Microbiological quality of fresh chicken meat products from different farms

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ABSTRACT

The purpose of this paper was to verify the shelf life of fresh chicken meat and offal (grilled chicken, drumsticks, wings, and liver, hearts and gizzards) by determining the microbiological status and the presence of Salmonella bacteria, and the total number of aerobic mesophilic bacteria, Listeria monocytogenes, Escherichia coli and Enterobacteriaceae during the nine days of storage, as well as to determine the correlation between microbial growth of certain bacteria with respect to the test interval and established pH values. The obtained results served to determine farm's rating and classification in relation to the established microbiological status. All products tested in relation to their microbiological quality maintained their hygienic safety within their 7-day shelf life. Only grilled chicken sample remained within the prescribed microbiological criteria limits on the ninth day after production. We established positive correlation with variable degree of correlation between the total number of bacteria isolated from chicken meat samples originating from all farms and the test interval, as well as the positive and negative correlation with variable degree of correlation, and the complete absence of correlation between the total number of bacteria isolated from samples and resulting pH values. Because the highest counts of certain bacteria on the seventh and ninth day for individual farms that were the subject of the study differed even though we examined the same type of product, we carried out a classification of individual farms. The conditions for keeping, transporting and slaughtering broilers, as well as the adequate storage of related products, have a significant impact on the microbiological status, hygienic safety and shelf life of fresh chicken meat.

Key words: *chicken meat, shelf life, microbiological status, farm*

INTRODUCTION

Keeping chicken meat fresh for as long as possible is imperative for the majority of producers and processors of this type of meat. However, alongside the economic aspect, this type of production has to comply with a whole range of mandatory provisions laid down by national and EU regulations on hygiene standards in order to protect the health of consumers and fulfil prerequisites of free trade with other countries. The determination of shelf life is an important component of food safety. It is defined as a period in which chicken meat remains safe for consumption and maintains specified quality under expected conditions for storage and usage. Shelf life determines the durability of product and is reported in accordance with EU Regulation 1308/2013 (Anon, 2013).

One of the conditions to be fulfilled refers to microbiological criteria for poultry regarding microorganisms, tested toxins and metabolites, sampling plan (the number of units in the sample (n) and the number of food units (c) used for calculating limit values between ("m and M"), including the lower ("m") and upper ("M") limit value), the reference test method and the stage at which the criterion applies,

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all in accordance with Regulation (EC) No 2073/2005 (Anon., 2005).

In accordance with Regulation (EC) No 852/2004 on the hygiene of foodstuffs, the main responsibility of food safety lies with food business operators who must ensure food safety throughout the food chain, starting from the primary production, processing and distribution to export, whereas the obligation to determine the product shelf life when placing it on the market is imposed subsequently (Anon., 2004). The shelf life of chicken meat depends on the initial number of microorganisms. This emphasises the importance of hygienic conditions and controls during various stages of production process (Yoshida et al., 2001) and is determined based on scientific knowledge, as well as experience from one's own practice. A shelf life within which consumers' health is not compromised is determined by combining these two methods. This, in turn, implies compliance with all legal standards and by-laws based on scientific evidence.

In addition to the importance of meeting mandatory measures during the slaughter and the processing of chicken meat, a significant factor is also the origin of the product, that is, a farm from which animals originate. The development of sustainability study, including a specific number of collected samples that were properly stored and adequately tested in laboratory, is one way to comprehend the overall characteristics of the product once it has been placed on the market and properly determine its shelf life.

The aim of this study was to verify a predetermined shelf life of fresh chicken meat (7 days) by monitoring the microbiological growth of certain bacteria in certain products with regard to the test interval and measured pH values, as well as to identify mutual correlations. This paper also addresses the microbiological status of products just before their expiry dates and their relationship with the farm of origin, as well as the rating and classification of included farms, based on obtained results.

MATERIALS AND METHODS

Materials

All chicken meat samples for microbiological analysis were collected on the same day from five cooperating farm. They included the following categories of products: "grilled meat", "drumsticks", "wings", and "liver, hearts and gizzards". Each sample consisted of five subunits and each subunit was collected from a different farm. Each product category was represented by five separate samples that were subsequently tested in laboratory on the day of sampling +1, +3, +6, +7 or +9 days. The sampling and the dynamics of laboratory tests are presented in Table 1.

All samples of chicken meat for pH measurements were collected and tested with the same dynamics as the samples used in the microbiological examination. The sampling and the dynamics of laboratory tests are presented in Table 2.

Every individual sample was collected in the quantity sufficient to comply with laboratory procedu-

Table 1 Sampling method and laboratory test dