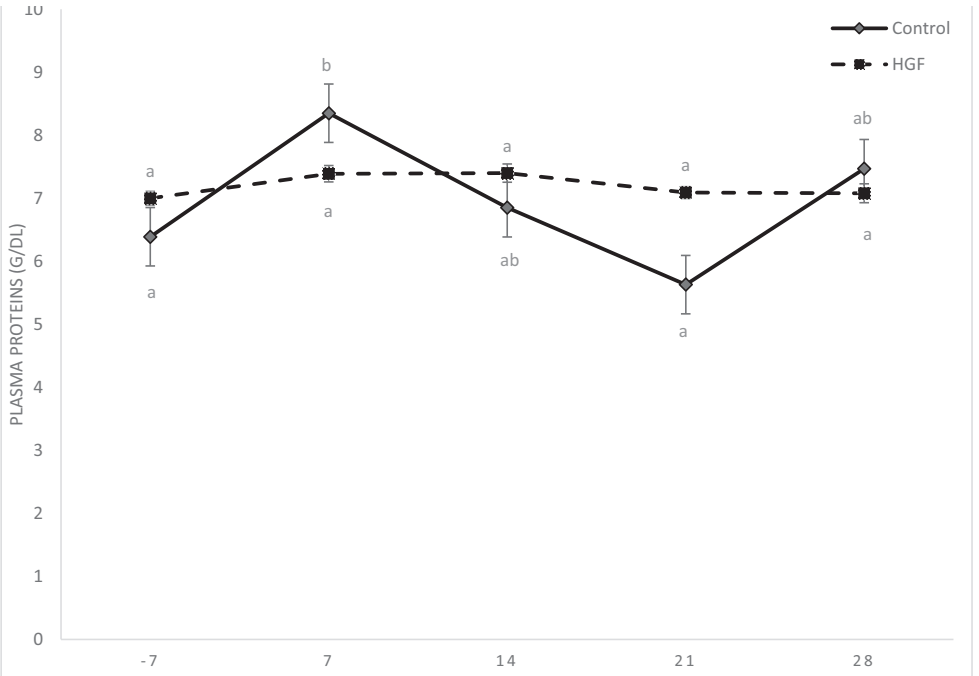

Fig. 2. Mean ± SEM of albumin blood concentration in control and HGF supplemented diet. Values with different letters indicate significant ($P < 0.05$) differences between days.

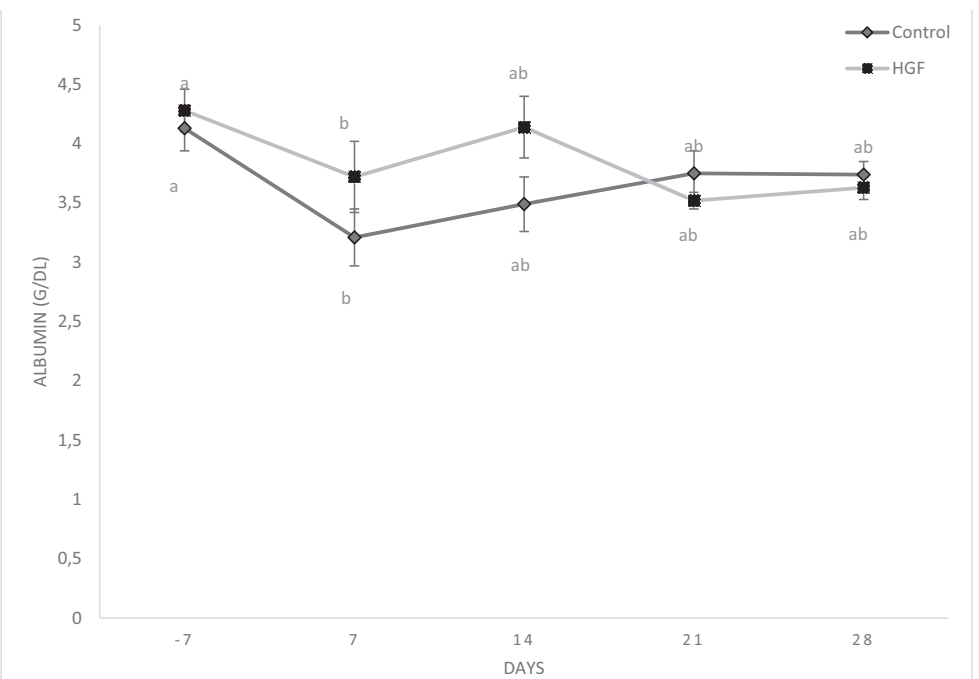

Fig. 3. Mean ± SEM of glucose blood concentration in control and HGF supplemented diet. Values with different letters indicate significant ($P < 0.05$) differences between days.

feed intake (Vadmand et al., 2015). All the nutrients are directed towards the mammary glands, which is reflected in a higher weight gain of the litter (Koketsu et al., 1997). Interestingly, this effect could not be due to the high feed intake, as the additional feed intake during lactation did not appear to be converted into milk production as no effect in the weight gain on the litter was observed (Mallmann et al., 2018). Although the feed supply was not ad libitum in our study, we observed that piglets from sows with HGF supplementation presented higher body weight at 60 days than those from sows of the control group.

Fibre contains substances such as cellulose that is not easily digested by non-ruminant animals. However, in pigs some of the fibre digestion takes place in the cecum and the colon due to the action of cellulolytic bacteria. The metabolism of these substances produces volatile fatty acids, which can provide up to 28% of the energy balance in piglets and even more in sows (Noblet & Le Goff, 2001). According

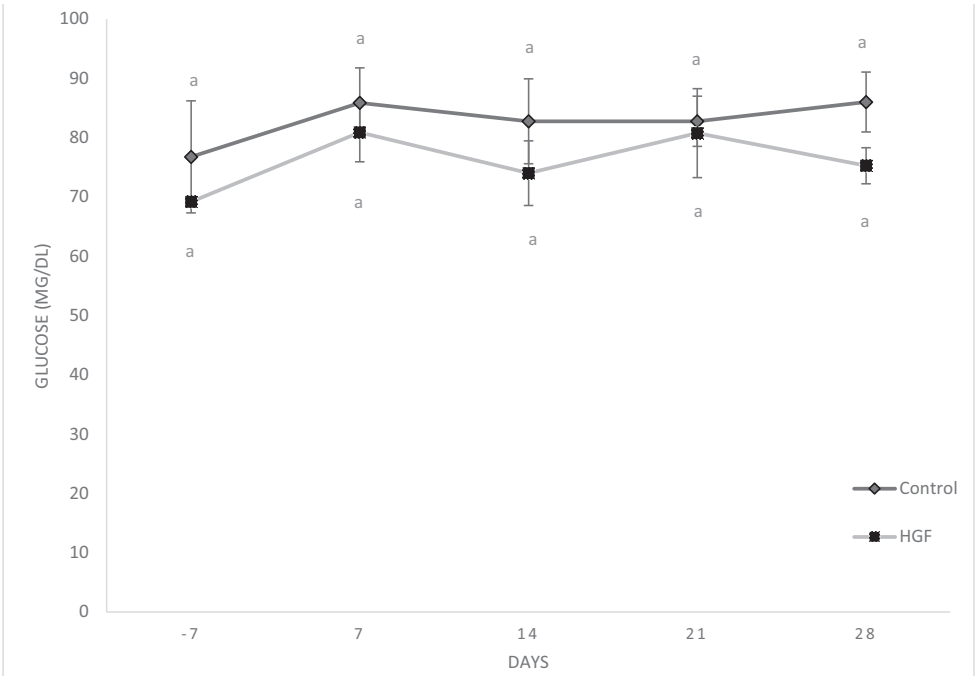

Fig. 4. Mean ± SEM of urea blood concentration in control and HGF supplemented diet. Values with different letters indicate significant ($P < 0.05$) differences between days.

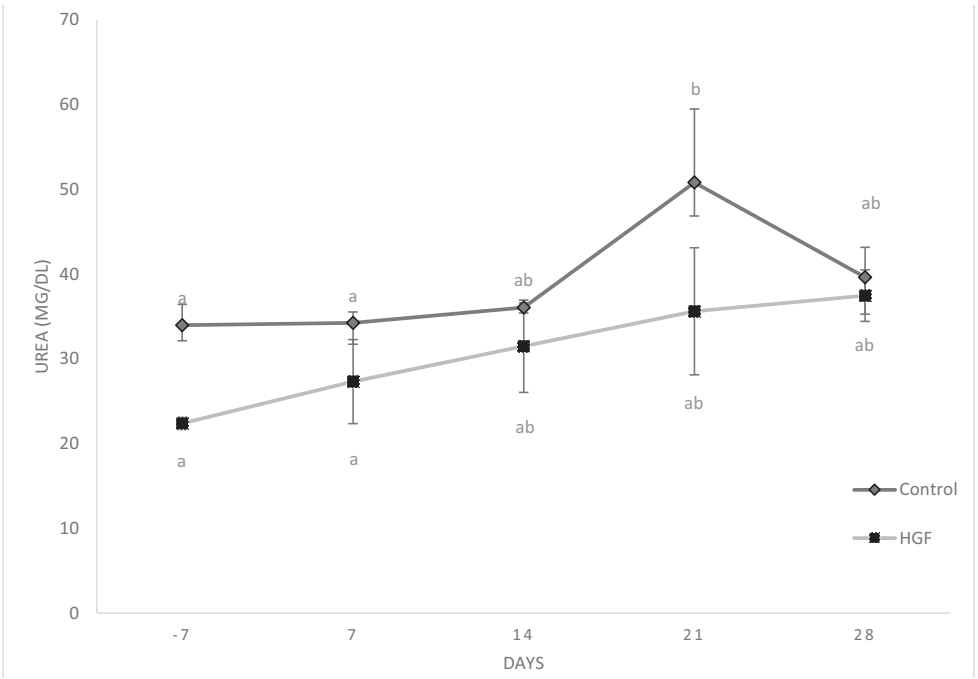

Fig. 5. Mean ± SEM of total creatinine blood concentration in control and HGF supplemented diet. Values with different letters indicate significant ($P < 0.05$) differences between days.

to this, the supplementation with HGF did not affect the body condition of the lactating sows, as the same behaviour was observed in the back fat thickness with a decrease during lactation without differences between treatments. Nevertheless, a low feed intake during lactation and a great body mobilization can have a negative effect on the following reproductive cycle by increasing the weaning-to-oestrus interval (Baidoo et al., 1992). In our study, no increase of this interval was observed reinforcing the idea that HGF supplementation does not affect body condition.

The biochemical values found in the animals included in the study were in accordance with the reference values (Friendship et al., 1984). These parameters are of a great value for the clinical and productive interpretation and necessary to resolve any changes that may be observed. The effect of fibre supplementation on metabolic parameters is not fully elucidated. Some studies showed that the high fibre supplemented diets present lower levels of insulin-like growth factor-1 (IGF-1), leptin and lower plasma concentrations of ß-hydroxybutyrate, glucose, insulin and urea (Jégou et al., 2016; Weaver et al., 2013). In contrast, other studies have found no clear effect of dietary fibre or glucose and insulin responses overall. Moreover, the high fibre diet resulted in increased plasma short-chain fatty acids and non-esterified fatty acids (Yde et al., 2011). In this study, the total plasma protein concentration increased in the postpartum but only in the control treatment, whereas the HGF group presented no differences during the lactation period. It is known that the higher fibre content of feed promoted a higher water intake, which could stimulate the sows to drink more (Oliviero et al., 2009). The increase of total plasma proteins observed could be due to this slight dehydration observed in the control group. Plasma concentration of albumin throughout the study was similar in both groups, suggesting that the availability of proteins in the HGF group is suitable. Albumin production occurs in the liver and decreased concentrations are indicative of protein deficiency (Jahhor et al., 1996). Protein availability measured by albumin has been positively correlated with the ovulation rate and negatively correlated with the weaning-to-oestrus interval (Rempel et al., 2018), which proposes a favourable effect of protein availability on reproductive performance. Lactation diminishes glucose due to a greater absorption of it by the mammary gland for lactose synthesis (Dourmad et al., 2000). The lack of response of glucose concentration could be due to analytical techniques or diet composition as insulin concentration was higher in sugar beet pulp fed pigs compared with potato pulp and pectin (Yde et al., 2011). The greater circulation of urea during lactation is related to the increased protein intake or the increased catabolism of endogenous protein (Quesnel et al., 2009) as milk production has a great impact on protein metabolism (Strathe et al., 2017). Regarding plasma urea, our results showed that in both groups its concentration increased during lactation, largely due to the increased consumption of nutrients in general and protein in particular. The increase in the HGF group was slightly superior with respect to the control group, possibly, owing to less endogenous protein catabolism as suggested in the results of the determination of plasma creatinine. During lactation, the increase in feed consumption in the sow can be hampered and, as milk production increases, many sows could become catabolic in this period (Hansen et al., 2012). Plasma creatinine is the most efficient indicator of muscle catabolism since it is a direct product of creatinine metabolism (Mitchell & Scholz, 2001). Slightly different results observed in the HGF group could indicate a better energy balance related to biochemical parameters and could suggest an indirect effect of the diet on muscle catabolism.

Conclusions

Our findings indicate that supplementation of the diet with HGF did not affect productive performance in the sow or in the litter. Adding to these results, no negative effects on energy balanced related biochemical parameters were found. The higher weight of piglets observed at 60 days suggests that the use of HGF could be an option to take into account in porcine production with economic and environmental benefits.

Acknowledgements

This work was supported by the University of Buenos Aires, Research project UBACyT 20020170200051BA "Performance of pigs fed with hydroponic green fodder. Evaluation of productive parameters and analysis of metabolic and immunological profiles". We are deeply grateful to Santiago Pillado for the handling of the animals during the entire study.

References

1. Baidoo S.K., Aherne F.X., Kirkwood R.N., Foxcroft G.R. Effect of feed intake during lactation and after weaning on sow reproductive performance. Canadian Journal of Animal Science. 1992. T. 72. P. 911-917.
2. Boulot S., Quesnel H., Quinou N. Management of high prolificacy in French herds; can we alleviate side effects on piglet suvival? Proceedings of the Banff Pork Seminar. Advances in Pork Production, University of Alberta. 2008. T. 19. P. 213-220.
3. Courboulay V., Gaudre D. Is it necessary to give fibrous diets to group-housed sows? Journées Recherche Porcine. 2002. T. 34. P. 225-232.
4. Dourmad J., Ettiene M., Noblet J. Reconstitution of body reserves in multiparous sows during pregnancy: Effect of energy intake during pregnancy and mobilisation during the previous lactation. Journal of Animal Science. 1996. T. 74. P. 2211-2219.
5. Dourmad J., Matte J., Lebreton Y., Fortin M. *Influence du repas sur l'utilisation des nutriments et des vitamins par la mamelle chez la truie en lactation*. Journal Recherche Porcine. 2000. T. 32. P. 265-273.
6. Friendship R., Lumsden J., McMillan I., Wilson M. Hematology and biochemistry reference values for Ontario swine.

Canadian Journal of Comparative Medicine. 1984. T. 48. P. 390-393.
7. García-Carrillo M., Salas-Pérez L., Esparza-Rivera J., Preciado-Rangel P., Romero-Paredes J. Producción y calidad fisicoquímica de leche de cabra suplementadas con forraje verde hidropónico de maíz. Agronomía mesoamericana. 2013. T. 24(1). P. 169-176.
8. Hansen A.V., Lauridsen C., Sørensen M.T., Bach Knudsen K.E., Theil P.K. Effects of nutrient supply, plasma metabolites, and nutritional status of sows during transition on performance in the next lactation. Journal of Animal. Science. 2012. T. 90. P. 466-480.
9. Jahoor F., Bhattiprolu S., Del Rosario M., Burrin D., Wykes L., Frazer M. Chronic protein deficiency differentially affects the kinetics of plasma proteins in young pigs. The Journal of Nutrition. 1996. T. 126. P. 1489-1495.
10. Jarrett S., Anshworth C. The role of dietary fibre in pig production, with a particular emphasis on reproduction. Journal of Animal Science and Biotechnology. 2018. T.9. P. 59.
11. Jégou M., Gondret F., Lalande-Martin J., Tea I., Baéza E., Louveau I. NMR-based metabolomics highlights differences in plasma metabolites in pigs exhibiting diet-induced differences in adiposity. European Journal of Nutrition. 2016. T. 55. P. 1189-1199.
12. Koketsu Y., Dial G.D., Pettigrew J.E., King V.L. Influence of feed intake during individual weeks of lactation on reproductive performance of sows on commercial farms. Livestock Production Science. 1997. T. 49.P. 217-225.
13. Mallmann A.L., Betiolo F.B., Camilloti E., Mellagi A., Ulguim R.R., Wentz I., Bernardi M.L., Gonçalves M., Kummer R., Bortolozzo F.P. Two different feeding levels during late gestation in gilts and sows under commercial conditions: impact on piglet birth weight and female reproductive performance. Journal of Animal Science. 2018. T. 96(10). P. 4209-4219.
14. Meunier-Salaün M., Eduards S., Robert S. Effect of dietary fibre on the behavior and health of the restricted fed sow. Animal Feed Science and Technology. 2001. T. 90. P. 53-69.
15. Mitchell A.D., Scholz A.M. Techniques for measuring body composition of swine. In Swine nutrition (Ed. S. L. Lewis AL). CRC Press USA. 2001. P. 32-37.
16. Noblet J., Le Goff G. Effect of dietary fibre on the energy value of feeds for pigs. Animal feed Science and Technology. 2001. T. 90. P. 35-52.
17. Oliviero C., Kokkonen T., Heinonen M., Sankari S., Peltoniemi O. Feeding sows with high fibre diet around farrowing and early lactation: impact on intestinal activity, energy balance related parameters and litter performance. Research in Veterinary Science. 2009. T. 86. P. 314-319.
18. Pandey H., Pathak N. Nutritional evaluation of artificially grown barley fodder in lactating crossbred cows. Indian Journal of Animal Nutrition. 1991. T. 8(1). P. 77-78.
19. Quesnel H., Meunier-Salaün M.C., Hamard A., Guillemet R., Etienne M., Farmer C., Dourmad J.Y., Père M.C. Dietary fiber for pregnant sows: Influence on sow physiology and performance during lactation. Journal of Animal Science. 2009. T. 87. P. 532-543.
20. Rempel L., Wallet J., Nonneman D. Characterization of plasma metabolites at late gestation in early parity sows on production and post-weaning reproductive performance. Journal of Animal Science. 2018. T. 96. P. 521-531.
21. Strathe A.V., Bruun T.S., Hansen C.F. Sows with high milk production had both a high feed intake and high body mobilization. Animal. 2017. T. 11(11). P. 1913-1921.
22. Vadmand C.N., Krogh U., Hansen C.F., Theil P.K. Impact of sow and litter characteristics on colostrum yield, time for onset of lactation, and milk yield of sows. Journal of Animal Science. 2015. T. 93.P. 2488-2500.
23. Weaver A.C., Kelly J.M., Kind K.L., Gatford K.L., Kennaway D.J., Herde P.J., Van Wettere W.H. Oocyte maturation and embryo survival in nulliparous female pigs (gilts) is improving by feeding a lupin-based high fibre diet. Reproduction, Fertility and Development. 2013. T. 25. P. 1216-1223.
24. Yde C.C., Bertram H.C., Theil P.K., Knudsen K.E. Effects of high dietary fibre diets formulated from by-products from vegetable and agricultural industries on plasma metabolites in gestating sows. Archives of Animal Nutrition. 2011. T. 65.P. 460-476.

Received 19 July 2021
Accepted 29 December 2021

Investigations on Antimicrobial Resistance in Commensal *Escherichia Coli* Isolates from Waterfowl (Ducks) and Turkeys

Valentina Urumova[1], Radostina Stefanova[1], Mihni Lyutskanov[1]
[1]*Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora-6000, Bulgaria*

Keywords: *Escherichia coli, resistance to antimicrobial agents, waterfowl, turkeys*

Abstract. *The aim of the present study was to investigate the prevalence of the phenotypic profiles and some genetic determinants of antimicrobial resistance to third generation cephalosporins, i.e., cefotaxime and ceftazidime, fluorinated quinolones and tetracyclines in commensal E. coli isolated from waterfowl (ducks) and turkeys.*

According to the requirements of the European Commission (Directive 2003/99), Bulgaria provides data on the phenotypic manifestations of antimicrobial resistance in Salmonella spp. and commensal Escherichia coli strains from the farm animals, but data on the genetic characteristics of resistant commensal Escherichia coli are limited. Due to limited data on resistance in commensal Escherichia coli isolates from ducks and turkeys in Bulgaria, we attempted to analyze data related to some genetic factors-determined resistance to chemotherapeutic drugs. In our study, we highlighted some of the most common genetic factors of resistance to cefotaxime and ceftazidime, bla $_{CTX-M-1}$ gene in poultry isolates in Europe. Also, plasmid-mediated resistance to fluorinated quinolones in Escherichia coli strains from poultry is often realized with the participation of genes determining resistance to cefotaxime, ceftazidime, gentamicin, tetracycline, etc.

From October 2020 to May 2021, 220 cloacal swab samples were collected in Stuart transport medium: 110 from waterfowl and 110 from turkeys. Ninety-three E. coli strains were isolated from the 110 waterfowl cloacal swabs, while 78 E. coli strains were isolated from the 110 turkey swabs. The E. coli isolates resistant to cefotaxime and ceftazidime were examined for the presence of bla$_{CTX-M-1}$ gene. Bacterial strains resistant to ciprofloxacin were examined for presence of plasmid-determined genes qnrS, qnrA and qnrB1, whereas those resistant to tetracycline were examined for tetA and tetB genes.

The highest percentage of waterfowl E. coli isolates exhibited resistance to tetracycline (81.7%), followed by resistance to ampicillin (75.3%). A high resistance was also observed with respect to ciprofloxacin (66.7%). The coli bacterial isolates from turkeys were most frequently resistant to tetracycline (71.8%) and ampicillin (70.5%), followed by ciprofloxacin (58.9%). As resistance genes were concerned, a significant prevalence was noted for tetA gene (81.7% and 71.8% in waterfowl and turkey strains) and qnrS gene (26.9% and 26% in waterfowl and turkey strains).

Introduction

The broad use of antimicrobial drugs creates appropriate conditions for the emergence and spread of resistance both among pathogenic bacterial microflora and commensal bacteria. The spread of antimicrobial resistance among zoonotic and commensal bacteria may pose risks related to a compromise of efficient therapy of human infectious diseases. In men, there are different mechanisms for transfer of zoonotic and commensal enterobacteria resistant to antimicrobial drugs, e.g. consumption of processing of contaminated foodstuffs, direct contact with animals and various environmental sources (soil, water, manure, etc.) (Argudìn et al., 2017). The monitoring of antimicrobial resistance in commensal enterobacteria, which are ubiquitously spread, is a good background for analysis of the selective pressure and early indicators for distribution of genetic determinants of resistance in different sectors of intensive livestock husbandry (EFSA, 2008).

The opinion of FAO/WHO/OIE (2008) is that the efficiency of third and fourth generation cephalosporins, as well as fluoroquinolones, which are all critically important for medicine, should not be compromised by their inadvertent use in the animal breeding and the agrarian sector. A negative example is the increasing spread of *E. coli* strains producing extended-spectrum beta-lactamases with a connection to the risk from horizontal transfer of genetic determinants of resistance to third and fourth generation cephalosporins (Saliu et al., 2017; Madec et al., 2017). Thus, for instance, the EFSA report (2020) discusses the very high prevalence of resistance to ciprofloxacin (73.5%) in commensal *E. coli* isolates from birds, i.e., the averagely high prevalence of *E. coli* from turkeys (34.8%). The report affirms that multi-drug resistant *E. coli* from broiler chickens were resistant to ciprofloxacin in 78.9% of cases, whereas isolates from turkeys were resistant in 71.7% of cases.

Correspondence to Valentina Urumova Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora-6000, Bulgaria. E-mail: valentina_62@abv.bg

On the other hand, tetracyclines are among the antimicrobial drugs that are most commonly used in veterinary medicine. What is more, the resistance to tetracyclines among commensal *E. coli* isolated from poultry and pigs is evaluated as high (Sengelov et al., 2003; ESVAC, 2019).

The aim of the present study was to investigate the prevalence of the phenotypic profiles and some genetic determinants of antimicrobial resistance to third generation cephalosporins, i.e., cefotaxime and ceftazidime, fluorinated quinolones and tetracyclines in commensal *E. coli* isolated from waterfowl (ducks) and turkeys.

Material and methods

From October 2020 to May 2021, 220 cloacal swab samples were collected in Stuart tranpsort medium: 110 from waterfowl and 110 from turkeys. Fifty cloacal swabs were obtained from 9-day-old turkey poults and 60 swabs from 12-month-old turkeys. The waterfowl samples originated from 2 farms located in South Bulgaria. In one of the farms, eggs were imported from France. The turkey samples originated from one farm from the central region of the country.

For the isolation of *E. coli*, we used MacConkey agar, and for the preliminary identification procedure, we used triple sugar iron agar. Respectively, biochemical identification of suspicious colonies was performed with the IMViC test (indole+/MR+/VP–/citrate–). *E. coli* isolates were identified by means of the semi-automated system Crystal (Becton, Dickinson, USA).

For phenotype analysis of *E. coli* resistance to antimicrobial drugs, the disc diffusion method and the MIC determination approach were used. The concentrations of chemotherapeutics for the disc diffusion method were as followed: ampicillin (10 μg), ampicillin/clavulanic acid (20/10 μg), ceftazidime (10 μg), cefotaxime (5 μg), gentamicin (10 μg), tetracycline (30 μg) and ciprofloxacin (5 μg) (manufactured by Himedia Biosciences, India). For MIC determination, the E-test was performed with test gradient strips manufactured by Liofilchem (Italy). For analysis of *E. coli* strains' resistance to cefotaxime and ceftazidime, a confirmatory test for MIC determination with the combinations cefotaxime+clavulanic acid and ceftazidime+clavulanic acid was done. The results were interpreted as per EUCAST criteria. Statistical data processing was performed with a Graph Pad program.

Resistant *E. coli* isolates were investigated for presence of the $bla_{CTX\text{-}M\text{-}1}$ gene. Bacterial strains resistant to ciprofloxacin were examined for presence of plasmid-determined genes *qnrS*, *qnrA* and *qnrB1*. The strains resistant to tetracyclines were examined for presence of *tetA* and *tetB* genes.

DNA was extracted with DNeasy Blood Tissue kit (Qiagen, Germany), and for detection of genes encoding resistance to tested antimicrobial drugs, real-time PCR was performed based on TaqMan hydrolysis probes (DNA Assay kits, Qiagen, Germany). The temperature regime of the amplification reaction comprised initial activation step, 1X at 95°C for 10 min, the second stage included 2 steps with 40 cycles of denaturation and annealing/elongation, 40X denaturation at 95°C for 15 s, annealing/elongation at 60°C for 2 min.

The positive DNA control had a cut-off of $C_T \leqslant 34$, and the positive control for amplification reaction: $C_T = 22 \pm 2$.

Results

Ninety-three *E. coli* strains were isolated from the 110 waterfowl cloacal swabs, while 78 *E. coli* strains were isolated from the 110 turkey swabs. From the latter, 34 strains were from 9-day-old turkey poults and 44 from 12-month-old turkeys.

Table 1 presents the results for the prevalence of resistance to tested antimicrobial drugs among waterfowl and turkey *E. coli* isolates. Table 2 presents separately the data on antimicrobial resistance in turkey isolates according to the age (9-day-old and 12-month-old birds).

The highest proportion of *E. coli* isolates from waterfowl were resistant to tetracycline (81.7%),

Table 1. Resistance distribution among indicator *Escherichia coli* strains isolated from waterfowl and turkeys (October 2020 – May 2021)

Antimicrobial	Resistant *E.coli* strains isolated from waterfowl (n = 93)	Confidence Limits (CL)	Resistant *E.coli* isolated from turkeys (n = 78)	Confidence Limits (CL)
Ampicillin	70 (75.3%)	66.0÷83.4	55 (70.5%)	60.0÷80.0
Amoxicillin/clavulanic acid	17 (18.3%)	11.1 ÷26.7	32 (41.0%)	30.4÷52.0
Cefotaxime	–	–	2 (2.6%)	0.4÷7.2
Ceftazidime	–	–	2 (2.6%)	0.4÷7.2
Gentamicin	16 (17.2 %)	10.2÷25.4	14 (17.9%)	10.3÷27.1
Tetracycline	76 (81.7%)	73.2÷88.9	56 (71.8%)	61.5÷81.1
Ciprofloxacin	62 (66.7%)	56.7÷75.7	46 (58.9%)	47.9÷69.5

followed by those resistant to ampicillin (75.3%). The waterfowl isolates were also outlined with a broad resistance to ciprofloxacin (66.7%). Among them, no resistance to cefotaxime and ceftazidime was found out; however, among turkey isolates, such a resistance was detected in 2 strains (2.6%), isolated form 9-day-old birds. *E. coli* isolates from turkeys also demonstrated a higher spread of resistance to tetracycline (71.8%) and ampicillin (70.5%), with higher percentages among the 9-day-old group (73.5%) compared with birds at 12 months of age (70.4%, 68.2%). Turkey isolates showed resistance more commonly to ciprofloxacin (58.9%), and again, the values were higher in 9-day-old turkey poults (70.5%). The broader spread of resistance to the combination amoxicillin/clavulanic acid among turkey isolates should be also noted (41.0%), as compared with waterfowl isolates (18.3%).

Table 3 and 4 present the MIC of tested antimicrobial drugs for waterfowl and turkey *E. coli* isolates. For waterfowl strains, MIC_{90} for ampicillin was 8 μg/mL, and higher values – 16 μg/mL – were determined for turkey strains. For both groups of *E. coli* strains, MIC_{90} of 4 μg/mL was observed with respect to amoxicillin/clavulanic acid. The detected MIC_{90} values for cefotaxime and ceftazidime among turkey isolates were 0.125 μg/mL and 0.25 μg/mL, respectively. The MIC_{90} for ciprofloxacin (0.5 μg/mL) and for gentamicin (2 μg/mL) were the same in both groups of strains. Higher MIC_{90} of 16 μg/mL for tetracycline was determined for turkey isolates; the respective MIC_{90} for tetracycline in waterfowl strains was 8 μg/mL.

Table 5 presents the spread of some specific genetic factors encoding resistance to cephalosporins, ciprofloxacin and tetracycline in *E. coli* isolated from waterfowl and turkeys. In 61.3% of waterfowl *E. coli* isolates (respectively 29.5% of turkey isolates), a multi-resistance profile including ampicillin, tetracycline and ciprofloxacin was determined. In 16.1% of waterfowl *E. coli* strains, the resistance profile including tetracycline and ciprofloxacin was observed. In 17.9% of turkey strains, the multi-resistance profile including ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, tetracycline and ciprofloxacin was found out.

In tested waterfowl and turkey *E. coli* isolates, the genes *tetA* and *qnrS* were the most prevalent: the *tetA* gene was found out in 81.7% of waterfowl strains while *tetB* was found in 48.4%. The *tetA* gene was detected in 71.8% of turkey isolates, but the prevalence of *tetB* gene was lower (17.9%). None of resistant *E. coli* isolates carried the *qnrA* gene. On the other hand, the prevalence of *qnrS* was observed in 26.9% of waterfowl and 26.0% of turkey isolates.

Table 2. Resistance distribution among indicator *Escherichia coli* strains isolated from turkeys at different ages (October 2020 – May 2021)

Antimicrobial	Resistance *E.coli* isolated from turkeys 9 days old (n = 34)	Confidence Limits (CL)	Resistance *E. coli* isolated from turkeys 12 months of age (n = 44)	Confidence Limits (CL)
Ampicillin	25 (73.5%)	57.6÷86.7	30 (68.2%)	53.8÷84.7
Amoxicillin/clavulanic acid	15 (44.1%)	28.1÷60.8	17 (38.6%)	24.9÷53.3
Cefotaxime	2 (5.9%)	0.6÷16.1	–	–
Ceftazidime	2 (5.9%)	0.6÷16.1	–	–
Gentamicin	11 (32.3 %)	17.8÷48.7	3 (6.8%)	1.3÷16.0
Tetracycline	25 (73.5%)	57.6÷86.7	31 (70.4%)	56.2÷82.8
Ciprofloxacin	24 (70.5%)	54.3÷84.3	22 (50.0%)	35.4÷64.5

Table 3. Minimum inhibitory concentration (MIC) distribution among indicator *Escherichia coli* strains isolated from waterfowl (n = 93)

	MIC μg/mL										
Antimicrobial	0.125	0.25	0.5	1	2	4	8	16	32	128	256
Ampicillin				4	9	10	17*	21	29	2	1
Amoxicillin/clavulanic acid			3	12	31	30	12*	4			
Gentamicin		1	3	34	39	16*					
Tetracycline				3	3	11	35*	30	8	1	
Ciprofloxacin	2	29	59*	1	2						

Legend: clinical breakpoints are marked with asterisks

Also, the *qnrB1* gene was more prevalent among turkey isolates, i.e., in 12.8% vs only 3 strains (3.2%) from waterfowl. The presence of $bla_{CTX\text{-}M\text{-}1}$ gene was detected in 2.6% of turkey *E. coli* isolates.

Figure 1 presents amplification plots of *qnrS* gene determined in *Escherichia coli* strains isolated from turkeys and Figure 2 presents amplification plots of *qnrS* gene determined in *Escherichia coli* strains isolated from waterfowl. Figure 3 presents amplification plots of *tetA* gene determined in *Escherichia coli* strains isolated from turkeys and respectively Figure 4 presents amplification plots of *tetA* gene determined

Table 4. Minimum inhibitory concentration (MIC) distribution among indicator *Escherichia coli* strains isolated from turkeys (n = 78)

	MIC μg/mL											
Antimicrobial	0.06	0.125	0.25	0.5	1	2	4	8	16	32	128	256
Ampicillin					5	3	15	23*	9	17	2	4
Amoxicillin/clavulanic acid				2	3	10	31	25*	7			
Cefotaxime	45	4	25	2		2*						
Ceftazidime	40	2	25	7	2		2*					
Gentamicin			4	10	5	45	12*	2				
Tetracycline					2	3	17	32*	21		3	
Ciprofloxacin		5	27	43*	3							

Legend: clinical breakpoints are marked with asterisks.

Table 5. Resistance phenotypes and genes determining resistance to antimicrobial agents in indicator *Escherichia coli* strains from waterfowl and turkeys (n = 171)

Genes determining resistance to beta-lactams, tetracycline and quinolone (n/%)							
Number of isolates	Resistance phenotypes	$bla_{CTX\text{-}M\text{-}1}$ gene	*tetA* gene	*tetB* gene	*QnrS* gene	*QnrA* gene	*QnrB1* gene
Waterfowl (n = 93)	Amp,T, CIP (57)	–	57 (61.3%)	40 (43.0%)	14 (15.1%)	–	3 (3.2%)
	T, CIP (15)	–	15 (16.1%)	3 (3.2%)	10 (10.7%)	–	–
	Amp, T (3)	–	3 (3.2%)	1(1.1%)	-	–	–
	Amp, G, T, CIP (1)	–	1 (1.1%)	1 (1.1%)	1 (1.1%)	–	–
Total:			76 (81.7%)	45 (48.4%)	25 (26.9%)	–	3 (3.2%)
Turkeys (n = 78)	Amp,T, CIP (23)	–	23 (29.5%)	13 (16.7%)	8 (10.2%)	–	5 (6.4%)
	Amp, AMC, T (14)	–	14 (17.9%)	1 (1.3%)	2 (2.6%)	–	
	Amp, AMC, T, CIP (14)	–	14 (17.9%)	–	3 (3.8%)	–	5 (6.4%)
	CIP (4)	–	–	–	2 (2.6%)	–	
	T, CIP (2)	–	2 (2.6%)	–	1 (1.3%)	–	
	Amp, G, T, CIP (2)	–	2 (2.6%)	–	2 (2.6%)	–	
	Amp, AMC, CAZ, CTX, T, CIP (1)	1 (1.3%)	1 (1.3%)	–	1 (1.3%)	–	
	Amp, AMC, CAZ, CTX (1)	1 (1.3%)	–	–	–	–	–
Total:		2 (2.6%)	56 (71.8%)	14 (17.9%)	19 (26.0%)		10 (12.8%)

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

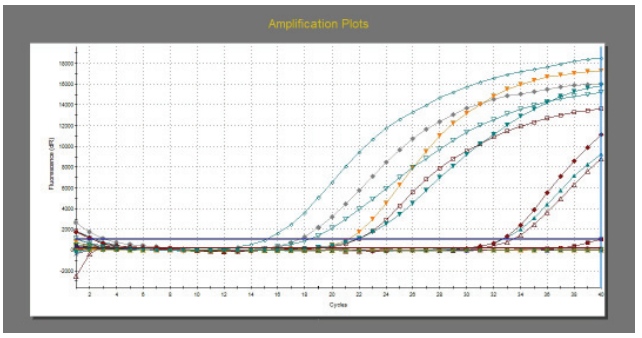

Fig. 1. Amplification plots of *qnrS* gene determined in *Escherichia coli* strains isolated from turkeys

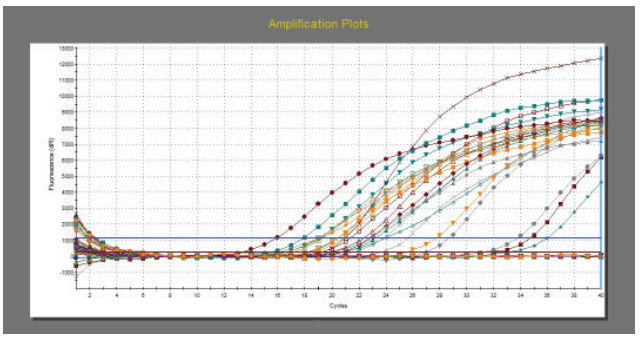

Fig. 2. Amplification plots of *qnrS* gene determined in *Escherichia coli* strains isolated from waterfowl

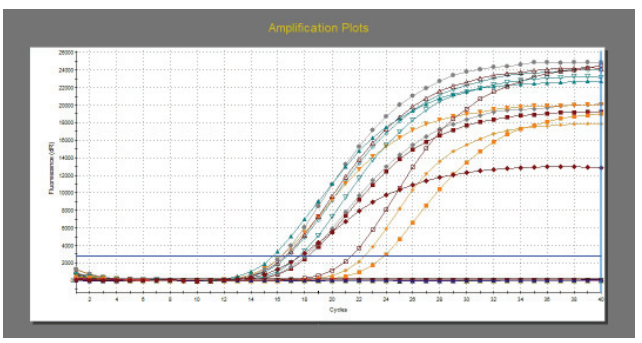

Fig. 3. Amplification plots of *tetA* gene determined in *Escherichia coli* strains isolated from turkeys

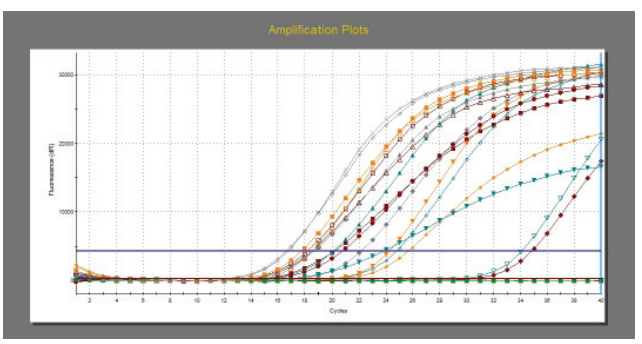

Fig. 4. Amplification plots of *tetA* gene determined in *Escherichia coli* strains isolated from waterfowl

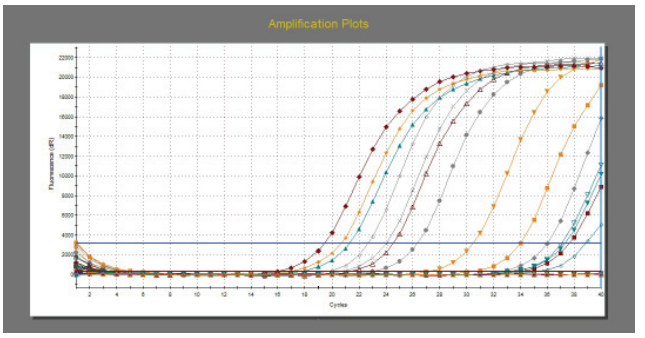

Fig. 5. Amplification plots of *tet B* gene determined in *Escherichia coli* strains isolated from waterfowl

in *Escherichia coli* strains isolated from waterfowl. Figure 5presents amplification plots of *tet B* gene determined in *Escherichia coli* strains isolated from waterfowl.

Discussion

The EFSA (2020) report declared a broad prevalence of resistance to ampicillin (66.8%), ciprofloxacin (56.5%) and tetracycline (61.2%) in commensal *E. coli* isolates from turkeys. According to the experts, the use of antimicrobial drugs at a population level in poultry farming was an argument in support of facts. On the other side, the report of ESVAC (2019) discusses data about the use of antimicrobial drugs in livestock husbandry, with a higher rate of increase for Bulgaria with respect to tetracycline (46.5 mg PCU) and lower levels for fluoroquinolones (5.7 mg PCU). The next report of EFSA and ECDC (approved in 2021) also mentioned that, in most EU member states, indicator *E. coli* isolates from broiler chickens and turkeys demonstrated rather high prevalence of resistance to ampicillin, sulfamethoxazole, trimethoprim and tetracycline. Data provided by Bulgaria for resistance to tetracycline in commensal *E. coli* isolates showed a tendency towards a decrease under 60.0% as compared with the data included in the preceding report from 2016. The multi-resistance profiles with the participation of tetracycline were found in 43.4% of tested *E. coli* isolates from broilers and 45.7% of isolates from turkeys (EFSA, 2021).

In confirmation of the EFSA data on distribution of resistance to tetracycline and ampicillin in commensal poultry E.coli isolates (annual report, 2020), in our study, we found a broader distribution of resistance to tetracycline, i.e., prevalence of genetic factors *tetA* gene (81.7%) and *tetB* gene (48.4%) in waterfowl commensal *E. coli* isolates. Similar data were reported for the presence of *tetA* (71.8%) in turkey strains, with lower prevalence of *tetB* gene (17.9%). Moreover, the phenotype analysis exhibited higher resistance to ampicillin in waterfowl strains (75.3%) as well as a high proportion of *E. coli* isolates from turkeys resistant to ampicillin (73.5%) and amoxicillin/clavulanic acid (44.1%). For example, in the Czech Republic, R derova et al. (2017) also

reported high percentages of resistance to ampicillin (96%) and tetracycline (90%) in commensal *E. coli* strains from turkeys. The authors also stated that ciprofloxacin-resistant isolates had MIC values from 0.25 μg/mL to 16 μg/mL and presence of *qnrS1* and *qnrB19* genes. In our study, MIC values higher than 2 μg/mL were not observed in resistant *E. coli* isolates with MIC_{90} values for strains from both species of 0.5 μg/mL. Also, the *qnrB19* gene was not detected. Another study from Norway (Slettemeås et al., 2019) also presented data about the high levels of MIC for ciprofloxacin (8 μg/mL) among 41% of *Escherichia coli* isolates from turkey meat. The authors take this fact into account as a result of imports of breeding animals in the country. In the present study we did not find statistically significant differences ($P \leqslant 0.001$) between ciprofloxacin resistant commensal *Escherichia coli* isolates from waterfowl in one of the 4 farms surveyed, which were imported Hungarian breeding animals.

In the present study, the resistance to ciprofloxacin was high: in 70.5% of isolates from turkeys and 66.7% from waterfowl. A study from the Czech Republic (Hricovà et al., 2017) presented data about the levels of resistance to fluorinated quinolones in commensal *E. coli* isolates from turkeys with considerably lower prevalence of resistance to ciprofloxacin: in 45% of strains. The authors observed the spread of *qnrB* and *qnrS* genes in 19% and 52% of turkey *E. coli* strain, respectively. In the UK, Gosling et al. (2012) reported a significantly lower spread of *qnrB* and *qnrS* genes among ciprofloxacin-resistant commensal *E. coli* isolates from turkeys: 3.7% and 1.4%, respectively. The authors also commented on the fact that in 88% of surveyed farms, isolates had multi-drug resistant profiles with the participation of ciprofloxacin. The authors also analyzed the broader spread of resistance to ampicillin and ceftazidime among commensal *E. coli* isolates from turkeys (84.9%; 4.7%) compared with the values obtained in the present study for both groups of *E. coli* bacteria (75.3%, 70.5%, 2.6%). With regard to the presence of the *qnrS* gene, the present study found out higher percentages among waterfowl and turkey strains (26.9%, 26.0%). A lower prevalence of the *qnrB* gene was observed among waterfowl isolates (3.2%). According to Gosling et al. (2012), the broad spread of multi-resistance profiles included up to 88.1% of turkey strains. We also found out a high percentage of multi-resistant strains in both waterfowl (62.4%) and turkeys (70.5%). As far as the beginning of this century, Van den Bogaard et al. (2001) reported multi-resistance profiles involving 5 and more chemotherapeutics among commensal *E. coli* isolates from turkeys.

Of interest is also the study of Skarzynska et al. (2021) in Poland, which established the distribution of a number of genes determining resistance to antimicrobial agents, including *qnrS* gene, *tetA* gene and *tetB* gene among wild birds, which the authors identify as a possible reservoir of resistance in environment. According to the authors, resistant profiles in *Eschericia coli* often refer to antimicrobials widely used in human and veterinary medicine.

Chuppava et al. (2018) observed high rates of resistance to ampicillin (42.0%) and enrofloxacin (48.0%) in day-old turkeys. For example, in the present study, the prevalence of resistance to aminopenicillins (73.5%) and ciprofloxacin (70.5%) was higher in *Escherichia* coli isolates from 9-days-old turkeys compared with the observed levels of resistance in isolates from turkeys 12 months of age. Multi-drug resistant profiles involving ceftazidime and cefotaxime were also observed in isolates from 9-days-old turkeys, respectively, related to $bla_{\text{CTX-M-1}}$ gene, *tetA* gene, and *qnrS* gene propagation. In accordance with the survey we conducted with farmers before our study, on the farm only ciprofloxacin was used in 9-days-old turkeys for prophylactic purposes. This fact may in some ways serve as an argument for the wider prevalence of ciprofloxacin resistance in commensal *E. coli* strains from 9-days-old turkeys. Of interest is also the fact that in commensal *E. coli* isolates from birds in this age category we found resistance to cefotaxime and ceftazidime, respectively, distribution of $bla_{\text{CTX-M-1}}$ gene. Probably the explanation for this fact can be found in the argument for the spread of plasmid-determinated genetic factors in multi-resistant *E. coli* strains.

Conclusion

The present study documented higher prevalence of resistance to tetracycline, ampicillin and ciprofloxacin among commensal *E. coli* isolates from turkeys and waterfowl (ducks) in comparison with average values for EC member states included in the EFSA report from 2020. The resistance to cefotaxime and ceftazidime was demonstrated in 2.6% of tested *E. coli* isolates from turkeys, as a part of multi-drug resistant profiles with the participation of aminopenicillins, third-generation cephalosporins, and one profile with tetracycline and ciprofloxacin at the same time. In our opinion, the wider prevalence of resistance to tetracycline, ciprofloxacin and ampicillin in commensal *E. coli* isolated from turkeys and ducks is probably based on the wider use of these chemotherapeutic drugs in our country than other countries in EU.

References

1. Argudìn MA., Deplano M., Meghraoui A., Dodémont M., Heinrichs A., Denis O., Nonhoff C., Roisin S. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics. 2017. 6 (12). P 1-38.
2. Chuppava B., Keller B., El-Wahab A.A., Meißner J., Kietzmann M., Visscher C. Resistance of *Escherichia coli* in turkeys after therapeutic or environmental exposition with enrofloxacin depending on flooring. International Journal of

Environmental Research and Public Health. 2018. 15. P1993. doi:10.3390/ijerph15091993.
3. Directive 2003/99EC of the European Parliament and of the Concil of 17 November 2003 on the monitoring of zoonoses and zoonotic agents. Official Journal of the European Union. 2003.
4. European Food Safety Authority (EFSA). Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commencal *Escherichia coli* and *Enterococcus* spp. from food animals. EFSA Journal. 2008. P 141:1-44.
5. European Food Safety Authority (EFSA). European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA Journal. 18, 6007. https://doi:10.2903/j.efsa.2020. 6007. 2020.
6. European Food Safety Authority and European Centre for Disease Prevention and Control.The European Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal. doi: 10.2903/j.efsa. 2021.6490. 2021.
7. ESVAC Ninth Report. Sales of veterinary antimicrobial agents in 31 European countries in 2017 Trends from 2010 to 2017. https://www.ema.europa.eu/en/ documents/other/answers-requests-scientific-advice-impact-public-health-animal-health-use-antibiotics-animals_en.pdf. 2019.
8. FAO/WHO/OIE. Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Report of a meeting held in FAO, Rome, Italy, November 2007, Geneva, Switzerland. http://www.who.int/foodborne_disease/resources/Report_CIA_Meeting. pdf. 2008.
9. Gosling R.J., Clouting C.S., Randall L.P., Horton R.A., Davies R.H. Ciprofloxacin resistance in *E.coli* isolated from turkeys in Great Britan. Avian Pathology. 2012. 41 (1). P 83-89.
10. Hricovà K., Röderova M., Pudova V., Hanulik V., Halova D., Julinkova P., Dolejska M., Popoušek I., Bardon J. Quinolone-resistant *Escherichia coli* in poultry farming. Central European Journal of Public Health. 2017. 25 (2). P 163-167.
11. Madec J.Y., Haenni M., Nordmann P., Poirel L. Extended-spectrum β-lactamase/AmpC and carbapenemase – producing *Enterobacteriaceae* in animals: a threat for humans. Clinical Microbiology and Infection. 2017. 23. P 826-833.
12. Saliu Eva-Maria, Wahjen W., Zentec J. Types and prevalence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in poultry. Animal Health Research Reviews. 2017. 18 (1). P 46-57.
13. Sengelov G., Halling-Sorensen B., Aarestrup F.M. Susceptibility of *Escherichia coli* and *Enterococcus faecium* isolated from pigs and broiler chickens to tetracycline degradation products and distribution of tetracycline resistance determinants in *E. coli* from food animals. Veterinary Microbiology. 2003. 95 (1-2). P 91-101. doi: 10.1016/S0378-1135(03)00123-8.
14. Skarzynska M., Zajac M., Bomba A., Bocian L., Kozdrun W., Polak M., Wiacek J., Wasyl D. Antimicrobial resistance glides in sky-free living birds as a reservoir of resistant *Escherichia coli* with zoonotic potential. Frontiers in Microbiology. 2021. 12. Article 656223. doi: 103389./fmicb.2021.656223.
15. Slettemeås J.S., Sunde M., Ulstad C.R., Norström M., Wester A.l., Urdahl A.M. Occurrence and characterization of quinolone resistant *Escherichia coli* from Norwegian turkey meat and complete sequence of an Incx1 plasmid encoding *qnrS1*. PLOS ONE. 2019. https://doi.org/10.1371/journal.pone.0212936.
16. Röderova M., Halova D., Papousek I., Dolejska M., Masarikova M., Hanulik V., Pudova V., Broz P., Htoutou-Sedlakova M., Sauer P., Bardon J., Cizek A., Kolar M., Literak I. Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals, and environment in the Czech Republic. Frontiers in Microbiology. 2017. 7. Article 2147. https://doi.org/10.3389/fmicb.2016.02147.
17. Van den Bogaard A.E., London N., Driessen C., Stobberingh E.E. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy. 2001. 47. P 763-771.

Received 19 November 2021
Accepted 28 December 2021

Evaluation of Fatty Acids Composition and Quantity in Raw and Processed Salmon, Herring and Mackerel Products in Lithuanian Market

Gintarė Zaborskienė, Artūras Stimbirys, Gintarė Zakarienė, Ignė Juknienė
Department of Food Safety and Quality, Faculty of Veterinary Medicine, Veterinary Academy, Lithuanian University of Health Sciences

Keywords: *fish products, fatty acids, omega 6 to omega 3 ratio, atherogenic index, thrombogenic index.*

Abstract. *Fatty acids (FA) and their composition are very important for human nutrition. Fish products are one of the most beneficial sources of FA for human health; therefore, it is very important to know which products in the Lithuanian market are the most suitable for consumers.*

The aim of the study was to determine the content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), the quantity and ratio of omega 6 and omega 3 fatty acids(n-6/n-3) in the raw material of salmon, herring and mackerel and their salted and smoked products, and to calculate the atherogenic (AI) and thrombogenic (TI) indices of these products.

The study was carried out in 2019–2020. A test sample for FA analysis was prepared according to LST EN ISO 12966-2:2011 standard. The content of FA was determined by gas chromatography using a flame ionization detector. Chromatographic analysis of fatty acid methyl esters was performed with Shimadzu GC - 2010 (Japan) gas chromatograph, using a BPX - 70, 120 m column according to the LST EN ISO 15304:2003/AC:2005 standard.

Compared with heat-treated products, fish raw material (fillets) had a higher content of PUFA ($P < 0.05$), higher levels of omega 3 FA (n-3) ($P < 0.05$), and their n-6/n-3 ratio was reliably lower. Salmon products had the healthiest FA composition for humans, the highest amount of omega 3 FA and the most suitable for human diet n-6/n-3 ratio.

The most favourable indices of atherogenicity and thrombogenicity (AI – 0.13, TI – 0.1) for human diet in tested fish product samples were calculated in the raw material of Atlantic salmon fillet and were slightly higher than in cold smoked mackerel (AI – 0.23, TI – 0.25). Moreover, the assessment of correlation relationships between individual indicators showed that the ratio n-6/n-3 decreased with increasing quantity of PUFA (coefficient r =–0.602 in salmon; r = –0.628 in mackerel; r = –0.831 in herring products).

Introduction

Fish and seafood product consumption is very important in human nutrition. According to Food and Agriculture Organization (FAO) data, global world fish production in 2018 year was 179 million tonnes, of which approximately 156 million tonnes were used for human consumption (FAO, 2020), and 3 billion people around the world consume fish and other marine organisms as a source of proteins (Tveteras et al., 2012) and fats.

Usually, fish fats are used in the human diet as a concentrated form of energy which helps to protect body from cold (Payne et al., 2018), regulate body cholesterol metabolism (Chiu et al., 2018; Hirako et al., 2010) and protect body tissues and organs. They also play an essential role in carrying fat-soluble vitamins, and take part as saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the human diet. According to scientific literature, PUFA, especially omega-3 fatty acids – eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) – are very significant for human health in both disease prevention and health status improvement (Oscarsson & Hurt-Camejo, 2017; Murillo et al., 2014).

The role of omega n-3 fatty acids in prevention and management of cardiovascular disease is evident; it reduces low-density lipoprotein, inhibits cholesterol production (Pedro-Botet et al., 2019), has a positive effect on brain function and neurodevelopment, reduces inflammation and plays a role on psychological and cognitive function (Scotio & Mjos, 2012).

The biological effect of omega-6 fatty acids is largely mediated during physical activity and inflammation by their conversion to n-6 eicosanoids that bind to diverse receptors found in every tissue of the body. Since n-3 and n-6 fatty acids compete for the same enzymes for desaturation and elongation, and each class of PUFA has a different effect on human health, an appropriate ratio of both FA is crucial. Some studies indicate that human beings evolved on the diet with n-6 to n-3 fatty acids ratio of approximately 1:1 (Simopouls, 2008). Other authors

Correspondence to Artūras Stimbirys, Department of Food Safety and Quality, Faculty of Veterinary Medicine, Veterinary Academy, Lithuanian University of Health Sciences.
E - mail. arturas.stimbirys@lsmuni.lt

state that a ration between 1:1 and 5:1 is beneficial to human health (Strobel et al., 2012, Gebauer et al., 2006), while nowadays this ratio can reach up to 20:1 in Western diets.

Fatty acids can have an impact in preventing heart diseases, lower the atherosclerotic processes, high blood pressure, inflammation, mental health disorders (Mozafarian et al., 2006), diabetes, digestive disorder, autoimmune disease, cancer (de Roos al., 2013), and have a positive effect in foetus development and adult health (Chowdhury et al., 2020; Gale et al., 2008).

The research interest related to trans fatty acids isomers (TFAI) in food and their significance to human health increases every year. According to researchers, TFAI have been implicated in the aetiology of various metabolic and functional disorders (Trattner et al., 2015). The main concern about its health effects arose due to the structural similarity of these isomers to saturated fatty acids, the lack of specific metabolic functions, and their competition with essential fatty acids. The metabolic effect of trans isomers is the main question for biochemists, nutrition specialists and epidemiologists. It is known that fish processing methods have different effects on nutritional, physical and chemical compositions, including fat and fatty acids (Abraha et al., 2018).

Fish fatty acid profiles, quantity and relationships are very important to human health. The other significant lipid quality indicators of food products are atherogenic (AI) and thrombogenic (TI) indices, which depend on the relative contents of particular fatty acid groups and may indicate total lipid quality in food and their potential effect on the development of coronary disease (Ulbricht et al., 1991). These rates can be used to compare the influence of fat fraction wellness in various foods.

So, the aim of the study was to determine the content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), the quantity and ratio of omega 6 and omega 3 fatty acids(n-6/n-3) in the raw material of salmon, herring and mackerel and their salted and smoked products, presented in the Lithuanian market, and to calculate the atherogenic (AI) and thrombogenic (TI) indices of these products.

Materials and methods

During this study, fatty acids SFA, MUFA, PUFA, TFAI, n-3, n-6, their ratio (n-6/n-3), AI and TI indexes were determined in raw and processed products of salmon (*Salmo salar*), herring (*Clupea harengus)* and mackerel (*Scomber scombrus),* presented in the Lithuanian retail market.

All types of fish products (3 samples from each raw fillets, salted, hot and cold smoked fish), were randomly purchased in supermarkets, shops and market places of Kaunas city, Lithuania. In total, 36 products, belonging to 4 assortment (raw fillets, salted, cold and hot smoked) groups, were taken for investigation.

Sample preparation. The composition of fatty acids was detected in intramuscular fish fat. The investigations were carried out in the Food Institute of Kaunas Technology University. All samples were homogenized with a homogeniser (Heidolph, Germany), and stored at +6–8°C in the refrigerator until further investigation. The samples were prepared according to the LST EN ISO 12966-2:2011 standard, where fatty acids were methylated using anhydrous 2 mol/l KOH methanol solution. The number of fatty acids was determined by the gas chromatography method using a flame ionization detector. Chromatographic analysis of fatty acids methyl esters was performed using gas chromatograph Shimadzu GC 17 A (Japan), using BPX – 70, 120 m column following methodology described in LST EN ISO 15304:2003/AC: 2005 standard. The analysis was done under the following conditions: column primary temperature was kept at +60°C, after 2 min., using 20°C/min speed was increased to +230°C and maintained for 45 min; injector temperature was +250°C; flame ionization detector temperature was +270°C; carrier gas was nitrogen.

Supelco 37 Component FAME Mix (Merck, USA) was used for fatty acids identification. The fatty acids tetradecen (C14:2) and hexadecen (C16:2) were identified by the means of interpolation. Each group of fatty acids in fish samples was calculated as a percentage (%) of the total amount (sum) of all FA (100%). The ratio of n-6/n-3 was calculated by dividing their total values. The atherogenic (AI) and thrombogenic (TI) indices were calculated according to Ulbricht and Southgate (1991) using the following formulas:

AI = [C12:0 + (C14:0 × 4) + C16:0] / (total unsaturated fatty acids), where:

C12 – the percentage of lauric acid in relation to TFA; C14 = the percentage of myristic acid in relation to TFA; and C16 = the percentage of palmitic acid in relation to TFA.

TI = $\sum$ (C14:0 + C16:0 + C18:0)/[0.5 × cis C18:1 + 0.5 × $\sum$ MUFA + 0.5 × $\sum$ (n-6) + 0.5× $\sum$ (n-3) + (n-3/n-6], where:

n-6 is fatty acids containing omega-6

n-3 is fatty acids containing omega-3.

Statistical analysis

All gathered data were analysed using statistical package SPSS for Windows 2.0 version (IBM Corp NY, USA). Significance of differences between treated samples was evaluated using Duncan's multiple range tests at a 5% confidence level. The Shapiro-Wilk test revealed the normal distribution of variables as well as TBC and TCC, which are expressed as mean ± standard error (SE). Correlation was analysed by Microsoft Excel statistical software (Microsoft Office Excel 2016, Microsoft Corp., Redmond, WA, USA).

Results

During this study, the composition and quantity

of fatty acids SFA, MUFA, PUFA, TFAI, n-3, n- 6, their ratio (n-6/n3), atherogenic and thrombogenic indexes in salmon, herring and mackerel raw, salted and smoked products were investigated.

Fatty acids composition and quantity in Atlantic salmon, herring and mackerel raw fillets

According to this study results, the highest quantity of SFA was determined in raw herring fillets and this amount was 1.5 times higher than in salmon and 1.2 times than in mackerel fillets, respectively (Fig. 1). Moreover, the obtained results among all investigated groups were statistically significant ($P < 0.05$).

MUFA quantity in Atlantic salmon raw fillets was 2.21 times ($P < 0.05$) higher than in herring and 2.24 times ($P < 0.05$) than in mackerel fillets.

The study results indicated that PUFA dominated in herring products, and this amount was 1.56 times higher than in Atlantic salmon and 1.24 times higher than detected in mackerel fillets. The results between all tested groups were statistically significant ($P < 0.05$).

According to our study results, the best n-6/n-3 ratio (1.38:1) was found in raw salmon, although the n-3 acids quantity in herring and mackerel fillets was higher compared with n-6 fatty acids. Due to this reason, the ratio between omega 6 to omega 3 fatty acids in these products was 0.17:1 and 0.57:1, respectively.

There were no statistically significant differences determined in TFAI between the samples obtained from raw salmon and mackerel groups, while in herring fillets, this quantity was 1.03 times ($P > 0.05$) higher than in mackerel products.

The calculated atherogenic and thrombogenic indices in raw fish fillet samples are presented in Table 1.

The data presented in Table 1 showed significant differences of atherogenic and thrombogenic indices related to fish species. According to our study results, their atherogenic indices ranged from 0.13 to 0.79, whereas thrombogenic indices values variated from 0.17 to 0.74, respectively. Therefore, Atlantic salmon raw fillet samples were most suitable for human diet.

Fatty acid composition and quantity in salted fish products

In order to compare fatty acids composition and quantity of salted herring, mackerel and salmon fillet products were evaluated. The highest amount of SFA was detected in the samples obtained from salted mackerel, and this amount was 1.33 times ($P < 0.05$) higher than in salmon, and 1.09 times ($P > 0.05$) than in herring, respectively (Fig. 2).

The statistically significant MUFA differences ($P < 0.05$) were found among all tested salted fish product groups. Moreover, the MUFA amount detected in salmon samples was 2.1 times higher than

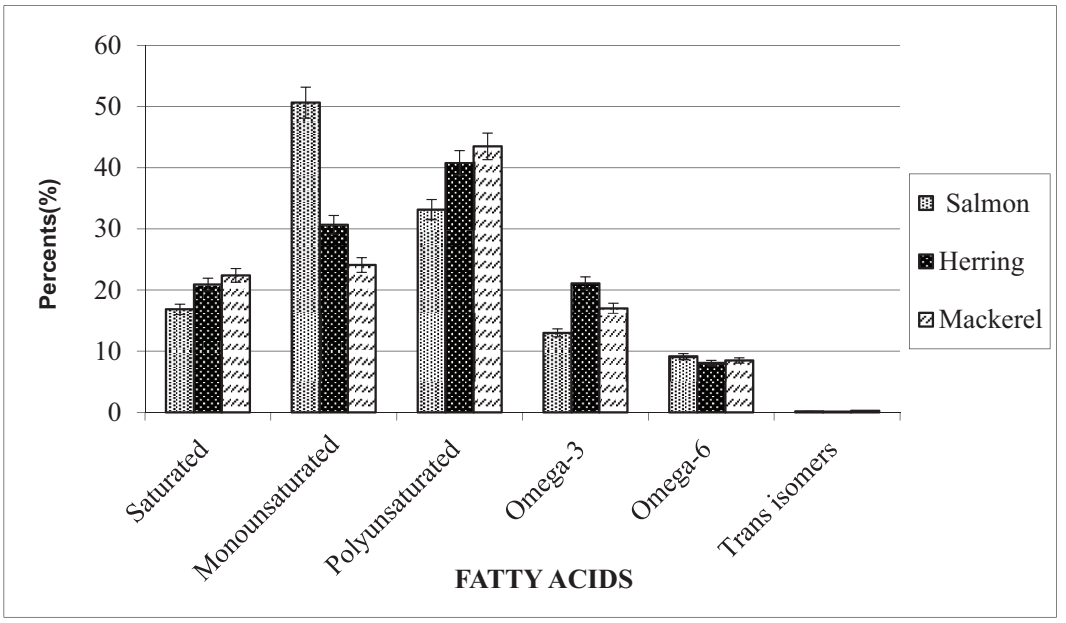

Fig. 1. Fatty acid composition in raw materials (salmon, herring, and mackerel fillets).

Table 1. Atherogenic (AI) and thrombogenic (TI) indices in Atlantic salmon, herring and mackerel raw fillets

Indices	Raw fillet		
	Salmon	Herring	Mackerel
Atherogenic	0.13*±0.02	0.77* ± 0.08	0.79* ± 0.07
Thrombogenic	0.17*±0.03	0.24 ± 0.06	0.74* ± 0.1

*Statistically significant values between groups ($P < 0.05$)

in mackerels and 1.65 times higher than in herrings' products, respectively.

The data presented in Fig. 2 showed that the highest amount of PUFA was detected in salted mackerel fillets. In comparison with herring and salmon product samples, the quantity of PUFA in salted mackerel products was 1.08 ($P > 0.05$) and 1.31 ($P < 0.05$) times higher than in previously mentioned products.

The higher amount of n-3 fatty was dominated in the samples of all tested salted fish products in comparison with the quantity of n-6 fatty acids. Moreover, the highest n-3 amount was detected in mackerels and the lowest in salted salmon products. Whereas, the higher percent of n-6 acids were dominated in salted salmon products, where their amount was 1.12 ($P > 0.05$) and 1.07 ($P > 0.05$) times higher than in herring and mackerel products, respectively.

The n-6/n-3 ratio depended on fish species in salted fish products, as it was 0.50:1 in mackerel, 0.38:1 in herring, and 0.70:1 in salmon samples.

It is important to note that the amount of TFAI in all tested salted fish products samples ranged from 0.10% to 0.24%. The study results indicated that the highest amount of TFAI was detected in mackerel and it was 2.4 times higher than in herring and 1.6 times higher than in salmon salted products. Moreover, the differences between tested fish species samples were statistically significant ($P < 0.05$).

The study results showed that the best AI and TI composition for human health was detected in salted salmon products (Table 2).

Statistically significant data were detected between the values of atherogenic and thrombogenic indices in Atlantic salmon and mackerel salted products.

Fatty acid composition and quantity in smoked fish products

In order to compare the impact of the smoking method on FA composition, the samples obtained from cold and hot smoked Atlantic salmon, herring and mackerel were investigated. It is important to note that there are some FA differences, related with fish species and the smoking method.

No significant SFA quantity differences ($P > 0.05$) were detected comparing cold and smoked fish product samples obtained from the same fish species. The SFA values of products, processed from various fish species ranged from 1.02 (in salmon) to 1.12 times (in mackerel). Therefore, the smoking method had no influence on the amount of SFA between the same fish species (Fig. 3).

Despite previous results, the statistically significant differences among the tested samples groups were detected in products processed from dissimilar fish species. According to this study results, the highest

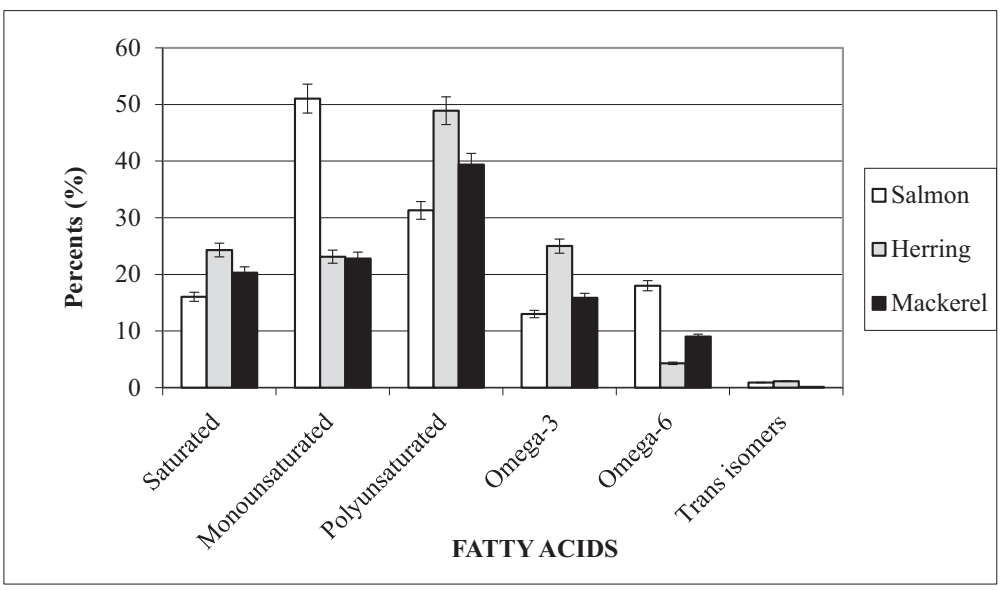

Fig 2. Fatty acid composition in salted fish products.

Table 2. Atherogenic (AI) and thrombogenic (TI) indices in Atlantic salmon, herring and mackerel salted products

Indices	Salted fish products		
	Salmon	Herring	Mackerel
Atherogenic	0.27* ± 0.03	0.64* ± 0.06	0.70* ± 0.07
Thrombogenic	0.21* ± 0.03	0.24 ± 0.04	0.69* ± 0.02

*Statistically significant values between groups ($P < 0.05$).

SFA amount was determined in mackerel and the lowest in salmon products. The biggest differences were determined between mackerel and smoked salmon products groups, where the results among groups ranged from 1.46 in cold smoked products to 1.53 times in hot smoked products, and were statistically significant ($P < 0.05$).

Contrary to previous results related to SFA quantity, the highest MUFA amount was detected in smoked salmon, and the lowest in mackerel products. There were no significant differences comparing the amount of MUFA between cold smoked and hot smoked products of the same fish species. The differences between them ranged from 1.11 (herring) to 1.35 times in salmon products ($P > 0.05$). However, the highest percent of MUFA was determined in salmon hot smoked products and the lowest in herring cold and hot smoked production.

The data presented in Fig. 3 showed that the highest amount of PUFA was detected in cold and hot smoked mackerel, and the lowest in salmon smoked products. Their differences among groups ranged from 1.44 to 1.48 times ($P < 0.05$).

In conclusion, it was determined that cold smoked fish products had higher PUFA amount than hot smoked products. The study results showed that the quantity of n-3 in all tested cold smoked fish products was 2.39 times ($P < 0.05$) higher than in the hot smoked samples, where differences ranged from 2.12 times in mackerel to 2.84 times in salmon. The results between tested groups were statistically significant ($P < 0.05$).

It is important to note that the quantity of n-3 depends on the smoking method in products, processed from different kinds of fish. A higher amount of n-3 was found in cold smoked fish than in hot smoked products. The quantity of TFAI determined in smoked fish product samples ranged from 0.97% (hot smoked mackerel) to 3.10% (cold smoked salmon) products. There were no statistically significant data found comparing the amount of TFAI between different fish smoking methods, except from salmon products as the difference between cold and hot smoked fish groups was 2.21 times ($P < 0.05$).

The omega 6 to omega 3 ratio between smoked fish products is presented in Table 4.

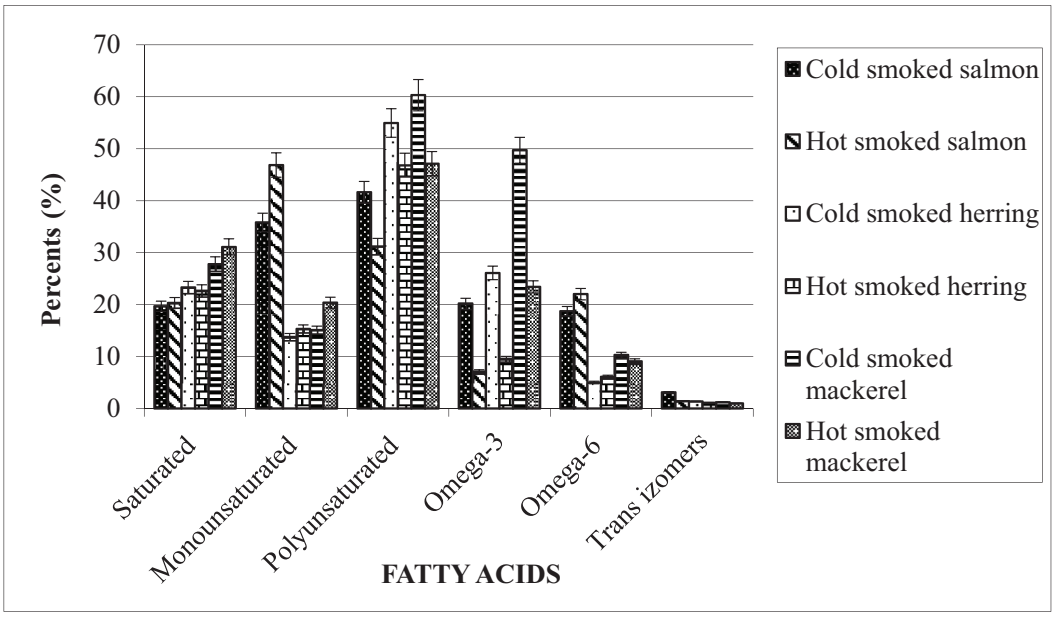

Fig. 3. Fatty acid composition in cold and hot smoked fish products.

Table 4. Omega 6 to omega 3 ratio in smoked fish products

Smoked product	Omega 6 / omega 3 ratio
Cold smoked salmon	$0.93 \pm 0.05^{a*}$
Hot smoked salmon	$3.10 \pm 0.13^{b*}$
Cold smoked herring	$0.19 \pm 0.02^{a*}$
Hot smoked herring	$0.64 \pm 0.08^{b*}$
Cold smoked mackerel	$0.21 \pm 0.034^{a*}$
Hot smoked mackerel	0.39 ± 0.04^{b}

Statistically significant values between groups. a – $P < 0.05$; b* – $P < 0.001$.

According to the data presented in Table 4, the most suitable n-6/n-3 ratio for the human diet was found in cold smoked salmon products.

It is important to note that the highest atherogenic index was detected in hot smoked herring, while the lowest in cold smoked mackerel products, and the difference between the groups was 2.8 times ($P < 0.05$).

This study results indicated (Table 5) that the thrombogenic index between all tested smoked fish product groups showed very similar values and no statistically significant differences were detected ($P > 0.05$).

The relationship between the quantities of PUFA, n-3 and n-6 FA were determined by calculating the correlation coefficients for each fish product. The produced data showed that the correlation between PUFA and the n-6/n-3 ratio of salmon products (raw, salted, smoked) was moderate negative ($r = –0.602$), between n-3 and the n-6/n-3 ratio it was negative strong ($r = –0.899$), and between n-6 and the ratio n-6/n-3, the relationship was moderate ($r = 0.639$).

Strong negative relationships were found in various processed herring products between PUFA and the n-6/n-3 ratio ($r = –0.83$), and strong positive between n-6 FA and the n-6/n-3 ratio ($r = 0.888$). A strong negative relationship was found between n-3 and the n-6/n-3 ratio ($r = –0.721$).

A negative moderate relationship was found between PUFA and the n-6/n-3 ratio ($r = –0.628$) in mackerel products, whereas a very weak correlation was noticed between n-6 and the n-6/n-3 ratio ($r = –0.038$). It is important to note that a very strong negative relationship was found between n-3 and the n-6/n-3 ratio ($r = –0.781$).

Discussion

The demand for healthy and functional foods in the world is steadily increasing. Fish products play an important role in human diet. It is important to note that a clear correlation exists between the expectation of a healthy life and the consumption of fish and sea food products (Sampels, 2015). Moreover, it is important to consider that fish products are rich in PUFA, especially n-3 and n-6 and their ratio (n-6/n-3) play the main the role in the human health (Ellulu et al., 2015; Pickova, 2009).

According to this study results, the highest amount of PUFA was detected in herring and mackerel fillet samples and mackerel products (salted, cold and hot smoked) in comparison with salmon samples. Moreover, FA profiles and quantity of PUFA in products depend on fish species and their quality is also affected by fish diet (Moini et al., 2012).

Our study data correlate with findings of other researchers who investigated mackerel (Orban et al., 2011) and salmon products (Gladyshev et al., 2009).

Contrary to the above, the tendency of significantly increasing PUFA values was determined in all tested cold smoked products in comparison with the samples obtained from raw material and other processed fish product groups. There are no similar data presented by other researchers. It is clear that cold smoking fish products had a significantly higher amount of n-3 in comparison with hot smoked products. This result can be related with high temperature (over 68°C), which has an influence on PUFA oxidation during the hot smoking process (Stolyhwo et al., 2006).

Omega 3 FA play a very important role in the prevention of human diseases associated with chronic inflammation. In molecular studies, omega-3 FA have direct effects in reducing the inflammatory state by reducing the level proinflammatory cytokines like interleukin -6 (IL-6) and tumour necrosis factor alpha, TNF-α, C reactive protein (CRP) and many other factors (Ellulu et al., 2015).

This study results showed that the highest quantity of n-3 fatty acids was found in herring and cold smoked fish products retailed in the Lithuanian market, whereas in farmed salmon products, this value was lower. These results can be explained by a different aquaculture (farmed) and wild marine fish nutrition. The n-3 quantity in fish and mammals mostly depends on the diet and on the ability to elongate and desaturate plant driven alfa linoleic acid to their longer C_{20} and C_{22} derivates (Ghioni et al., 1999). Feeding has an influence on the total fat content and fatty acids composition in all species of aquaculture fish. Salmon and rainbow trout, especially, get a lot of vegetable oils in their feed, such as sunflower, soybean, rapeseeds and linseeds to achieve intensive growth as well as faster lipid deposition during a short time period. Therefore, a high number of n-6 deposits in their fat can be detected due to diet. Contrary to the above, in marine fish the basic fatty acids content, cumulated in marine food chain, depends on marine phytoplankton (Jónasdóttir, 2019; Ruiz-Lopez et al., 2012). They can effectively synthesize LC PUFA

Table 5. Atherogenic (AI) and thrombogenic (TI) indexes in smoked fish products

Indices	Smoked fish products					
	Cold smoked Atlantic salmons	Hot smoked Atlantic salmon	Cold smoked herring	Hot smoked herring	Cold smoked mackerel	Hot smoked mackerel
AI	0.33	0.51	0.85*	0.82*	0.23*	0.30
TI	0.19	0.28	0.21	0.22	0.25	0.27

*Statistically significant values between groups ($P < 0.05$).

from ALA and LA using desaturation and elongation reactions.

More recently, a special focus was placed on the ratio n-6/n-3, because a very high intake of n-6 acids is less desirable due to excessive amounts of n-6 PUFA and very high n-6/n-3 ratio diets promoting the pathogenesis of many diseases. However, in some cardiovascular disease, cancer, inflammatory and autoimmune diseases, an increased level of omega-3 PUFA (a low n-6/n-3 ratio) exerts suppressive effect on illnesses associated with chronic inflammation (Simopulos, 2008).

This study results were in agreement with WHO recommendations as the most suitable for human health n-6/n-3 ratio was found in Atlantic salmon products and their values ranged from 0.70:1 in salted products up to 1.38:1. Similar results associated with salmon products were described by Strobel et al. (2012), although Regulska – Ilow et al. (2016) stated that ratio n-6/n-3 in raw mackerel was lower.

TFAI are created when liquid fish oil is hydrogenated; this is frequently done to increase their plasticity and chemical stability for subsequent food processing. TFAI also have been implicated in aetiology of various metabolic functional human disorders, related to cancer risk (Valenzuela & Morgado, 1999), and may provoke cardiovascular diseases (Dawczynski & Lorkowski, 2016).

According to this study results, the content of trans isomers in fish products was low and ranged from 0.11% in raw mackerel fillet up to 3.1% in cold smoked products. It is important to note that other researchers (Roe et al., 2013; Regulska – Ilow et al., 2013) found similar results in fish, presented in United Kingdom and Poland markets.

It is known that two processes, atherosclerosis and thrombosis, have influence on the coronary and heart diseases, including ischaemic heart disease (IHD). Moreover, the dietary fat consumed has influence on both of them. SFA with a chain length of 12, 14, 16 atoms have a cholesterol raising effect and thus are atherogenic (Keys et al., 1965; Bonamone & Gundy, 1988).

It is known that SFA with a chain length of 14, 16, 18 C are thrombogenic (Hornstra & Lussemberg, 1975). On the opposite, the MUFA and n-6 PUFA may reduce plasma cholesterol and low-density lipoprotein cholesterol (LDL–C) concentration (Gurr et al., 1989).

The atherogenic index is anti-atherogenic, inhibiting the aggregation of plague diminishing levels of some parts of components. The thrombogenic index shows the tendency to form clots in blood vessels. This is defined as the relationship between the thrombogenic saturated and anti-thrombogenic fatty acids. The atherogenic and thrombogenic indices, proposed by Ulbricht Southgate (1991), are related to the composite diet or a single food intake in prevention of atherosclerosis and platelets (Orban et al., 2011). However, other factors are important: low density lipoprotein (LDL), platelet activation factor (PAF), LDL oxidation, which may influence the inflammatory response to atherogenesis. Additionally, single fatty acids might have a harmful effect on human health due to atheroma and thrombus formation (Garaffo, 2011). According to our study results, the most favourable atherogenic (AI) and thrombogenic (AI) indices were found in most salmon products. These results are similar to the results described by other authors (Krešić et al., 2019), although our calculated AI and TI indices were slightly higher than the results presented in Fernandes et al.'s (2014) study about mackerel products.

Conclusions

The results of this study showed that a higher amount of PUFA ($P < 0.05$) was found in raw (fillets) and cold smoked fish products in comparison with heat-treated samples, retailed in the Lithuanian market. The most favourable FA composition for human health was detected in mackerel products, which had the highest content of n-3. Also, it is very important to note that a higher amount of n-3 ($P < 0.05$) was also found in raw (unprocessed) fish samples compared them with processed. Therefore, their n-6/n-3 ratio was also significantly lower. The most favourable atherogenic (AI – 0.13) and thrombogenic (TI – 0.17) indices for human health were detected in raw Atlantic salmon fillets, while slightly worse indices were calculated in cold-smoked mackerel (AI – 0.23, TI – 0.25) samples.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Bhuiyan A.K.M A., Ratnayake W.M., Ackman R.G. Stability of lipids and polyunsaturated fatty acids during smoked in Atlantic mackerel (*Scomber scombrus*). Journal of the American oil chemists' society. 1986. T. 63. P. 324-328.
2. Bonamone A., Gundy S.M. Effect of dietary stearic acids on plasma cholesterol level and lipoprotein level. New England journal of medicine. 1988. T. 318. P.1244-1248.
3. Burr M.L. Fish and the cardiovascular system. Progress in Food and Nutrition Science. 1989. T.13(3-4). P. 291-316.
4. Chiu C.Y, Wang L.P., Liu S.H., Chiang M.T. Fish oil supplementation alleviates the altered lipid homeostasis in blood, liver, and adipose tissues in high-fat diet-fed rats. Journal of agricultural food chemistry. 2018. T. 66(16). P. 4118-4128.
5. Chowdhury M.H., Ghosh S., Kabir M.R., Mamun M.A.A., Islam MS. Effect of supplementary omega-3 fatty acids on pregnant women with complications and pregnancy outcomes: review from literature. The journal of maternal-fetal & neonatal medicine. 2020. T. 9. P. 1-17.
6. Dawczynski C., Lorkowski S. Trans-fatty acids and cardiovascular risk: does origin matter? Expert review of cardiovascular therapy. 2016. T. 14(9). P.1001-1005.
7. de Roos B. Mechanisms of fish oil-modulated inflammation and health. Bioactive food as dietary interventions for arthritis and related inflammatory diseases. Academic Press. 2013. P. 545-553.
8. Ellulu M.S., Khaza'ai H., Abed Y., Rahmat A., Ismail P., Ran-

neh Y. Role of fish oil in human health and possible mechanism to reduce the inflammation. Inflammopharmacology. 2015. T. 23(2-3). P. 79-89.
9. FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.
10. Fernandes E.C., Vasconcelos S.A.M., Ribeiro A. M., Sarubbo A. L., Andrade C. A.S., Filho M.B.A. Nutritional and lipid profiles in marine fish species from Brazil. Food Chemistry. 2014.T. 160. P. 67-
11. Gale R. C., Robinson M. S., Godfrey M. K., Law M. C., Schlotz W., O'Callaghan J. F. Oily fish intake during pregnancy – association with lower hyperactivity but not with higher full-scale IQ in offspring. Journal of Child Psychology and Psychiatry. 2008. T. 49. P. 1061-1068.
12. Garaffo M.A., Vassallo-Agius R., Nengas Y., Lemmbo E., Rando R., Maisano R., Dugo G., Giuffrida D. Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (*Thunnus thynnusLl.)* and their salted product "Bottarga" Food and Nutrition, 2011. T. 2. P. 736-743.
13. Gebauer S.K., Psota T.L., Haris W.S., Kris-Etherton P.M. n -3 fatty acids dietary recommendations and food sources to achieve essentiality and cardiovascular benefit. The American Journal of Clinical Nutrition. 2006. T. 83. P. 1526-1535.
14. Ghioni C., Tocher D.R. Bell M.V. Dick R., Sargent J.R. Low C18 to C20 fatty acids elongase activity and limited conversation of stearidonic acid 18:4(n-3) in a cell line from the turbot *Scophtalmus maximus*. Biochimica et Biohysica Acta. 1999. T. 1437. P. 170-171.
15. Gladyshev M.I., Sushchik N.N., Makhutova O.N. and Kalachova G.S. Content of essential polyunsaturated fatty acids in three canned fish species. International journal of food sciences and nutrition. 2009. T. 60 (3). P. 224-230.
16. Gladysshev M.I. Sushchik N.N., Gubanenko G., Demirchieva S. M., Kalachova G.S. Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*). Food chemistry. 2006. T. 96. P. 446-451.
17. Gurr M. J., Borlak M., Ganatra S. Dietary fat and plasma lipids. Nutritional research reviews. 1989. T. 2 (1). P. 63-86.
18. Hirako S., Kim H.J., Arai T., Chiba H., Matsumoto A. Effect of concomitantly used fish oil and cholesterol on lipid metabolism. Journal of Nutritional Biochemistry. 2010. T. 21(7). P. 573-9.
19. Hornstra G., Essential fatty acids in mothers and their neonates. The American journal for clinical nutrition. 2000. T. 71. P.1265-1269.
20. Hornstra G., Lussenburg R.N. Relationship between the type of dietary fatty acid and arterial thrombosis tendency in rats. Atherosclerosis. 1975. T. 22(3). P. 499-516.
21. Jónasdóttir S.H. Fatty acid profiles and production in marine phytoplankton. Marine Drugs. 2019. T.17(3). P. 151.
22. Keys A., Anderson J.T., Grande F. Serum cholesterol response to changes in diet IV. Particular saturated fatty acids in the diet metabolism. 1965. T. 14. P. 776-787.
23. Krešić G., Vulić A., Dergestin Bačun L., Lešić T., Želježić D., Pleadin J. Nutritive composition and lipid quality indices of commercially available filleted fish. Hrana u zdravlju i bolesti. 2019. T. 8 (1). P. 67-73.
24. LST EN ISO 12966-2:2011. Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids (ISO 12966-2:2011).
25. LST EN ISO 15304:2003/AC:2005. Animal and vegetable fats and oils - Determination of the content of trans fatty acid isomers of vegetable fats and oils - Gas chromatographic method. (ISO 15304:2002/Cor.1:2003).
26. Mazaffarian D., Rimn E.B. Fish intake contamination and human health -evaluating the risks and benefits. 2006. Journal of American medical association. T. 296. P. 1885-1899.
27. Moini S., Khoshkhoo Z.N., and Hemati Matin R. The fatty acids profile in mackerel (*Scomberomorus guttatus*) and its shelf life in cold storage at -18 °C. Global veterinari. 2012. T. 8(6). P. 665-668.
28. Murillo E., Rao K.S., Durant A. A. The lipid content and fatty acid composition of four eastern central Pacific native fish species. Journal of food composition and analysis. 2014. T. 33. P. 1-5.
29. Orban E., Di Lena G., Nevigato T., Masci M., Casini I., Caproni R. Proximate, unsaponifiable lipid and fatty acid composition of bogue (*Boops boops*) and horse mackerel (*Trachurus trachurus*) from the Italian trawl fishery. Journal of food composition and analysis. 2011. T. 24(8). P. 1110-1114.
30. Oscarsson J., Hurt-Camejo E. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. Lipids in health and disease. 2017. T.16(1):149.
31. Payne S., Macintosh A., Stock J. Body size and body composition effects on heat loss from the hands during severe cold exposure. American journal of physical anthropology. 2018. T. 166(2). P. 313-322.
32. Pedro-Botet J, Pintó X. LDL-cholesterol: The lower the better. Clínica e investigación en arteriosclerosis. 2019. T. 31. P.16-27.
33. Pickova J. Importance of knowledge of lipid composition of foods to support development toward consumption of higher levels of n-32 fatty acids via freshwater. Physiological research / Academia Scientiarum Bohemoslovaca. 2009. T. 58. P. 39-45.
34. Regulska – Ilow B., Ilow R., Konikowska K., Kawicka A., Rozanska D., Bocchinska A. Fatty acid profile of the fat in selected smoked marine fish. Roczniki Państwowego Zakładu Higieny. 2013. T. 6494. P. 299-307.
35. Roe M., Pinchen H., Church S., Elahi S., Walker M., Farron-Wilson M., Buttriss J., Finglas P. Trans fatty acids in a range of UK processed foods. Food Chemistry. 2013. T. 40. (3). P. 427-431.
36. Ruiz-López N., Sayanova O., Napier J.A., Haslam R.P. Metabolic engineering of the omega-3 long chain polyunsaturated fatty acid biosynthetic pathway into transgenic plants. Journal of experimental botany. 2012. T. 63(7). P. 2397-2410.
37. Sampels S. The effects of processing technologies and preparation on the final quality of fish products. Trends of food science and technology. 2015. T. 44. P. 131-137.
38. Scotio K., Mjos S.A. Trans isomers of EPA and DHA in omega 3 products on European market. Lipids. 2012. T. 47. P. 659-667.
39. Shirai N., Higuchi T., Suzuki H. Analysis of lipid classes and the fatty acid composition of the salted fish roe food products. Food chemistry. 2006. T. 94. P. 61-67.
40. Simopouls A. P. The importance of omega -6 /omega 3 fatty acids ration in cardiovascular disease and other chronic diseases Experimental biology and medicine. 2008. T. 233. P. 674-688.
41. Stolyhwo A., Kołodziejska I., Sikorski E. Z. Long chain polyunsaturated fatty acids in smoked Atlantic mackerel and Baltic sprats. Food Chemistry. 2006. T. 94. P. 589-595.
42. Strobel C., Jahreis G., Kuhnt K. Survey of –3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids in health and disease. 2012. T. 11. P. 1-144.
43. Tocher D. R. Metabolism and foundation of lipids and fatty acids in Teleost fish. Reviews in Fisheries science. 2003. T.1192. P. 107-184.
44. Trattner W., Wretling S., Öhrvik, V., Mattisson I. Fatty acid composition of Swedish bakery products, with emphasis on *trans*-fatty acids. Food Chemistry. 2015. T. 175. P. 423–430.
45. Tveteras S., Asche F., Marc F., Bellemare M., Smith M., Guttormsen A., Lem A., Lien K., Vannuccini, S. Fish is food – the FAO's fish price index. PLoS One. 2012. *7*(5), e36731.
46. Ulbricht T. L., Southgate D. A. Coronary heart disease: Seven dietary factors. Lancet. 1991. T.338. P. 985-992.
47. Valenzuela A, Morgado N. Trans fatty acid isomers in human health and in the food industry. Biological research. 1999. T. 32(4). P. 273-87.

Received 28 October 2021
Accepted 30 December 2021

Veterinarija ir Zootechnika

Volume 79(2), 2021
Supplement

The international scientific conference is dedicated to the 75th anniversary of Lithuanian University of Health Sciences Veterinary Academy Faculty of Animal Sciences.
2021 November 11. Kaunas

LIVESTOCK PRODUCTION: RECENT TRENDS AND FUTURE PROSPECTS

Abstracts

Comparison Between Pork Quality Traits When Pigs Were Reared Under Intensive and Extensive Conditions

Asta Racevičiūtė-Stupelienė[1], Vilma Vilienė[1], Monika Nutautaitė[1], Jolita Klementavičiūtė[1], Vilma Šašytė[2], Deimantė Lymontaitė[1]

[1]Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: asta.raceviciutestupeliene@lsmuni.lt
[2]Dr. L. Kriaučeliūnas Small Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania

Pig production is one of the most important livestock sectors globally (1). Primarily, domesticated livestock has been farmed extensively. However, the overall objectives of the development of intensive livestock production have been improved: animal nutrition, feed efficiency, health management, environmental control, reproduction management, genetic selection for better performance, and consistency of product quality and delivery to the marketplace (2). So, this study was aimed at performing a comparative analysis of pork quality from pigs raised under intensive and extensive conditions. From each group, 6 pigs were selected, for a total of 12 pigs. After slaughter, the samples were collected post-mortem and taken from the longest back muscle (*Longissimus dorsi*) between the 12th and the last rib. The following analyses of pork quality were performed 48 hours after slaughter: physical and chemical characteristics, biogenic amine and fatty acid profiles, cholesterol and malondialdehyde concentrations were determined. When analysing pork colour characteristics, it was found that pork reared under extensive conditions had 6% lower brightness (L*), but 15% higher redness (a*) and 39% higher yellowness (b*), compared with pork reared intensively. Pork from extensively reared pigs showed 1.9% higher water binding, 0.94% higher tenderness and 16.66% higher water content, but 11.41% lower cooking losses and 3.26% lower pH compared with pork raised under intensive conditions. However, the results obtained for physical properties, as well as chemical features, were not statistically significant ($P > 0.05$). Assessing the profile of biogenic amines, the meat of extensively reared pigs showed a 35.54% lower phenylethylamine content than the meat of intensively reared pigs ($P < 0.05$). Pork from pigs reared under extensive conditions was found to have 3.52% lower cholesterol and 0.1 μmol/kg lower malondialdehyde, as well as higher levels of linoleic, elaidic and total monounsaturated and polyunsaturated fatty acids contents compared with meat from intensively grown pigs. Nevertheless, the results obtained were found to be not significant ($P > 0.05$). In conclusion, we can claim that the meat of pigs reared under both intensive and extensive circumstances is of similar quality because we observed no significant differences between analysed pork reared under different conditions.

Keywords: intensive, extensive, pigs, pork quality, comparative analysis.

References

1. *Food Outlook: Biannual Report on Global Food Markets, June 2021; 2021 IIS 3410-S41.49219; ISBN 978-92-5-134334-0 (Internet). 2021.*
2. *Maes DGD, Dewulf J, Piñeiro C, Edwards S, Kyriazakis I. A critical reflection on intensive pork production with an emphasis on animal health and welfare. Journal of animal science 2020, 98(Supplement_1), S15–S26.*

Possibility to Use Mechanical Vibrations for Prevention and Treatment of Bovine Mastitis

Antanas Sederevičius[1], Vaidas Oberauskas[1], Rasa Želvytė[1], Judita Žymantienė[1], Kristina Musayeva[1], Joris Vėžys[2], Algimantas Bubulis[3], Vytautas Jūrėnas[3], Juozas Žemaitis[4]

[1]Department of Anatomy and Physiology Research Center of Digestive Physiology and Pathology, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania
[2]Department of Mechanical Engineering, Faculty of Mechanical Engineering and Design, Kaunas University of Technology, Lithuania, E-mail: joris.vezys@ktu.lt
[3]Institute of Mechatronics, Kaunas University of Technology, Lithuania
[4]UAB "Grūduva"

The aim of the study is to develop a mechanical vibration excitation device for the improvement of udder and teat blood flow in cattle (cows), prevention of subclinical mastitis and treatment of clinical mastitis.

There are known devices developed by foreign scientists that expose the udder by acoustic or electromagnetic waves of a certain frequency (1). This induces harmonic and directional oscillations of different frequencies directed to a certain area of the excited object, e.g. local udder location (2) (Device "ARMENTA", Israel, 2018)). The udder and teat contain an extensive network of blood vessels and capillaries, which reduces the efficiency of the milking process and increases the risk of developing mastitis. The mechanical vibration device developed by LSMU and KTU researchers is mounted on the milker, and while operating during the milking process, it analyses input parameters such as temperature, amplitude of oscillations and pulse using the oximeter; the system is able to adapt the vibration frequency and duration of action according to measured parameters. This device is designed to improve the blood flow to the udder and teats of cows, reduce the incidence of mastitis and increase the effectiveness of therapy. The device has a feedback control system, by which the data can be stored in a database, which allows changing the technological parameters of the device with the help of artificial intelligence.

Experimental studies were performed with a cow udder training model and Laval milkers, each of which was excited by low-frequency directional harmonic oscillations (frequency 10–45 Hz, amplitude 2–5 mm). Vibrations were measured with a laser vibration sensor (KEYNCE LK-G82) both on the milking machine body and on the teats and at various locations in the udder. The mechanical vibrations caused by the vibrator in the milker propagate through the teats to the entire udder and produce a physiotherapeutic effect, activating its blood circulation. Tests were performed when the test udder model was filled with fluid. In this way, the dynamic characteristics of the udder subjected to vibrations change, thus approaching the real conditions when the cow's udder is full of milk. Further research will be done with real animals, thus confirming the results of our training model.

Keywords: mechanical vibration, shockwave therapy, mastitis

References

1. *Wang CJ, Schaden WKo J-Y: Shockwave Medicine, TransI Res Biomed. Basel, Karger, 2018, vol.6, pp. 107–108.*
2. *Russian Federation Patent RU 2644826, 14.02.2018*

Effect of Humic Substances Supplement on Growth Performance, Gastrointestinal Tract and Meat Quality Parameters of Broiler Chickens

Agila Daukšienė[1,2], Modestas Ružauskas[1,2,3], Romas Gružauskas[5], Vytaute Starkutė[1,4], Dovilė Klupšaitė[1], Vita Lėlė[1,4], Paulina Zavistanavičiūtė[1,4], Eglė Zokaitytė[1,4], Erika Mozurienė[1], Gražina Juodeikienė[5] and Elena Bartkienė[1,4]

[1]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Lithuania, E-mail: agila.dauksiene@lsmuni.lt
[2]Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Lithuania
[3]Institute of Microbiology and Virology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Lithuania
[4]Department of Food Safety, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Lithuania
[5]Department of Food Science and Technology, Faculty of Chemical Technology, Kaunas University of Technology, Lithuania

The rapid spread of antimicrobial-resistant bacterial communities is a threat to human, animal, and environmental health. Humic substances (HS) are a promising feed additive as an alternative to reduce the use of growth promoter antibiotics in poultry feed (Domínguez-Negrete et al., 2019). Active ingredients of HS consist of humus, humin, humic acid, ulmic acid, fulvic acid, and certain microelements (Arif et al., 2019). The objective was to evaluate the growth performance, caecum microbial profiles and physico-chemical meat quality parameters of broiler chickens (BC). A total of 60 ths. *Ross 308* cross broilers were randomly divided into two groups and fattened for 40 days. The dietary treatments consisted of the basal diet feed (control group n = 30 000) without supplementation and the diet supplemented with HS at the level of 2 g/kg of feed (experimental group (HS group), n = 30 000). A corn-soybean meal-based diet was formulated according to the nutritional requirements prescribed in the *Ross* nutrition specification (2019) and NRC (1994). SPSS software version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Differences were classified by the Duncan multiple comparison test. Results were considered statistically significant at $P \leqslant 0.05$. The results showed that the addition of HS had a positive impact on the broilers' growth performance: the body weight by 1% and the feed conversion ratio (FCR) by 0.6% were decreased ($P \geqslant 0.05$). It was found that the caecum of 1-day old BC was colonised with bacteria; however, the variety of microorganisms was low, with the most prevalent *Escherichia*, *Clostridium*, and *Enterococcus*. In the gut of 40-day old BC, predominant bacteria were *Bacteroides*, *Bransiella*, *Lactobacillus*, *Faecalibacterium*, and *Blautia*. As the results showed, the addition of HS increased the amount of caecal probiotica bacteria (particularly *Bifidobacterium* spp.). An addition of HS caused an increase in breast meat yield by 1.5% and leg meat yield by 0.8%, compared with the control group ($P \geqslant 0.05$). Meat tenderness, cooking and drip loss, protein and fat content of the HS group was higher, but differences in these indicators were not statistically significant ($P \geqslant 0.05$). HS group meat samples showed lower dry matter, water holding capacity, redness, and pH (24 h), compared with the control group ($P \geqslant 0.05$). Overall, these results show that HS had positive tendencies on poultry production, caecal probiotica bacteria colonisation and meat quality.

Keywords: humic substances, productivity, microbiota, meat quality, broiler chickens.

References

1. *Domínguez-Negrete A, et al., Animals 2019, 9, 1–15.*
2. *Arif M, et. al., Iran Journal of Veterinary Research 2019, 20(3), 167–172.*

Impact of Extruded and Fermented Wheat Bran on Production Quality of Broiler Chickens

Agila Daukšienė[1,2], Romas Gružauskas[4], Vita Lėlė[1,3], Vytautė Starkutė[1,3], Dovilė Klupšaitė[1], Paulina Zavistanavičiūtė[1,3], Erika Mozurienė[1], Eglė Zokaitytė[1,3], Gražina Juodeikienė[4] and Elena Bartkienė[1,3]

[1]Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Lithuania, E-mail: agila.dauksiene@lsmuni.lt
[2]Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Lithuania
[3]Department of Food Safety, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Lithuania
[4]Department of Food Science and Technology, Faculty of Chemical Technology, Kaunas University of Technology, Lithuania

Wheat bran (WB) is a cereal by-product of the milling industry, which can be used as valuable feed ingredient in feed of poultry (1). WB is also considered to be a source of dietary fibers or non-starch polysaccharides, which cannot be directly utilized by broiler chickens (2). However, WB can be converted into value-added products and also animal feeds by using the process of extrusion and fermentation. To study the impact of extruded and fermented WB on meat quality of broiler chickens, a total of 42 ths. broilers (1-day old, *Ross 308* cross) were divided into two groups for 40 days of fattening. The control group (CON group) was fed the basic compound feed. The 3% of basal were replaced by WB extruded and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* in the diet of treatment group (WB group) and fed for 15 days of fattening. After 15 days, the broilers of the WB group received the basic compound feed. A soybean meal and corn based diet was formulated according to NRC (1994) and the *Ross* nutrition specification (2019). SPSS software version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Differences were classified by the Duncan multiple comparison test. Results were considered statistically significant at $P \leqslant 0.05$. Results showed that the breast and leg meat yield was improved, compared with the control ($P \geqslant 0.05$). There was a significant ($P \leqslant 0.05$) increase in breast meat water binding capacity (WHC), cooking losses (CL), amount of fat and ashes, compared with the control. The leg meat CL, lightness and redness were increased, compared with the control group ($P \leqslant 0.05$). The results of meat sensory attributes showed that WB had positive tendencies on breast meat overall acceptability, leg meat total odour intensity, juiciness, fatness and softness, compared with the control ($P \geqslant 0.05$). It was found that WB in the feed of broilers had an effect on chicken meat emotional acceptability: predominant emotions of taste were happy, surprised and neutral, compared with the control ($P \geqslant 0.05$). In summary, the use of extruded and fermented WB in the feed of broiler chickens had no adverse effect on broiler meat physical and chemical parameters.

Keywords: valorization of wheat bran, meat quality, broiler chickens.

References

1. *Luo Y, et al., Food and Function 2021, **12**, 2962–2971.*
2. *Semjon B, et al., Animals 2020, 10(6), 942.*

Rojal Jelly Supplementation Can Improve Boar Semen Motility and Viability Parameters during Liquid Storage

Iljenkaitė Aistė[1], Sonata Trumbeckaitė[2], Kerzienė Sigita[1], Sutkevičienė Neringa[1]

[1]Large Animal Clinic, Animal Reproduction Laboratory, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: neringa.sutkeviciene@lsmuni.lt
[2]Laboratory of Biochemistry, Neuroscience Institute, Medicine Academy, Lithuanian University of Health Sciences, Lithuania

Royal jelly is gluten secreted by hypopharingeal and submandibular glands of young worker bees and has different types of biological activity in various cells and tissues of animal models, and serves as an antioxidant source (1, 2, 3). A successful effect of royal jelly supplementation on sperm quality and fertilizing ability has been reported in domestic animals (4, 5, 6). The current study was carried out to investigate the protective effects of the royal jelly supplementation on the sperm kinematics and plasma membrane functionality during the liquid storage of boar semen at 16°C and 4°C, at various periods of time (0, 24, 48, 72 and 96 h). Semen samples were collected from 11 boars, diluted with a long-term extender and supplemented with different concentration of raw royal jelly (control – 0%, 0.5%, 1% and 2%) at a final concentration of 50×10^6 sperm/mL. Only those samples having more than 75% motility and more than 75% normal sperm were used for further experiments. In the laboratory, the semen was assessed for sperm morphology, viability (eosin-nigrosin staining), subjective motility and objective sperm motility by the sperm class analyzer (SCA). Sperm viability and motility in two ways were checked after 24, 48, 72 and 96 h of incubation. In total, 396 tests for sperm viability and motility were performed. The longer storage time and the lower incubation temperature showed the lower sperm motility and viability results in the all treated and non-treated samples. The results showed that royal jelly supplementation at lower concentration (0.5% and 1%) for storage time at 16°C temperature resulted in a protective effect on cell membrane integrity; however, the liquid storage of semen supplemented with 2% royal jelly had a negative effect on sperm plasma membrane integrity. The highest viability values were found in 1% royal jelly concentration at all storage times at 16°C temperature. Sperm subjective and objective motility SCA results in the samples stored at 4°C decreased with a higher royal jelly concentration and a longer storage time and differed significantly compared with results in the samples stored at 16°C ($P < 0.05$). Our data showed that the royal jelly supplementation at lower concentrations can improve boar semen motility and viability parameters during liquid storage at 16°C for 96 h and no protective effect was observed for sperm functionality and kinematics at 4°C temperature.

Keywords: royal jelly, boar, sperm viability, sperm motility.

References

1. *Kamakura M, et al., Bioscience, Biotechnology, and Biochemistry 2001, 65, 277–284.*
2. *Petelin A, et al., Evidence-Based Complement Altern Med 2019, Article ID 4969720, 11 p.*
3. *Moradi A, et al., Small Rum Res 2013, 113, 346–352.*
4. *Abd-Allah SM, J Buff Sci 2012, 1, 1–4.*
5. *Alcay S, et al., Cryobiology 2017, 74, 81–85.*
6. *Shahzad Q, et al., Anim Reprod Sci 2016, 167, 83–88.*

Effect of Extenders on Stallion Epididymal Sperm Motility and Viability after 72 Hours of Storage

Anna Ellung Lehmann[1], Indrė Poškienė[2], Lina Anskienė[3], Jūratė Sabeckienė[4], Neringa Sutkevičienė[1]

[1]Animal Reproduction Laboratory, Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: neringa.sutkeviciene@lsmuni.lt
[2]Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania
[3]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania
[4]Department of Veterinary Pathobiology, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania

Collection of epididymal stallion sperm offers the opportunity to retain and use genetic material from the males after elective castration or even post mortem (1, 2). Epididymal semen can be used either cryopreserved or fresh, and stored for some days according to the extender used (3). The aim of the present study was to evaluate the effect of the extender on stallion epididymal sperm motility and viability during liquid storage for 72 h at 5°C in EquiPlus extender and Ringer B. Braun solution. For this study, 8 stallions (3–10 years old) of different breeds were used. Sperm from the cauda epididymis was harvested immediately after routine castration and diluted in a prepared EquiPlus semen extender and in Ringer B. Braun solution. The semen was assessed for primary (immediately after collection) semen quality: sperm viability (eosin–nigrosine staining), progressive subjective motility and motility by sperm class analysis (SCA). The samples were stored at 5 ± 1°C for 72 h. Sperm viability, progressive subjective motility and SCA motility were checked after 24, 48 and 72 h of incubation of the samples at 5 ± 1°C. The motility assay showed that the longer the storage time the lower the sperm motility results in both diluents. Results of subjective sperm motility in EquiPlus after 72 h of storage were 12.2% ± 3.61% higher than those in Ringer solution ($P < 0.05$), although subjective sperm motility 1 h after dilution was 2.5% ± 1.49% higher in Ringer solution ($P > 0.05$). SCA motility results after 72 h were higher in EquiPlus (37.05% ± 2.01%, $P < 0.001$). Analysis of sperm viability during liquid storage at 5°C in the different diluents showed better results in EquiPlus extender after 24 h (4% ± 6.03%), 48 h (4.35% ± 2.45%) and 72 h (10.15% ± 0.92%), although primary sperm viability results were better (90.8% ± 5.57%) in Ringer solution than those in EquiPlus (88.45% ± 7.05%, $P > 0.05$). We found statistically significant differences in epididymal sperm motility results in the two extenders after 72 h liquid storage. The highest percentages of progressive motile epididymal spermatozoa (22.00% ± 10.59%) and viable sperm (80.35% ± 11.2%) after 72 h were found in sperm diluted with EquiPlus extender ($P < 0.05$); meanwhile, for short term storage (1–24 h), epididymal semen can also be stored in Ringer B. Braun solution.

Keywords: stallion, semen storage, semen quality, Ringer B solution.

References

1. *Monteiro GA, Guasti PN, Rocha AS, Martin I, Sancler-Silva YFR, Dell'Aqua CPF, Dell'Aqua JA, Papa FO. Journal of Equine Veterinary Science 2013, 33, 169–173.*
2. *Gloria A, Carluccio A, Petrizzi L, Noto F, Contri A. Theriogenology 2016, 85(2), 345–350.*
3. *Stawicki RJ, McDonnell SM, Giguère S, Turner RM. Theriogenology 2016, 85(4), 698–702.*

Micro- and Macroelements in Seminal Plasma Influence Diluted Boar Semen Quality for Seven Days

Barbora Paulavičiūtė, Audronė Rekešiūtė, Antanas Banys, Neringa Sutkevičienė

Animal Reproduction Laboratory, Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: neringa.sutkeviciene@lsmuni.lt

Growing evidence shows that macro- and microelements in the seminal plasma of domestic animals are of great importance due to their roles in sperm metabolism, function, survival and oxidative stress (1, 2). The current study was carried out to investigate the effect of micro- and macroelements in fresh boar seminal plasma and their association with sperm quality parameters after 7 days of liquid storage at 16°C. Semen samples were collected from 40 boars and diluted with a long-term extender at a final concentration of 35×10^6 sperm cells/mL. In the laboratory, the semen was assessed for sperm morphology, viability (eosin-nigrosin staining), pH, subjective and objective sperm motility by sperm class analyzer (SCA). Only those samples having more than 75% motility and more than 75% normal sperm were used for further experiments. Sperm viability, motility and pH were checked after 24, 48, 72 and 168 h of storage at 16°C. Seminal plasma was separated and the concentration of macroelements (Na, K, Ca, Mg, P) and microelements (Cu, Zn, Fe) was determined. The longer storage time showed the lower sperm motility and viability results in all samples. Motility measured subjectively dropped by 21% ($P < 0.01$) and by 12.58% ($P < 0.01$) measured objectively. Viability decreased by 21.98% ($P < 0.01$) and pH value dropped by 0.21 ($P < 0.01$) after 7 days of incubation. Elements correlated with sperm quality parameters. Iron and copper negatively correlated with sperm tail abnormalities ($P < 0.05$) and showed a positive effect on sperm motility and viability results ($P < 0.05$) after 7 days. With the increase of potassium, sperm viability after 7 days declined ($P < 0.01$). After 7 days of storage, pH values strongly correlated significantly with sperm viability ($P < 0.01$). Our data showed that micro- and macroelements in seminal plasma have an effect on diluted boar semen quality parameters for 7 days of incubation and could be as a predictive value of boar semen fertility.

Keywords: boar, semen plasma, microelements, macroelements, semen quality.

References

1. *Pipan MZ, Mrkun J, Strajn BJ, Vrtač KP, Kos J, Pišlar A, Zrimšek P. Acta Veterinaria Scandinavica 2017, 11(59), 1–9.*
2. *Wu Y, Guo L, Liu Z, Wei H, Zhou Y, Tan J, Sun H, Li, Jiang S, Peng J. Theriogenology 2019, 132; 88–94.*

The Effect of Diets with Different Ratios of Arginine and Lysine on Immune Status, Oxidative and Epigenetic Changes in Tissues of Turkeys

Ewelina Cholewińska[1], Dariusz Mikulski[2], Magdalena Krauze[1], Anna Stępniowska[1], Paulius Matusevicius[3], Katarzyna Ognik[1], Jan Jankowski[2]

[1]University of Life Sciences in Lublin, Lublin, Poland
[2]University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
[3]Department of Animal Nutrition, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

The results of our long-term feeding trials indicate that the inclusion levels of methionine (Met) in turkey diets should be higher than those recommended by the National Research Council (NRC 1994). In the present experiment, it was assumed that the appropriate dietary ratio of arginine (Arg) to lysine (Lys) can improve the immune status and growth performance of turkeys. The aim of this study was to evaluate the effects of two inclusion rates of Arg relative to Lys (95% and 105%) in turkey diets with Lys content consistent with NRC recommendations (1994) or 10% higher on the immune status of birds and indicators of protein and DNA damage due to oxidation, nitration or epigenetic changes. Another goal was to determine which dietary Arg:Lys ratio stimulates the immune response of turkeys vaccinated against *Ornithobacterium rhinotracheale* (ORT). The experiment was performed on 576 female turkeys that were randomly assigned to 32 pens. The experiment had a completely randomized design with four dietary treatment groups, 8 replicate pens per group and 18 birds per pen. Two dietary inclusion levels of Lys were analyzed, low (L_L = NRC) and high (L_H = NRC+10%). In diets with the low level of Lys, L-Lysine HCl was added to the basal diet to obtain 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in 4 successive feeding periods, according to NRC guidelines (1994). L-Arginine HCl was added to the basal diet to obtain 95% and 105% Arg relative to the content of dietary Lys (low and high, A_L and A_H, respectively). The effects of 4 experimental diets, with 2 levels of Lys and 2 levels of Arg (L_LA_L, L_LA_H, L_HA_L, L_HA_H), were compared in the study. The inclusion rate of Met in experimental diets exceeded that recommended by the NRC (1994), and DL-Methionine was added to obtain 0.62, 0.59, 0.51 and 0.39 g of Met per 100 g of feed in 4 successive feeding periods. The dietary treatments had no influence on the growth performance of turkeys. It was found that the Lys content of turkey diets should be 10% higher than that recommended by the NRC (1994). The increased Lys content should be combined with the higher Arg level (105% of Lys content). Although the above Arg:Lys ratio did not improve the growth performance of birds, it stimulated their immune system (in particular the immune response following vaccination) and reduced protein nitration as well as protein and DNA oxidation. The present findings can be used to revise and update the existing nutritional recommendations for the optimal levels and ratios of Arg and Lys in turkey diets.

This work was supported by the National Science Center, Grant No. 2017/27/B/NZ9/01007.

The Effect of Different Bedding Materials on Hygiene in Calf Housing

Dorota Witkowska[1], Aneta Ponieważ[1], Katarzyna Rychlica[1], Daria Murawska[2], Paulius Matusevičius[3]

[1]Department of Animal and Environmental Hygiene, Faculty of Animal Bioengineering, University of Warmia and Mazury, Olsztyn, Poland, E-mail: dorota.witkowska@uwm.edu.pl
[2]Department of Commodity Science and Animal Improvement, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
[3]Department of Animal Nutrition, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Kaunas, Lithuania

The type and quality of bedding material significantly influence the health and welfare of farm animals that are kept in buildings. The basic function of bedding in calf housing is to absorb moisture and toxic gases, keep the floor dry and shape a beneficial microclimate (1). One of the most popular bedding materials in many countries is straw. Some research indicates that dust levels can be significantly higher in facilities using straw in comparison with alternative bedding materials, whereas dust is an important carrier of microorganisms. Given that calves' immunity builds for a relatively long time, this group of animals may be especially exposed to diseases caused high microbiological contamination. The choice of high-quality bedding material may improve hygiene in calf housing. For this reason, the aim of our study was to determine the effect of classic straw and 6 alternative bedding materials (light pellets, medium sawdust, peat, chopped straw, flax, hemp) on the levels of microbial contamination of air. The calves were kept in accordance with the Polish standards (Journal of Laws 2010, No. 56, item 344) in identically equipped and separated boxes (8 calves on each type of bedding). Quantitative analyses of mesophilic aerobic bacteria and fungi were conducted every 2 days for a period of 2 weeks. Air samples (5 L) were collected with an air sampler MAS-100 Eco® (Merck Corp.) at 5 locations in each box. Bacteria and fungi were cultured on solid media (TSA and Sabouraud) and incubated at 35°C for 24 h (bacteria) or 25°C for 120 h (fungi). Microbial counts were expressed in cfu/m^3 of air after correction (Feller's formula). After logarithm transformation, the data were normally distributed (Kolmogorov-Smirnov test) and analyzed by ANOVA (StatSoft). Considering bacterial contamination of air, the best quality ($P < 0.01$) characterized peat, light pellet, and sawdust (4.57, 4.59 and 4.64 $\log_{10}$ cfu/m^3, respectively). The most contaminated air was registered using traditional straw ($P < 0.01$; 4.79 $\log_{10}$ cfu/m^3). Chopped bedding materials (straw, flax, and hemp) had an intermediate effect. Whereas in the case of fungi, chopped flax and hemp had the best impact on the quality of air ($P < 0.01$; 3.96 and 4.09 $\log_{10}$ cfu/m^3). The fungal contamination levels were the highest using light pellet and peat ($P < 0.01$; 4.50 and 4.47 $\log_{10}$ cfu/m^3). It could be concluded that bedding material significantly influences microbial air quality and hygiene standards in calf housing.

Key words: calf health and welfare, bedding materials, microorganisms, air quality.

References

1. *Kwiatkowska-Stenzel A, et al. Research in Veterinary Science 2017, 115, 523–529.*

Dairy Cows Stress Assessment by Using Innovative Biomarkers of Herd Management Systems

Dovilė Malašauskienė, Mindaugas Televičius, Mingaudas Urbutis, Ramūnas Antanaitis

Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania

The objectives of these studies were to examine the option of using automatic health tracking systems biomarkers as a form of stress indicator and to determine the relationship between biomarkers, blood cortisol levels, and lactate concentration. Ninety cows within 1–30 days were selected and categorized into 3 groups: group 1 – 1–7 days after parturition (dpp) (n = 30); group 2 – 8–14 dpp (n = 30), and group 3 – 15–30 dpp (n = 30) after calving. The cows were milked using Lely Astronaut® A3 milking robots. The pH and temperature of the contents of cow reticulorumen were measured using specific smaX-tec boluses manufactured for animal care. The blood samples were tested for cortisol, lactate concentration. Data about rumination time (RT), milk yield (MY), milk composition, milk somatic cell count (SCC), milk electrical conductivity (EC), consumption of concentrate (CC), weight (BW) were collected from the Lely T4C management program. The RT increased during all of the exploratory periods (with readings between 1.12%–4.90%). A decrease was also observed in the lactate levels (by 1.10 times) and cortisol levels (by 1.98 times, $P < 0.05$) of cows in group 2 (8–14 dpp) compared with an average of group 1 (1–7dpp) in the previous study period (15–30dpp). However, lactate concentrations increased (by 1.84 times, $P < 0.05$) as well as cortisol levels (by 2.09 times, $P < 0.01$) when compared with group 2 on average. The results obtained indicate that RT increased during all exploratory periods, while a decrease of 1.10 times and 1.98 times was observed in lactate levels and cortisol levels, respectively. RT positively correlated with the lactate concentration levels and negatively correlated with cortisol levels during the entire study period. According to this study, there was a positive correlation with milk lactose (ML) during stress, which tends to increase the risk of mastitis and decrease CC, RT, BW, MY, reticulorumen ph, and fat/ protein ratio F/P.

Keywords: biomarkers, stress, rumination time, cortisol, lactate.

References

1. *Soriani N, Trevisi E, Calamari L (2012) Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. J Anim Sci 90: 4544–4554.*
2. *Bristow DJ, Holmes DS (2007) Cortisol levels and anxiety-related behaviors in cattle. Physiol behav 90: 626–628.*
3. *Isehunwa GO, Oladun OT, Akpan JE, Alada ARA (2015) Effect of Cortisolon Plasma Lactate Levels following Cortisol-induced Hyperglycaemia in Common African Toad, Bufo regularis. Niger J Physiol Sci 32: 21–25.*
4. *Mishra M, Martz FA, Stanley RW, Johnson HD, Campbell JR and Hilderbrand E. Effect of diet and ambient temperature-humidity on ruminal pH, oxidation reduction potential, ammonia and lactic acid in lactating cows. Journal of Animal Science, 1970 30(6), 1023–1028. 47.*
5. *Lefcourt, AM, Huntington JB, Akers RM, Wood DL, Bitman J. Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. Domestic animal endocrinology, 1999 16(1), 41–55.*

Evaluation of Genetic Correlation Between Lameness and Productivity of Cows

Gediminas Urbonavičius[1], Ramūnas Antanaitis[2], Vida Juozaitienė[1]

[1]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: gediminas.urbonavicius@lsmuni.lt
[2]Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

The literature suggests that severe lameness is associated with poor general health, a weak cow immune system and decreased milk production (1, 2, 3). The aim of our study was to evaluate the genetic relationship between lameness and milk traits in fresh dairy cows (n = 4726). Lameness (during 2018–2020) was diagnosed on the visual locomotion scale (4) in 7.2% fresh dairy cows. Parameters such as milk yield, milk lactose, milk fat, milk protein and somatic cell count (SCC) were registered with the help of Lely Astronaut® A3 milking robots. Analysis of somatic milk cells was carried out with the logarithmic expression of this indicator: SCS = (log2 (SCC/100)) + 3 (5). Genetic correlations (r_g) were calculated using programs: PEST 4.2 (Multivariate Prediction and Estimation, 12 March 1999, Linux 2.0.36. Groeneveld E., Kovac M., Wang T. Department of Animal Sciences, University of Illinois) and VCE 4.2.5 (8 December 1998, Linux 2.0.34 i586, written by E. Groeneveld).

The results of phenotypic studies showed that the milk yield of healthy cows (28.36 ± 0.122 kg) was by 1.24 kg more, milk protein (3.48 ± 0.006%) was by 0.04 percentage points higher than in the group of lame cows ($P < 0.05$). The average concentration of lactose in milk in the group of healthy cows (4.67% ± 0.004%) was by 0.10 percentage points higher than in the group of sick cows ($P < 0.05$), and the quality of milk on the SCS scale was better (1.04 times) in non-lame cows (SCS = 1.89 ± 0.007, $P < 0.05$).

After genetic evaluation of the data, we found a positive genetic correlation between lameness scores and somatic cells in milk ($r_g = 0.220$). These results were found to indicate a higher genetic predisposition for cows with higher levels of SCC in milk to lameness. An unfavorable negative genetic correlation was revealed between the assessment of cows for signs of lameness and their milk yield, as well as the content of milk fat, protein and lactose ($r_g = –0.098–0.300$). This research shows the genetic links between cow health and productivity, as well as the potential for genetic herd improvement.

Keywords: dairy cows, heath, lameness, productivity, genetic correlation.

References

1. *Refaai W, Gad M, Mahmmod Y. Association of claw disorders with subclinical intramammary infections in Egyptian dairy cows. Veterinary World. 2017, 2231.*
2. *Hagiya K, Yamazaki T, Nagamine Y, Togashi K, Yamaguchi S, Gotoh Y, Kawahara T, Masuda Y, Suzuki M. Genetic correlations between production and disease traits during first lactation in Holstein cows. Animal. 2014, 8, 217–223.*
3. *Sato K, Bartlett PC, Alban L, Agge JF, Houe H. Managerial and environmental determinants of clinical mastitis in Danish dairy herds. Acta Vet. Scand. 2008, 50, 4.*
4. *Sprecher DJ, Hostetler DE, Kaneene JB. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 1997, 47, 1179–1187.*
5. *Ali AKA, Shook GE. An optimum transformation for somatic cell concentration in milk. J Dairy Sci. 1980, 63, 487.*

Heritability of Lameness in Dairy Cows

Gediminas Urbonavičius[1], Ramūnas Antanaitis[2], Vida Juozaitienė[1]

[1]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
E-mail: gediminas.urbonavicius@lsmuni.lt
[2]Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

In the last few decades, greater attention has been focused on improving animal welfare in the European Union and globally. Lameness is an important health and welfare concern in dairy farming and has negative implications on productivity and behavior (1, 2, 3). The aim of our study was to evaluate the heritability of lameness and to compare it with heritability of milk traits in fresh dairy cows. The experiment was carried out on 10 commercial dairy farms. Cows were kept in free housing system farms, milking with Lely Astronaut A3 milking robots. A lameness diagnosis (from calving till 30 days after calving) was performed on the visual locomotion scale (VLS) by trained staff (by the same person) according to the standard procedure described by Sprecher et al. (4): 1 = normal, 2 = presence of a slightly asymmetric gait, 3 = the cow clearly protects one or more limbs (moderately lame), 4 = severely lame, and 5 = extremely lame (non-weight-bearing lame). For the study of heritability (h^2), PEST 4.2 (Multivariate Prediction and Estimation, 12 March 1999, Linux 2.0.36. Groeneveld E., Kovac M., Wang T. Department of Animal Sciences, University of Illinois) and VCE 4.2.5 (8 December 1998, Linux 2.0.34 i586, written by E. Groeneveld) programs were used. The following effects and their statistical interpretations were applied in the model: lactation number (fixed), year season (fixed), animal (additive genetic effect, random) and error (random). We found that the heritability of lameness was 0.23. The analysis showed that only a small part of the phenotypic changes in this indicator in the analyzed population was associated with genetic factors. On the other hand, the heritability of milk production and composition (h^2 = 0.20–0.25) was at a similar level, and these indicators are used in the breeding program for genetic improvement of dairy cattle.

Keywords: dairy cows, lameness, productivity, heritability.

References

1. *Zhang X, Ding J, Li Y, Song Q, Li S, Hayat MA, Zhang J, Wang H. The changes of inflammatory mediators and vasoactive substances in dairy cows' plasma with pasture-associated laminitis. BMC Vet. Res. 2020, 16, 119.*
2. *Donovan GA, Risco CA, Temple GD, Tran TQ, Van Horn HH. Influence of transition diets on occurrence of sub-clinical laminitis in Holstein dairy cows. J. Dairy Sci. 2004, 87, 73–84.*
3. *King M, Leblanc S, Pajor E, Devries T. Cow-level associations of lameness, behavior, and milk yield of cows milked in automated systems. J. Dairy Sci. 2017, 100, 4818–4828.*
4. *Sprecher DJ, Hostetler DE, Kaneene JB. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 1997, 47, 1179–1187.*

Influence of Seasonality on B-Hydroxybutyrate (Bhb) at Early Postpartum Period and Reproduction Performance in Dairy Cows

Indrė Mečionytė[1], Vytuolis Žilaitis[2], Lina Anskienė[1], Giedrius Palubinskas[1]

[1]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: indre.mecionyte@lsmuni.lt
[2]Large Animals Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

High-yielding dairy cows suffer from negative energy balance (NEB) during the first weeks of lactation (1). The NEB depends on zootechnical factors and seasonality. The effect of seasons on cows is controversial, some authors argue that seasons affect the health and reproductive performance of cows (2). The main sign to determine NEB can be evaluated by β-hydroxybutyrate (BHB) concentration in cows' blood. Therefore, the aim of this study was to determine the influence of seasonality on BHB at the early postpartum period and reproduction performance of dairy cows.

The experiment was carried out on 1200 dairy cows. Blood samples were taken from the ear vein at 7–10 DIM in the mornings after milking, and the level of BHB (mmol/L) was determined in different seasons. The number of cows with higher concentration of blood BHB (> 1.2 mmol/L) had a tendency to increase in cold seasons of the year: 28.57% of all cows had increased blood BHB more often in winter (χ^2 = 12.857, df = 1, $P < 0.001$) and 41.43% in autumn (χ^2 = 2.057, df = 1, $P > 0.05$). An average blood BHB concentration in cows was 11.14% higher in summer and autumn than in other seasons ($P < 0.05$). The number of inseminations depends on blood BHB concentration. Four times inseminated cows had 22.27% higher blood BHB concentration than single inseminated cows ($P < 0.001$), and 23.77% higher than double inseminated cows ($P < 0.001$). The first insemination time correlated with blood BHB (r = 0.176; $P < 0.001$).

In conclusion, the season affects the distribution of cows with elevated and average BHB concentration and has influence on insemination rate and first insemination time.

Keywords: cow, NEB, BHB, season, insemination.

References

1. *Xu W, et al. Scientific Reports. 2018, 8.*
2. *Turk R, et al. Reproductiom in domestic animals. 2015, 50(4), 595–603.*

Relationship of Milk Production and Somatic Cell Count in Purebred and Crossbred Cows of Lithuanian Red and Red and White Population

Renata Japertienė[1], Lina Anskienė[1], Sigitas Japertas[2]

[1]Department of Animal Breeding, Lithuanian University of Health Sciences, Faculty of Animal Sciences, Kaunas, Lithuania, E-mail: renata.japertiene@lsmuni.lt
[2]Public Institution Practical training and trial center of Lithuanian University of Health Sciences, Kauno distr., Lithuania

Cross-breeding of dairy cattle has been used as an alternative to pure breeding and for improvement of various traits. In many cases, crossbreds of the F1 genotype are better than of other genotypes, but the continuous breeding of animals of F1 genotype and the adaptation to the desired genetic combinations of different breeding environments still remain a major challenge. The cross-breeding method produces a heterosis effect, which increases the productivity of cows. This method is recommended for use in productive dairy farms. When using a cross-breeding method to improve dairy herds, it is very important to consider not only the breeds selected for mating, but also the breed of the mother whose characteristics we want to improve and which breed of the father to choose in order to get the desired result.

The aim of this study was to investigate the relationship of milk production and somatic cell count in purebred and crossbred cows of Lithuanian Red and Red and White population. The research was carried out in 7 farms in 2020 with dairy cows (n = 363) of different genotypes of Lithuanian Red cattle population. The conditions for keeping and feeding the cows were similar and they were fed equally balanced rations. The milk yield (MY), milk fat (MF), milk protein (MP), concentration of lactose (L), urea (U) and somatic cells count (SCC) were evaluated. Samples were performed each month of the year during control milking. SCC in milk of cows was divided into groups: (1 – <= 200; 2 – 200–400; 3 – > 400 thousands/mL). For the effects of season, estimation months were assigned (1 – winter, 2 – spring, 3 – summer and 4 – autumn). The statistical analysis of data was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software.

We observed that the highest MY and the lowest SCC were estimated in purebred Lithuanian Red cows; the highest milk protein content was detected in purebred Ayrshire cows; the highest milk fat content and urea concentration was estimated in crossbreds of Lithuanian Red x Danish Red; the lowest and below the norm lactose concentration was detected in crossbreds of Lithuanian Red x Swedish Red.

Analysis of different genotypes of cows according to the groups of SCC showed that with increasing SCC, we did not find a direct effect on milk yield and composition except on concentration of lactose. Lactose content in milk of group 1 of SCC was within the norm, while in group 2 and 3 of SCC, as the number of SCC increased, the lactose content decreased.

By using generalized linear model, we estimated that the dependent variables were linearly related to the factors; of all fixed effects, genotype had the impact on fat and urea content ($P < 0.001$); SCC group on milk yield, lactose content ($P < 0.001$) and urea content ($p<0.01$), season on milk yield, protein, lactose, urea content ($P < 0.001$) and fat content ($P < 0.01$); interaction of genotype with SCC group had an impact only on protein content ($P < 0.05$).

Keywords: genotype, milk production, somatic cell count, season.

In Search of New Potential Markers for Male Fertility in Farm Animals. Aquaporins in the Reproductive System and Metabolomic Profiling of Semen

Katarzyna Michałek[1], Patrycja Oberska[1], Paulina Małkowska[1], Marta Grabowska[2], Maciej Murawski[3], Elena Bartkienė[4], Maria Suciu[5], Dariusz Gączarzewicz[6], Małgorzata Szewczuk[7], Andrzej Syczewski[8]

[1] Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology in Szczecin, Poland, E-mail: kmichałek@zut.edu.pl
[2]Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland
[3]Department of Animal Nutrition, Biotechnology and Fisheries, University of Agriculture in Krakow, Poland
[4]Department of Food Safety and Quality, Lithuanian University of Health Sciences Kaunas, Lithuania
[5]Electron Microscopy Center, Babeş-Bolyai University, Cluj-Napoca, Romania
[6]Department of Animal Reproduction, Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Poland
[7]Department of Ruminant Science, West Pomeranian University of Technology in Szczecin, Poland
[8]Department of Genetic and Animal Husbandry, Szczecin, Poland

Male fertility disorders are a growing problem for both humans and animals. Examination of male reproductive organs and analysis of sperm quality are currently the basic methods for determining the reproductive potential of a male. However, it does happen in everyday animal husbandry practice that fertilization does not occur or its effectiveness is low despite the high functional value of an individual allowed for breeding (1, 2). Hence, it has long been postulated to seek new indicators enabling the precise determination of male reproductive potential and identification of individuals with reduced fertility (3, 4). Studies are conducted in response to the current needs related to animal breeding aimed at answering the question whether the measurement of AQPs in reproductive system and metabolomic sperm evaluation can become a modern, precise and effective indicator enabling the full determination of the male and female reproductive potential and semen quality assessment in the future. The study is carried out on 2 species of farm animals: (1) cattle, a male of the black and white Polish Holstein-Friesian breed, and (2) sheep of the Wrzosówka and Świniarka breeds. The experiment is carried out on the tissues of the male reproductive tract. Bovine tissue samples are collected from 3 age groups: calves 5 to 7 weeks of age, young cattle between 5 and 6 months of age and 1–3 years old reproductive bulls. Sheep tissue samples are collected from 2 age groups: 1–4 months old lambs and adult rams. The presented research involves AQP expression analysis in individual sections of the male reproductive system along with the assessment of animal growth and development and determination of their potential role in the proper course of reproductive processes. The ultrastructure of individual reproductive organs in adult cattle was analyzed. An analysis of AQPs in bovine sperm and metabolomic profiling of semen is also planned, as well as search for relationships between the studied indicators and the quality of sperm and male reproductive potential.

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

References

1. *Oberska P, Michałek K. Animal Reproduction Science 2021, 231, 106807.*
2. *Riberio et al., Molecular Basis of Disease 2021, 1867, 166039.*
3. *Pellavio G, Laforenza U. Biochimie 2021, 188, 45–51.*
4. *Yeste et al., Reproduction in Domestic Animals 2017, 52, 12–27.*

Effects of Beta-Casein Genetic Variants on Milk Composition in the Milk of Dual-Purpose Crossbreed Cows

Kristina de Vitte[1], Ramutė Mišeikienė[2], Sigita Kerzienė[3], Marius de Vitte[4], Rolandas Stankevičius[1]

[1]Department of Animal Nutrition, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: kristina.de.vitte@lsmu.lt
[2]Institute of Animal Rearing Technologies, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Department of Physics, Mathematics and Biophysics, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[4]Coventry University, Coventry, England

Milk is considered nutritious and healthy food for humans. The study aimed to calculate allelic and genotypic frequencies of beta-casein variants (A1 and A2 allele) in crossbred dual-purpose cows and analyze milk production traits of tested cows dependent on beta-casein genotypes. Altogether, genomic DNA of 116 crossbred dual-purpose Simmental cows was collected to estimate beta-casein using the Sanger sequencing method. In the population included in the study, there were homozygote genotype A1A1 (32 animals), A2A2 (33 animals), and heterozygote genotype A1A2 (51 animals). Allele A2 was observed with a frequency of 0.504, and allele A1 with a frequency of 0.496. The most frequent was heterozygous genotype A1A2 (44%), while the homozygous A1A1 genotype was the rarest (27.6%). A2A2 genotype was associated with lower protein content than A1A2 genotype by 0.1% ($P < 0.05$). A breeding program with crossbred dual-purpose Simmental cows could be achieved relatively quick for milk that only contains the beta-casein variant A2.

Keywords: cattle, CSN2 gene, A2 milk, A1A1, A1A2, A2A2.

Antibiotic Resistance Genes Prevalence in *Apis Mellifera* from Different Lithuania Regions

Kristina Morkūnienė, Renata Bižienė, Rūta Insodaitė, Nijolė Pečiulaitienė, Ramutė Mišeikienė, Laimutis Kučinskas

Institute of Biology Systems and Genetics, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: kristina.morkuniene@lsmuni.lt

The honey bee is a major producer of honey. In addition, honey bees are important plant pollinators (1). Genetic analysis of bee microbiota is one of the possible studies that can be used to assess the prevalence of genes leading to antimicrobial resistance in the environment (2). Samples of 111 bees from 4 different Lithuanian apiaries located in Kupiškis, Panevėžys, Prienai and Alytus districts were collected for the study. Group 5 was composed of free-living (wild) bees. Five gene fragments associated with antimicrobial resistance were examined: aminoglycosides (*aph*), beta-lactams (*blaZ*), tetracycline (*tetM*) and sulphonamides (*sul1* ir *sul2*). Four of them were found in bees raised in Lithuania, except for a fragment of the gene encoding aminoglycosides that was not found. The highest frequency of sulphonamide-resistant gene was found in bees. Besides, 43.2% of the samples were positive in more than one gene. The wild bee microbiota had the lowest number of antibiotic-resistant genes. A comparison of antibiotic resistance genes DNA sequences of a varroasis-treated and untreated bee was also performed. The significant difference was only in bees with the *sul2* gene in the microbiota. Bees treated with prophylactic medicinal products have a higher resistance to this group of antibiotics than untreated bees. Analysis of bee results showed no significant difference between particular breeds. We recommend a rational use of antimicrobials to reduce the transfer of genes responsible for antibacterial resistance in the bee microbiota and their possible transmission to the bees themselves, especially by avoiding sulphonamide preparations.

Keywords: honeybee, *Apis mellifera,* antibiotic-resistant genes, microbiota.

References

1. *Syromyatnikov MY, Borodachev AV, Kokina AV, Popov VN. A Molecular Method for the Identifcation of Honey Bee Subspecies Used by Beekeepers in Russia. Insects 2018, 9, 10; doi:10.3390/insects9010010*
2. *Cenci-Goga BT, Sechi P, Karama M, Ciavarella R, Pipistrelli MV, Goretti E, Elia AC, Gardi T, Pallottini M, Rossi R, Selvaggi R, Grispoldi L. Cross-sectional study to identify risk factors associated with the occurrence of antimicrobial resistance genes in honey bees Apis mellifera in Umbria, Central Italy. Environmental Science and Pollution Research. 2020, 27(9):9637–9645; doi: 10.1007/s11356-020-07629-3.*

Comparative Analysis of the Nutritional Value of Rabbit Meat: Differences Between Belgian Giants and Crossbreeds

Monika Nutautaitė[1], Vilma Vilienė[1], Asta Racevičiūtė-Stupelienė[1], Jolita Klementavičiūtė[1], Vilma Šašytė[2], Giedrė Milašienė[1]

[1]Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: monika.nutautaite@lsmuni.lt

[2] Dr. L. Kriaučeliūnas Small Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Due to rabbits' prolificacy and superior feed conversion efficiency, rabbit breeding is becoming more commercially successful as a source of meat (1). Breeders and meat producers have concentrated their attention on small mammals such as rabbits to match market expectations for healthier and leaner production (2). The differences in production across rabbit species are significant and must be studied scientifically. So, this study was aimed at investigating the nutritional value differences between Belgian giants and crossbreed rabbits' meat. A total of 14 rabbits (46–130 days old) were selected by weight and divided into 2 groups: crossbreeds (7 rabbits/group) and Belgian giants (7 rabbits/group). During the experiment, housing and feeding conditions were identical for both groups and complied with the statutory norms. And at the end of the feeding test, the following analyses were performed after the slaughter and collection of muscle samples post-mortem: the morphological composition of the carcass, physicochemical properties, lipid oxidation levels (fresh and stored samples) and biogenic amines profile. Morphological studies of rabbit carcasses revealed that crossbreed rabbits had slightly greater morphological indices (higher carcass and general muscles weight ($P < 0.05$); higher lumbar spine yield ($P < 0.05$)) than Belgian giants. When compared with the muscles of crossbreed rabbits, the muscles of Belgian giant rabbits exhibited with statistically lower inter-muscle fat content by 1.73% ($P < 0.05$). Other nutritional value markers of rabbit meat, such as colour intensity and physical qualities revealed no statistically significant differences across rabbit breeds ($P > 0.05$). Therefore, lipid oxidation levels in rabbits' thighs (24 hours after the slaughter) were higher in crossbreed ($P < 0.05$), compared with Belgian giants. However, after 3 months of storage, lipid oxidation levels in the lumbar spine of rabbits were 49% higher ($P < 0.05$) in Belgian giant muscles than in crossbreed. After determining biogenic amine profiles in both breeds' lumbar spine samples, the results showed that Belgian giants had significantly higher cadaverine and tyramine levels ($P < 0.05$) compared with crossbreeds. Therefore, higher total biogenic amine content was found in crossbreed samples. However, the observed results were not statistically significant ($P > 0.05$). The overall nutritional value of the two species examined did not differ significantly in many aspects, hence there was no evident trend between crossbreeds and Belgian giants.

Keywords: Belgian giants, crossbreeds, meat quality, nutritional value, comparative analysis.

References

1. *Gehad Seddik, Ahmed A. Aboughaba, Fatma A M, Doaa Salman, Motamed E. Mahmoud. A Comparative Study on Biochemical Parameters For Mature California And Chinchilla Rabbits In Sohag Governorate. Journal of Applied Veterinary Sciences 2019, 4(1), 43–47.*
2. *Króliczewska B, Miśta D, Korzeniowska M, Pecka-Kiełb E, Zachwieja A. Comparative evaluation of the quality and fatty acid profile of meat from brown hares and domestic rabbits offered the same diet. Meat science 2018, 145, 292–299.*

Suitability of Layer-Type and Dual-Purpose Male Chicks for Capon Production

Daria Murawska[1], Michał Gesek[2] Dorota Witkowska[3], Korina Michalska[2]

[1]Department of Commodity Science and Animal Improvement, University of Warmia and Mazury in Olsztyn, E-mail: Poland daria.murawska@uwm.edu.pl
[2]Department of Pathological Anatomy, University of Warmia and Mazury in Olsztyn, Poland
[3]Department of Animal and Environmental Hygiene, University of Warmia and Mazury in Olsztyn, Poland

The elimination of day-old cockerels of layer or dual-purpose breeds poses a problem for the poultry industry (1). Capon production could contribute to the rational management of unwanted male chicks (2). The aim of this study was to determine the effect of age and breeds on the growth and carcass quality characteristics of caponized cockerels.

The experiment was conducted on 420 cockerels (3 groups: Rhode Island Red RIR, Green-legged Partridge (GlP) and Leghorn Lh; 140 of each breed, 7 replications per group and 20 birds per replication). The birds were raised to 28 weeks of age and were fed commercial diets *ad libitum*. At week 8 of age, birds were surgically castrated in accordance with Commission Regulation (EC) No. 543/2008. The procedure was approved by the Local Ethics Committee in Olsztyn, Poland. From week 12 of age, at 4-weeks intervals, 21 birds (1 bird per replication) were selected randomly and slaughtered. The statistical analysis involved the determination of arithmetic means and SEM. The data were analyzed by two-way ANOVA. The significance of differences in mean values between age groups was determined by the Duncan test. Significance was set at $P \leqslant 0.05$.

At 8 weeks of age, the average body weight (BW) of all cockerels was similar (RIR – 599.9 g, Lh – 597.8 g, GLP – 565.8 g). Between week 12 and 28, the BW of RIR, Lh and GIP capons increased respectively from 1218.8 g to 3038.2 g ($P < 0.05$), 1084.4 g to 2323.6g ($P < 0.05$) and 1125.0 g to 2187.9 g ($P < 0.05$). The carcass weight of RIR, Lh and GIP capons increased respectively from 738.7 g to 2041.5 g ($P < 0.05$), 654.2 g to 1438.5 g ($P < 0.05$) and 675.5 g to 1418.5 g ($P < 0.05$). From week 16 of age, the RIR capon's BW was significantly higher compared with the BW of Lh and GIP capons ($P < 0.05$). The statistically confirmed increase in carcass weight was found in GIP and Lh capons up to the age of 24 weeks ($P \leqslant 0.001$). RIR capons had the highest dressing percentage at 28 weeks of age (67.2%). Lh and GlP capons achieved the highest dressing percentage at 24 weeks of age (66.7% and 64.8%, respectively).

Capons of the analyzed breeds, raised under identical conditions, should be slaughtered at different ages. GIP and Lh capons can be slaughter earlier (week 24) than RIR capons (week 28).

Keywords: capon, growth, body weight, carcass weight.

References

1. *Brümmer N, Christoph-Schulz I, and Rovers AK. 2017. Consumers' perspective on dual-purpose chickens. In: Proceedings in System Dynamics and Innovation in Food Networks. pp. 164–169, DOI:10.18461/pfsd.2017.1717.*
2. *Murawska D, Gesek M, Witkowska D. 2019. Suitability of layer-type male chicks for capon production. Poultry Science 98(8): 3345–3351. doi: 10.3382/ps/pez146*

GH Gene (2291A>C) Polymorphism and its Influence on the Economic Traits in Lithuanian Beef Cattle

Nijolė Pečiulaitienė, Ramutė Mišeikienė, Kristina Morkūnienė, Renata Bižienė, Laimutis Kučinskas

Institute of Biology Systems and Genetics, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania, E-mail: nijole.peciulaitiene@lsmuni.lt

Traditional trait improvement has centered on quantitative genetics, using statistical analysis of phenotypic data to determine animals with the highest genetic merit. This selection approach is most effectively implemented for highly heritable traits that are easily recorded before reproductive age. Genomic selection refers to the use of genome-wide genetic markers to predict the breeding value of selection candidates (1, 2). The growth hormone (GH) gene is a candidate gene for predicting growth and meat quality traits in animal genetic improvement since it plays a fundamental role in growth regulation and development (3, 4). The aim of this study was to investigate the prevalence of GH gene (2291A>C) polymorphism and to determine its influence on the growth rate in beef cattle. Cattle hair follicles samples were collected from 85 bulls consisting of Angus (41), Limousin (19), Galloway (19) and Simmental (6) cattle. Hair samples and the data on daily weight gain records were obtained from Šilutė control bulls feeding station. Bovine genomic DNA was extracted from hair follicles using Chelex DNA extraction method. Polymorphism of GH locus was identified using a PCR-RFLP method. Investigation of polymorphism 2291A>C of GH gene showed that allele A (frequency – 0.947) and genotype AA (frequency – 0.918) were the most common in the general population of beef cattle. Meanwhile, the homozygous CC genotype was the rarest, with a frequency of 0.024. When calculating the influence of GH gene polymorphism (2291A> C) on cattle productivity traits, it was observed that this polymorphism had a statistically significant ($P < 0.05$) effect on cattle live weight, which was determined before cattle slaughter. Evaluation of genotype influence data showed that cattle of AC genotype weighed more than animals of AA or CC genotype. However, after calculating the statistical reliability criterion *P* value, only a statistically significant result of two characteristics was determined: cattle overweight, kg (from the end of March to the end of June 2017) and live weight, kg. In conclusion, the results showed that polymorphism of the GH gene influences some of the productivity traits of beef cattle.

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

References

1. *Odzemir M, Topal M, Aksakal V. (2018). The relationships between performance traits and the bGH/Alu I and Pit-1/Hinf I polymorphisms in Holstein cows. Indian Journal of Animal Research 52:186-191. doi: cabdirect.org 20183092482*
2. *Pogorzelska-Przybyłek P, Nogalski Z, Sobczuk-Szul M, Purwin C, Kubiak D. (2018). Carcass characteristics and meat quality of Holstein-Friesian x Hereford cattle of different sex categories and slaughter ages. Arch Anim Breed 61(1): 253–61. doi: org/10.5194/aab-61-253-2018*
3. *Omer RMA, Marsi M, Jawasreh KI, Nour IA, Biraima ADA, Musa LMA, Ahmed MKA. (2018). Molecular detection of selected genetic polymorphisms in growth hormone and insulin like growth factor 1 genes in indigenous Sudanese Baggara cattle. Journal faculty Veterinary Medicine Kafkas University 24:187–194. doi: http://vetdergikafkas.org/uploads/pdf/pdf_KVFD_L_2334.pdf*
4. *Ruban SY, Fedota AM, Lysenko NG, Kolisnyk AI, Goraichuk IV. (2017). The effects of polymorphisms in calpain, calpastatin and growth hormone genes on growth traits in Angus cows. Journal Cytology and Genetics 51:352–360. doi: 10.3103/S0095452717050024*

Gut Response to High or Low Dietary Arginine, Methionine and Lysine Levels in Young Turkeys Applied to Different Challenge Models

Paweł Konieczka[1], Dariusz Mikulski[1], Jerzy Juśkiewicz[2], Katarzyna Ognik[3], Paulius Matusevicius[4], Jan Jankowski[1]

[1]University of Warmia and Mazury in Olsztyn, Poland, E-mail: pawel.konieczka@uwm.edu.pl,
[2] Institute of Animal Reproduction and Food Research PAS in Olsztyn, Poland
[3]University of Life Sciences in Lublin, Poland
[4]Department of Animal Nutrition, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Dietary levels of arginine (Arg), lysine (Lys) and methionine (Met) may be important for the overall health of turkeys. The aim of the study was to determine the influence of feeding diet of low levels (as recommended by NRC)[1] of Arg, Lys and Met (Low ArgLysMet) or diet of high levels (10% higher than recommended by NRC) of Arg, Lys and Met (High ArgLysMet) on performance and the functional status of the gut in turkeys reared either in the optimal conditions or infected with *C. perfringens* or *E. coli* lipopolysaccharide (LPS). A total of 192-day-old female Hybrid Converter turkeys were allocated to 48 pens with 4 birds per pen and 8 replicates per each of the 6 treatment groups. The treatment groups were as follow: (i) birds fed either Low ArgLysMet or High ArgLysMet diets, (ii) birds fed diets as above but orally challenged at day 25, 26 and 27 of age with *C. perfringens* bacteria, or (iii) birds fed as above but orally challenged in 25, 26 and 27 of age with LPS. At day 28 of age, 8 birds from each treatment were sacrificed for sample collection. Feeding birds diet of High ArgLysMet resulted in significantly increased body weight on day 25 of age (before challenge) and on days post-challenge (days 25–28), as well as increased body weight in overall experimental period (0–28). Birds fed diet of High ArgLysMet had significantly lower concentrations of IgA and 8-OHdG enzyme in the blood as well as increased activity of OGG1 enzyme in the jejunal tissue. In the case of the challenge response (*C. perfringens* or LPS), a higher blood or gut tissue concentrations of, among others, IgA, IgM, IgY, Casp 8, APEX 1, 8-OHdG and IL-6 indicate that infection with *C. perfringens* caused greater immunological pressure on the bird gut than LPS. The gut permeability test indicated that a higher concentration of the intestinal permeability marker (FITC-d) in the blood was found in birds infected with *C. perfringens* compared with the control group; however, irrespective to dietary Arg, Lys and Met levels. Our data indicate that feeding diet of increased Arg, Lys and Met levels was not associated either with excessive stimulation of the immune system nor with the increased level of DNA damage in the gut tissue. At the same time, the increased concentration of DNA-repair enzymes in the gut tissue as a result of the administration of High ArgLysMet diet may indicate its beneficial effect on the functional status of the gut-barrier function. This work was supported by the National Science Centre, Grant No. 2017/27/B/NZ9/01007.

Keywords: *C. perfringens*, *E. coli*, essential amino acid, turkeys.

References

1. *National Research Council. Nutrient Requirements of Poultry; The National Academies Press: Washington, DC, USA, 1994.*

Bovine colostrum modulates the intestinal homeostasis and diminishes the adhesion and invasion of enteropathogenic *Escherichia coli*

Ramunė Grigalevičiūtė[1], Povilas Kavaliauskas[1,2,3,4], Rita Plančiūnienė[5], Ieva Prikockytė[6], Vilma Zigmantaitė[1], Audrius Kučinskas[1], and Paulius Matusevičius[7]

[1]Biological research center, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: ramune.grigaleviciute@lsmuni.lt
[2]Institute of Infectious Diseases and Pathogenic Microbiology, prienai, Lithuania.
[3]Joan Sanford I. Weill Cornell Medical Colege, New York, United States
[4]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, United States
[5] Institute of Microbiology and Virology, Lithuanian University of Health Sciences. Kaunas, Lithuania
[6] Kaunas Hospital, Lithuanian University of Health Sciences. Kaunas, Lithuania
[7] Department of Animal Nutrition, Lithuanian University of Health Sciences. Kaunas, Lithuania

Bovine colostrum (BC) plays a critical role in the development of neonatal calves. The exact molecular mechanisms of BC and activated intestinal signaling pathway remains poorly understood. Therefore, we aimed to characterize the effects of BC on the intestinal homeostasis and gut permeability using chimeric calve-mice fecal microbiota transplant mice and neonatal rats *in vivo*. Furthermore, we determined the effects of BC on the *in vitro* adhesion and invasion of enteropathogenic *Escherichia coli* O157.

The pre-incubation of intestinal Caco-2 cells with BC (250 μg/mL) resulted in significantly decreased ($p<0.05$) burden of adhered *E. coli* O157 (2.1 $\log_{10}$CFU) in comparison to untreated control (UC) (2.9 log/CFU) or autologous milk control (MC) (3.0 $\log_{10}$CFU). The Caco-2 treatment with BC ameliorated *E. coli* O157 intracellular invasion (2.1 $\log_{10}$CFU) in comparison to UC (2.9 $\log_{10}$CFU) or MC (3.0 $\log_{10}$CFU). The BC activity on *E. coli* virulence was fraction-depended and peaked at T3 hour *post-partum*. The heat inactivation of BC resulted in significant loss of anti-virulence activity *in vitro*. The BC treatment (10 ml/Kg) of chimeric calve/mice fecal microbiota resulted significantly lower MC permeability in comparison UC. Neonatal rat feeding with BC resulted in greater layer of intestinal mucins, that was most evident when animals were fed with BC fractions collected at 1, 3 and 4 hours post-partum.

Collectively these results demonstrate host-directed anti-virulence properties of BC. BC decreases the intestinal permeability *in vivo* by upregulating mucus production. Further studies are needed to better understand the composition of BC and molecular signaling pathways induced by feeding with BC.

Key words: Bovine colostrum; intestinal permability, immunomodulation.

Association of Toll-like Receptor 4 Gene Polymorphisms with Cow Milk Production Traits

Ramutė Mišeikienė[1,2], Nijolė Pečiulaitienė[2], Renata Bižienė[2], Kristina Morkūnienė[2], Saulius Tušas[1], Laimutis Kučinskas[2]

[1]Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: ramute.miseikiene@lsmuni.lt
[2]Institute of Biology Systems and Genetic Research, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Toll-like receptor 4 (*TLR4*) is located on the cell surface and initiates innate immune responses (1). The *TLR4* gene in cattle about 3739-bp contains an open reading frame of 2526-bp encoded 841 amino acids (2). The bovine *TLR4* gene is highly polymorphic (2) and some SNPs in the TLR4 gene have been associated with milk traits (1, 3).

The research was aimed to investigate polymorphisms c.9421C>T and c.2021C>T and to evaluate their influence on milk fat, protein and lactose percent in Lithuanian dairy cows. Genotypes were determined using RFLP-PCR and detected by performing 2% agarose gel electrophoresis of PCR-RFIP samples and evaluating fragment sizes according to the molecular marker in UV light. The data concerning milk fat, protein, lactose were analyzed. The analysis was performed using program SPSS 22.0. The influence of gene and statistical significance of differences between different genotypes was evaluated by ANOVA (one-factor dispersion analysis). For the influence of the TLR4 gene polymorphisms on productivity traits in interaction with other factors was evaluated using the linear model. The following factors were analyzed in the model: gene, farm, lactation, duration of lactation.

Blood samples were collected from 150 Lithuanian dairy cows. Genotype and allele frequencies for the analyzed population of cows were calculated. Three genotypes of cows in both polymorphisms were analyzed: CC, CT and TT. The C and T alleles of the c.9421C>T (382 bp) and c.2021C>T (367 bp) polymorphisms were identified based on the amplification of specific primers, followed by digestion with the restriction enzymes *Alu*I and *Bsi*HKAI. Genotypic frequencies of the c.9421C>T for the CC and CT genotypes were 0.283 and 0.507, respectively, and the allele frequencies for C and T allele were 0.536 and 0.464; meanwhile, genotypic frequency of the c.2021C>T polymorphism for the TC genotype was 0.954, and for the CC genotype it was 0.03. Allele C frequency was 0.490 and allele T frequency was 0.510. Impact of polymorphisms on milk composition indicators in Lithuanian Holstein cows' milk was determined. Genotype TC of c.9421C>T showed higher fat and protein percentage than the other two genotypes, and the highest lactose percentage was established in TT genotype (4.62%). The results of c.2021C>T showed that cows homozygous for the C allele had the biggest influence on milk fat, protein and lactose percentage. After evaluating influence of the TLR4 gene polymorphisms on productivity traits in interaction with other factors, it has been determined that c.2021C>T polymorphism with lactation influenced milk fat by 3.9% ($P < 0.05$). Polymorphism c.9421C>T together with farm influenced milk fat by 2.5% ($P < 0.05$) and milk protein by 2.2% ($P < 0.05$). The highest effect on cow fat, protein and lactose percent was made by farm and lactation duration ($P < 0.001$).

Keywords: cow, *TLR4* gene, polymorphism, PCR-RFLP.

References

1. *Bilal MS, et al. High grain diet triggers inflammation in the goat uterus: A comprehensive regulation diet modulates the immune response. International Journal of Agriculture & Biology, 2017, 19, 34–40.*
2. *El-Domany WB, et al. Genetic Polymorphisms in LTF/Eco RI and TLR4/Alu I loci as candidates for milk and reproductive performance assessment in Holstein cattle. Reproduction in Domestic Animals, 2019, 54, 678–686.*
3. *Mariotti M, et al. Polymorphisms within the toll-like receptor (TLR)-2, -4 and -6 genes in cattle. Diversity, 2009, 1, 7–18.*

The Prevalence of Microorganisms in Milk Depending on Cow Lactation Number and Lactation Period

Ramutė Mišeikienė[1], Saulius Tušas[1], Jūratė Rudejevienė[2]

[1]*Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: ramute.miseikiene@lsmuni.lt*
[2]*Dr. L.Kriauceliunas Small Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

Mastitis is caused by a wide spectrum of pathogens and, epidemiologically categorized into contagious and environmental mastitis (1). Contagious pathogens include *Staphylococcus aureus, Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* (2), while environmental pathogens include *E. coli*, *Klebsiella* spp., *Strept. dysgalactiae* and *Strept. uberis* and the majority of infections caused by these pathogens are clinical and of short duration. The degree of the inflammatory response depends on the invading pathogen, and host factors such as stage of lactation, age, immune status of the cow, genetics, and nutritional status (3, 4). According to Goli et al. (5), younger cows in their first lactation period are more resistant to contagious causative agents and about 13% of first-lactation cows are already infected with *S. aureus*. Many of them remain infected during the whole lactation, unnoticed, and served as reservoirs for the spread of the infection to other cows in the herd. Research indicates that cows older than 7 years are at risk of udder infection.

The aim of this study was to evaluate the isolation frequency of mastitis causing-microorganisms depending on the cow lactation number and lactation period. The study was conducted in a dairy farm with 428 lactating cows. To identify distribution of main milk microorganisms (CNS, *Enterobacteria*, *Str. agalactiae*, *E. coli, S. aureus*), milk samples from cow teats were collected. Agents were identified according to standard operating procedures SDP 5.4.4.B.6 guide "Fundamental mastitis-causing bacteria evaluation in milk" developed by "Laboratory and field handbook on bovine mastitis" within 24 h after sampling.

First lactation cows compared with cows of older lactation produced milk with a lower amount of microorganisms (except *E. coli).* With an increasing cow lactation number, the amount of *Enterobacteriae* increased as well ($P < 0.05$), while with an increasing lactation period, a greater count of CNS, *Str.agalactiae* and *E.coli* was detected but the biggest number of *Enterobacteriaceae* was established in the first months of lactation.

After assessing the prevalence of microorganisms isolated at different stages of lactation, it was found that with an increasing lactation period a bigger amount of CNS, *Str.agalactiae* and *E.coli* was detected. The highest amount of *Enterobacteriaceae* was detected during the first months of cow lactation. In conclusion, after evaluating microorganisms established in cows with different lactations and lactation periods, no clearly visible trends were found. *E.coli* was more often detected at the end of the cow lactation period. A significant increase of CNS was observed at the end of the first lactation cows than in the cows with the middle lactation period ($P = 0.008$). At the end of the lactation period of the second and third lactation cows, *Enterobacteriaceae* and *Str. agalactiae* were identified more frequently.

Keywords: cow, lactation, microorganisms, milk.

References

1. *Cervinkova D, et al. Prevalence of mastitis pathogens in milk from clinically healthy cows. Vet Med. 2013, 58, 567–75.*
2. *Shoaib M, Agib AI, Naseer MA, Bhutta ZA, et al. Etiology of bovine mastitis. 2021. DOI: 10.5772/intechopen.98543.*
3. *Harmon R. Physiology of mastitis and factors affecting somatic cell counts. Journal of Dairy Science, 1994, 77, 2103–2112.*
4. *Heikkilä AM, Liski E, Pyörälä S, Taponen S. Pathogen-specific production losses in bovine mastitis. J. Dairy Sci. 2018, 101, 9493–9504.*
5. *Goli M, et al. Prevalence assessment of Staphylococcus aureus and Streptococcus agalactiae by multiplex polymerase chain reaction (M-PCR) in bovine sub-clinical mastitis and their effect on somatic cell count (SCC) in Iran dairy cows. Afr. J. Microbiol. Res. 2012, 6, 3005–3010.*

Aquaponics: The Symbiosis of Fish and Vegetable Growing Systems

Ramutis Klimas[1], **Asta Klimienė**[2], **Gintautas Narvilas**[1]

[1]*Marine Research Institute, Klaipėda University, Klaipėda, Lithuania, E-mail: klimas.ramutis@gmail.com*
[2] *Botanical Garden, Klaipėda University, Klaipėda, Lithuania*

During 2020–2023, Klaipėda University is implementing the project No. 14PA–KL-19-1-08701-PR001 "The use of innovative, environmentally friendly and pollution reducing aquaponics", by the measure of program for rural development in Lithuania "Communication of knowledge and outreach activities", activity field "Support for demonstration projects and outreach activities". An experiment is performed in 10 farms of different categories. The aim of this project is to introduce and promote development of this progressive method in Lithuania. The aquaponics system is a technology and a process during which fish and plants are grown together. It is symbiotic operation of two systems: plants live and grow owing to the fish, and fish grow partly owing to the plants. According to the experience of other countries, this technological system is beneficial both economically and environmentally (1–3). The aquaponics system guarantees an output of two valuable and high quality food products (certain fish and vegetable) in one space saving place. This system can be proceeded all year around, and does not require cultivation and fertilization of the soil and is in need of significantly less water. In an aquaponics system, water cleared by the roots of plants comes back to the pool of fish with less nitrogen compound, and just to fill up evaporated water is needed. This way, water resources are saved and a major part of nitrogen compound does not get in the environment. Plants do not need to be additionally fertilized; therefore, there is no use of herbicides. Electric power is used in the process of growing fish and plants only. The system can be used not only in rural but also in urban territories. Hence, aquaponics is a sustainable and pollution reducing innovative technological system, which has never been practiced in Lithuania before.

Keywords: technology, water, fish, plants, environmental.

References

1. *Kloas W, et al. Aquaculture Environment Interactions. 2015, 7, 179–192.*
2. *Goddek S, et al. Aquaponics Food Production Systems. Switzerland, 2019, 619 p.*
3. *Yep B, Zheng Y. Journal of Cleaner Production. 2019, 228, 1586–1599.*

Mobile Tigecycline Resistance (MTR) in Animals Aimed at Human Consumption

Raquel P. F. Guiné

CERNAS Research Centre, Polytechnic Institute of Viseu, 3504-510 Viseu, Portugal

Tigecycline (TIG) is a synthesized third-generation tetracycline antibiotic, which exhibits powerful in vitro activity against a wide spectrum of bacteria. It is one of the last available treatment options used to fight serious infections caused by multidrug-resistant pathogens (1). The increasing prevalence and widespread dissemination of antimicrobial resistance, especially the emergence of multidrug-resistant (MDR) micro-organisms, cause global concern (2). Infections caused by MDR pathogens are high burdens, because of the increased costs, longer hospital stay, and morbidity and mortality rates. The use of tetracycline both in humans and also in animals can, among other factors such as gene mutations, contribute to high-level tigecycline (TIG) resistance. Mobile TIG resistance (MTR) can be detected in diverse bacteria isolated from food animals; therefore, it is important to explore the potential sources as well as its impact in the livestock sector and the one health implications. Although TIG has not been used in animals, animals destined for human consumption can get colonized by TIG-resistant organisms through environmental transfer, consumption of contaminated feed or drinking water. Very recently, plasmid-mediated transmissible tet(X), tet(X3) and tet(X4) genes conferring high-level tigecycline (TIG) resistance were discovered in isolates from food animals, meat and environment in China. Hence, the clinical usefulness of TIG was being threatened by the mobile TIG resistance (MTR). The presence of MTR in the livestock sector is a threat to global food safety and security (3). Through international or domestic food animals/meat trade and travel, TIG-r organisms can be transported from one location to another, making this a worldwide problem. The MTR impacts globally on different chain links, the one health concept, with high economic implications.

Keywords: mobile tigecycline resistance, antibiotic resistance, one health, livestock sector.

References

1. *Marathe et al., Journal of Global Antimicrobial Resistance 2021, 27, 37–40.*
2. *Mohanty & Mahapatra, Annals of Medicine and Surgery 2021, 62, 228–230.*
3. *Fu et al., Journal of Hazardous Materials 2021, 409, 124921.*

Possibility to Use Sex-Sorted and Unsorted Sperm for *in Vitro* Fertilization

Renalda Juodžentytė[1], **Vytuolis Žilaitis**[1], **Giedrius Palubinskas**[2]

[1]*Large Animals Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: renalda.juodzentyte@lsmuni.lt*
[2]*Veterinary Center for Continuing Education and Counseling, Lithuanian University of Health Sciences, Kaunas, Lithuania*

The main goal of successful livestock farming is to quickly breed healthy offspring of good genetic breeds. This can be achieved through the application of innovative, advanced technologies. Embryo transplantation has become one of the most effective methods that can rapidly improve the genetic potential of breeding stock and thus rapidly increase the number of elite stock in the herd (1). As usual, the prevalent sex sorting techniques, even though being used commercially, need to be further refined for mass scale use of sexed sperm. Whereas sperm sorting techniques that are being used commercially have 90% accuracy (2). The problem is that the fertility when using sex-sorted sperm is lower than that when the unsorted sperm is used (3). Many methods of sperm separation (selective fractionation (centrifugation) and swim-up) have been developed to improve sperm quality. Changes in viability/motility and capacitation/acrosome reaction of sex-sorted sperm could be the reason for the reduced initial fertilization rates in in vitro and in vivo studies. The aim of this study was to investigate the changes of motility and acrosomal reaction during capacitation of sex-sorted and unsorted sperm and difference to cleavage of oocytes fertilization.

The ovaries of dairy cows were cut out immediately after slaughter and transported within one hour. The diameter (mm) of various follicles was measured. Quality grading (A, B, C, D) of the oocytes was performed on the basis of cumulus cell development and homogeneity of cytoplasm according to Chaubal et al. (2006) (4). A total of 94 COCs (cumulus cells) were aspirated from 29 ovaries. Only normal COCs were used for maturation. *In vitro* matured COCs were fertilized with frozen-thawed sex-sorted and unsorted sperm. Frozen sperm from a Holsteins bulls was thawed at 37°C for 40 s. The thawed first group sex-sorted sperm (n = 17 samples) and the second group of unsorted sperm (n = 17 samples) were capacitated 60 min by a swim-up method. After incubation Trypan blue stain was used to evaluate viability and the acrosome status of spermatozoa. Stained sperm was counted and divided in three groups: non-viable (the spermatozoa stained all), hyperactivated, and with acrosomal changes (changes of spermatozoa head membrane). After fertilization, the embryonic cleavage was evaluated within 48 h (cleavage rate).

The investigate results show that the sex-sorted sperm shows lower capacitation (10%) and the acrosome reaction (6%) rate than the unsorted sperm. Also, the evaluation of the motility after last centrifugation in the first (sex-sorted) group showed that sperm motility decreased by 15.1%, and in the second (unsorted) group it decreased by 12.5%. After fertilization, a lower cleavage rate was determined in oocytes from the sex-sorted sperm (A class oocytes – 37.63% and B class oocytes – 25.84%) and the unsorted sperm (A class oocytes – 47.36% and B class oocytes – 42.1%). In conclusion, this study shows that the unsorted spermatozoa were most suitable for in vitro fertilization of A and B grade oocytes. The comparison of oocytes group results shows that inferior quality oocytes require more motility spermatozoa to fertilize.

Keywords: capacitation, fertilization, in vitro, oocytes.

References

1. *Kennady V, et al., Journal of Entomology and Zoology Studies. 2018, 6(5), 2215–2218.*
2. *Holden SA, Butler ST. Animal. 2018, 12, 97–103.*
3. *Palma GA, et al., Anat Histol Embryol. 2008, 37(1), 67–73.*
4. *Chaubal SA, Molina JA. Theriogenology. 2006, 65, 1631–1648.*

Association Between *Mblk-1* Gene Polymorphisms and Resistance to *Varroa Destructor* Mites in Honey Bee

Renata Bižienė, Rūta Insodaitė, Kristina Morkūnienė, Laimutis Kučinskas

Institute of Biology Systems and Genetics, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania. E-mail: renata.biziene@lsmuni.lt

Varroasis is an invasive disease caused by *Varroa destructor* ticks that infect honey bees (*Apis mellifera*) at any stage of their development. The host–parasite interaction between honey bees and the mite *Varroa destructor* is unusual, as honey bee colonies are relatively poorly defended against this parasite. The interaction has existed since the mid-20th century, when *Varroa* switched host to parasitize *A. mellifera*. Infection with these ticks has reduced the viability and productivity of bees, disrupts normal development, reduces weight and resistance to viral diseases. Without acaricides, honey bee colonies typically die within 3 years of *Varroa* infestation (1). However, it has been observed that some bee colonies are able to defend themselves against these parasites without additional chemical or natural substances. The resistance of honey bees is associated with genetic factors that determine their behavior to protect against parasites. *Mblk-1* was identified from a transcriptomic non-hypothesis-driven approach focusing on the honeybee higher brain centers as a candidate that can be potentially important for the highly social behaviors of the honeybee (2). In this study, we aimed to test the contribution of three *Mblk-1* gene polymorphisms to the resistance to *V. destructor* mites. This case–control study involved 117 DNA samples which were genotyped for three single nucleotide polymorphisms using Real-Time Polymerase Chain Reaction method. The distribution of three polymorphisms located at 7454459 (Asn → Thr), 7454648 (Gln → Arg) and 7454648 (Leu → Pro) positions of the Mblk-1 gene were analyzed among three groups of honey bees: the domestic honey bees treated against *Varroa destructor* ticks, untreated domestic honey bees and wild bee families. Statistical analysis showed that distribution of mutant allele A of the polymorphism at 7454459 (Asn → Thr) position was significantly more common among untreated and wild bees compared with domestic, which are contagious infected with *Varroa destructor* (15.1% and 13.3% vs. 0.8%, $P = 0.004$). The distribution of genotypes was also statistically significant: the mutant AA genotype was more common among uninfected bees, and this genotype was not detected in families treated for the disease. The distribution of alleles and genotypes of the other *Mblk-1* gene polymorphisms at positions 7454648 and 7454648 that also altered the amino acid sequence of the protein was not statistically significant. However, there is a tendency for the mutant, resistance-causing T and A (respectively) alleles of these polymorphisms to be more common among domestic untreated against *Varroa destructor*, and wild bee families.

Keywords: Honeybee, *Apis mellifera, Mblk-1* gene, *Varroa Destructor.*

References

1. *Conlon BH, Aurori A, Giurgiu AI, Kefuss J, Dezmirean DS, Moritz RFA. A gene for resistance to the Varroa mite (Acari) in honey bee (Apis mellifera) pupae. Mol Ecol. 2019; 28(12):2958–2966.*
2. *Takayanagi-Kiya S, Kiya T, Kunieda T, Kubo T. Mblk-1 transcription factor family: its roles in various animals and regulation by nol4 splice variants in mammals. Int J Mol Sci. 2017; 18(2):246.*

Zoohygienic Conditions in a Chinchilla Farm and their Influence on Selected Production Indicators

Stanisław Łapiński, Barbara Tombarkiewicz

University of Agriculture in Krakow, Faculty of Animal Sciences, Department of Zoology and Animal Welfare, Poland, E-mail: stanislaw.lapinski@urk.edu.pl

Despite the fact that chinchillas have been farmed for a century, there are not many studies concerning their behavior, housing requirements or degree of domestication, all of which are important factors in the assessment of the welfare. Different guidelines for keeping and caring chinchillas are focused mainly on the dimensions of the cages, leaving aside the microclimate issues, whilst individual factors such as temperature, humidity, air velocity, cooling, concentration of harmful gases and room lighting determine the welfare of animals. Chinchillas are animals that are particularly sensitive to environmental factors. Unfortunately, to this day, they remain one of the few species of farm animals for which the standards of microclimatic parameters have not been established.

The aim of the study was to assess the influence of microclimatic conditions in a chinchilla farm on production indicators such as fertility and quality of the coat.

The research was carried out in the chinchilla breeding farm in Myślenice (Poland) in two periods: winter (stage I) and spring (stage II). Measurements were carried out in two breeding rooms, in each on 3 racks and 3 levels (level I – the highest cages, level II – medium cages, level III – the lowest cages), altogether in 54 measurement points (cages). The research included measurements of basic microclimatic parameters such as lighting, humidity, water vapor pressure, air movement, temperature, cooling, concentration of carbon dioxide, hydrogen sulphide and ammonia as well as ozone concentration measurements. The obtained results were compared with the assessment of the quality of the fur coat and the reproductive results of chinchillas.

The obtained results indicate that the average fertility (youngsters per year) of chinchillas at individual cage levels was distributed as follows: 1.83 at the 1st level of cages, 1.66 at the 2nd level and 1.82 at the 3rd level.

Based on the conducted microclimatic tests, it can be concluded that the assessed farm had a high hygienic standard. The best production effects (high fertility, the highest quality of fur coat) were found in chinchillas from cages located at the highest levels. The fertility of chinchillas depends on the illumination of the room (the lowest fertility was found in the least lit cages).

Summing up, it can be stated that it is necessary to elaborate zoohygienic standards for chinchilla farms in order to objectify the assessment of animal welfare and obtain high production effects.

Keywords: chinchilla, farm microclimate, fur quality, fertility, welfare.

Effect of a High-Fat Diet and Chromium on Hormones Level and Cr Retention in Rats

Anna Stępniowska[1], Ewelina Cholewińska[1], Magdalena Krauze[1], Paulius Matusevicius[2], Bartosz Fotschki[3], Jerzy Juśkiewicz[3], Katarzyna Ognik[1]

[1]University of Life Sciences in Lublin, Lublin, Poland
[2]Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Institute of Animal Reproduction and Food Research PAS in Olsztyn, Poland

High-fat diet (HFD), especially taken for a long time, can cause obesity as well as many civilization diseases such as diabetes and cardiovascular diseases. Scientific publications have shown that HFD is the reason for hypertriglyceridemia, hyperinsulinemia, and glucose intolerance. The adverse effect of HFD on the organism can be neutralized by adding chromium to the diet. We hypothesized that the addition of chromium to HFD would reduce the negative effect of that diet on the secretion of hormones regulating carbohydrate metabolism and physiologically important neurotransmitters. It was additionally postulated that chromium in the nanoparticle form will be more easily digestible and better retained in the body of rats than chromium in the organic form, and thus the regulatory effect of this form of Cr on hormonal metabolism will be more efficient. The aim of the study was to determine how the administration of HFD supplemented with various forms of chromium to rats affects the accumulation of this element in the tissues and levels of selected hormones (leptin, ghrelin, insulin, glucagon, serotonin, noradrenaline, and histamine). The experiment was conducted on 56 male Wistar rats, which were divided into 8 experimental groups. The rats received for 8 weeks a standard diet or high-fat diet with the addition of 0.3 mg/kg body weight of chromium(III) picolinate (Cr-Pic), chromium(III)-methioninate (Cr-Met), or chromium nanoparticles (Cr-NP). It was noted that chromium in organic forms was better retained in the organism of rats than Cr-NP. However, Cr-Pic was the only form that increased the insulin level, which indicates its beneficial effect on carbohydrate metabolism. In the blood plasma of rats fed HFD, an increased level of serotonin and a reduced level of noradrenaline were noted. The addition of Cr to the diet, irrespective of its form, also increased the serotonin level, which should be considered a beneficial effect. A high-fat diet was shown to negatively affect the level of hormones regulating carbohydrate metabolism (increasing leptin levels and decreasing levels of ghrelin and insulin). This work was supported by the National Science Centre, Grant No. 2020/39/B/NZ9/00674.

Modern Techniques Related to the Control of Veterinary Drug Residues in Meat Samples

Vadims Bartkevics[1], Laura Elina Ikkere[1], Iveta Pugajeva[1], Elena Bartkiene[2]

[1]Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latvia. E-mail: Vadims.Bartkevics@bior.lv
[2]Department of Food Safety and Quality, Lithuanian University of Health Sciences, Kaunas, Lithuania

Modern analytical techniques represent a high potential for the analysis of a wide range of food contaminants, especially such sensitive and selective instrumental methods as high-resolution mass spectrometry (HRMS). This approach not only significantly lowers the limit of detection, but also opens opportunities for retrospective analysis of the data obtained. Due to the high selectivity, it is also possible to detect compounds in the presence of interfering matrix components. This allows reduction of the total analysis time due to both faster sample preparation and shorter chromatographic program, as it is possible to identify and quantify compounds that are not completely chromatographically separated. However, the main advantage is the possibility to significantly increase the range of simultaneously detectable veterinary drugs.

This report describes the application of two analytical methods based on HRMS for the analysis of veterinary drug residues in animal origin products. The first study proposes an analytical method for simultaneous identification, screening and quantification of 164 residues and metabolites of pharmacologically active substances belonging to such therapeutic classes as anti-infectious (antibiotics and chemotheurapeutics), anti-inflammatory and antiparasitic agents (against protozoa, endo- and ectoparasites), corticoids and agents acting on the nervous and reproductive systems, substances with hormonal and thyreostatic action, and beta agonists. Different sample preparation procedures were compared and optimised for the detection of selected veterinary drugs in chicken, porcine and bovine meat by UHPLC coupled to Orbitrap HRMS. A total of 130 selected compounds in chicken meat, 127 compounds in bovine meat and 123 compounds in porcine meat samples could be quantified with an accuracy ranging from 70% to 120%. The method was successfully used to detect and quantify veterinary drug residues in real samples.

The main purpose of the second study was to develop a fast and reliable analytical method based on ion cyclotron resonance HRMS for the detection of quinolones in poultry meat. The sample preparation procedure was simplified by reducing the procedure to extraction and freezing out steps and the chromatographic separation step was excluded completely. As a result, the total analysis time was reduced to less than an hour. The validation study revealed that the method is capable of detection and confirmation of ten quinolone compounds in poultry indicating the compliance with MRL values. Analysis of treated chickens revealed that the developed method is suitable for the determination of ciprofloxacin and enrofloxacin. The proposed procedure could be one of the fastest quantitative confirmatory methods for the analysis of quinolones available so far.

Keywords: veterinary drug residues, mass spectrometry

References

1. *Ikkere LE, et al., Journal of Pharmaceutical and Biomedical Analysis 2020, 188:113389.*
2. *Pugajeva I, et al., Journal of Pharmaceutical and Biomedical Analysis 2019, 166, 252–263.*

Comparative Analysis of the Nutritional Composition of Eggs from Two Different Poultry Species from Lithuania

Vilma Vilienė, Asta Racevičiūtė-Stupelienė, Monika Nutautaitė, Jolita Klementavičiūtė, Nedas Bajarauskas

Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: vilma.viliene@lsmuni.lt

Eggs are one of nature's most nutritious foods, maintaining a low saturated fat content and a high protein content (1). Eggs are a rich source of energy, minerals, and vitamins, all of which are essential for a healthy diet. However, egg quality can be determined by poultry species and comparative data on this case is essential and can aid technological development (2). So, this study was designed to compare Guinea fowl and laying hen egg nutritional value. All the eggs were purchased at local Lithuanian markets, considering the egg laying time. The following indicators were determined when eggs were fresh and after 28 days of storage: egg, yolk, and albumin heights, Haugh unit, yolk colour intensity, eggshell thickness, the pH of the yolk and albumin, dry matter content of yolk, fat, ashes, and protein contents; a sensory evaluation and an analysis of consumers' acceptability by facial expressions were performed. Guinea fowl eggs were observed to have the greater redness of the yolk colour of fresh eggs by 9.69 points; the weight of the shell with film and its strength by 38%; the weight without film by 46%; the shell thickness at the pointed end by 48%, the middle by 58%, and the blunt end by 77%; the shell-to-egg weight ratio by 60% compared with laying hens ($P < 0.05$). Stored Guinea fowl eggs had higher yolk redness (a*) by 8.11 points, shell thickness at the pointed end by 40%, and the ratio of shell-to-egg weight by 45%, compared with lying hens ($P < 0.05$). No significant differences were found in the chemical composition of Guinea fowl and laying hen eggs. However, when evaluating egg yolk colour characteristics, the results showed that Guinea fowl yolks brightness (L*) was lower by 8%, the yellowness (b*) by 21%, but redness (a*) was more intense compared with the laying hens' yolks ($P < 0.05$). Sensory evaluation showed that consumers rated Guinea fowl eggs as having 18% greater protein colour uniformity, 59% more intense yolk colour, 38% greater total yolk flavour intensity, 21% more acceptable yolk, and 27% less yolk crumbness than laying hens ($P < 0.05$). The emotional expressions of the consumers' faces while tasting Guinea fowl eggs and comparing them with laying hens showed that the following emotions were expressed more: happiness, contempt, and less fear ($P < 0.05$). To sum up the obtained results, as an alternative to laying hens' eggs, Guinea fowl eggs can be consumed, considering better albumin and shell properties, storage retention, consumer acceptance and emotions expressed by tasting them.

Keywords: Guinea fowl, laying hens, egg quality, sensory analysis, comparative analysis.

References

1. *Kozelová D, Országhová D, Matejková E, Fikselová M, Horská E, Ďurdíková D, et al. Eggs and their consumption affected by the different Factors of purchase. Potravinarstvo 2018, 12(1), 570–577.*
2. *Sun C, Liu J, Yang N, Xu G. Egg quality and egg albumen property of domestic chicken, duck, goose, turkey, quail, and pigeon. Poultry science 2019, 98(10), 4516–4521.*

Complex Evaluation of Arabian Horses Bred on Lithuanian and German Stud Farms

Vilma Vilienė, Asta Racevičiūtė-Stupelienė, Monika Nutautaitė, Agnė Žukaitė

Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, E-mail: vilma.viliene@lsmuni.lt

In terms of animal welfare, the sport horse industries generally control themselves. Therefore, it can vary greatly between different countries. However, there are insufficient standards and rules in place for horse training (1). As a result, differences between countries are frequently evident. So, this study was designed to perform an Arabian horse breed complex evaluation by comparing feeding and keeping conditions in Lithuania X and Germany Y studs. Research was carried out with a total of 14 horses, each stud farm consisting of 7 Arabian breed horses, which were selected by gender, age, and similar weight. The horses were assessed on their conformation, temperament, movements, feeding, and keeping circumstances, as well as their body measurement indices. The conformation of the horses showed that statistically significantly higher measurements were obtained for Arabian horses bred in Germany: correspondingly longer shoulder length by 14%, anterior ankle length by 14%, anterior leg circumference height by 28%, pelvic length by 25%, lower leg length by 4%, hind ankle length by 6%, hind leg height by 29% compared to the measurements of horses bred in Lithuania ($P < 0.05$). Evaluation of horse body size indices (large body format, pelvis, pelvis-knee patella, shoulder-foreleg length from wrist joint to hoof) revealed that lower indices were obtained in Lithuania X stud farms compared with Arabian horses bred in Germany ($P < 0.05$). These statistically higher indices were obtained in Germany's Y stud farm horses: shoulder, hind ankle length, forearm–foreleg length from wrist joint to hoof, thighs ($P < 0.05$). After evaluation of temperament, it became clear that the horses of the Arabian breed bred in both Lithuania and Germany had positive characteristics in their manners: 7 horses bred in Lithuania and 6 bred in Germany were peaceful; after assessing the negative characteristics, there were no angry horses bred in Lithuania, and in Germany there were no cowards who did not trust a person. Therefore, in terms of mobility, the walk and trot of horses bred in Germany were of higher quality compared with horses bred in Lithuania. Regarding the feeding of horses in both countries, the norms were in line with the feeding recommendations for sport horses. After analysing the conditions of keeping Arabian horses in Lithuania and Germany, it became clear that horses are kept in different types of stables, but in individual pens, depending on the requirements. In summary, although the feeding and keeping conditions corresponded to the norms in both Lithuanian and German stables, considering the performed measurements and the calculated indices, the Arabian breed horses bred in German Y studs achieved higher indicators.

Keywords: Arabian breed, horse, Lithuania, Germany, conformation, body measurement indices.

References

1. *Witkowska-Piłaszewicz O, Grzędzicka J, Seń J, Czopowicz M, Żmigrodzka M, Winnicka A, et al. Stress response after race and endurance training sessions and competitions in Arabian horses. Preventive veterinary medicine 2021; 188, 105265.*

Influence of Puerperal Metritis on the Recovery of the Estrous Cycle After Calving in Modern Dairy Cows

Vilmantas Juodžentis[1],[2], Giedrius Palubinskas[2], Jūratė Šiugždaitė[3], Vytuolis Žilaitis[1], Henrikas Žilinskas[1]

[1]Large Animals Clinic, Faculty of Veterinary, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania. E-mail: vilmantas.juodzentis@lsmu.lt
[2]Department of Animal Breeding, Faculty of Animal Sciences, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Department of Veterinary Pathobiology, Faculty of Veterinary, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

The aim was to determine how puerperal metritis influences the recovery of estrous cycle in modern dairy cows. The study was carried out on lactating Holstein cows from a commercial dairy farm located in Lithuania. The cows were housed in free stall barns with access to fresh water *ad libitum* and were fed a total mixed ration supplemented with the concentrate based on milk yield. The cows were selected between day 5 to 14 after calving (day 0 = day of calving). The cows were divided into 2 different groups: multiparous cows after puerperal metritis treatment (M, n = 34) and multiparous cows without signs of puerperal metritis (H, n = 38). All 72 cows were divided into groups after their first ovulation: HSO (n = 29) – cows without signs of puerperal metritis and with a single ovulation; MSO (n = 21) – cows after puerperal metritis treatment with a single ovulation; HDO (n = 9) – cows without signs of puerperal metritis and with a double ovulation; and MDO (n = 13) – cows after puerperal metritis treatment with a double ovulation. The changes in ovaries were examined using a digital diagnostic ultrasound scanner (Dramiński iScan, Dramiński S.A., Olsztyn, Poland) at a frequency of 7.5 MHz, using a linear rectal transducer. The first dominance of the follicle postpartum was recorded when at least one of the follicles reached 8.5 mm in diameter. To detect follicle ovulation, the cows were monitored by ultrasound machine three times a week (Monday, Wednesday, Friday). Ultrasonography was started on day 5 postpartum and was continued until the follicle ovulation was diagnosed. The statistical analysis was performed using computer software SPSS 22. Data were statistically significant when $P < 0.05$.

The mean time to the first follicle deviation postpartum was longer in the MSO group compared with the HSO group, 8.9 ± 1.6 and 6.8 ± 1.8 days postpartum, respectively ($P < 0.002$). The same tendency was observed in the MDO and HDO groups, 9.5 ± 1.3 and 7.0 ± 1.4 days postpartum, respectively ($P < 0.002$). We found that in the HDO group the first dominant wave follicle's ovulation was more frequent when in the MDO group, 55.6% and 23.1%, respectively ($P < 0.027$). Moreover, the HDO group cows ovulated their follicle during the first follicular wave faster compared with the MDO group (11.4 ± 2.7 day and 20 ± 1 day, respectively, $P < 0.01$). Also, HDO group cows had a smaller diameter of the ovulatory follicles compared with the MDO group cows (15.3 ± 1.9 mm and 17.3 ± 1.7 mm, respectively, $P < 0.04$).

The conclusion can be drawn that dairy cows which have had puerperal metritis need more time until the first follicle deviation postpartum. Also, healthy cows have a higher frequency for double ovulation in the first dominant wave postpartum.

Keywords: metritis, postpartum, ovulation, follicle.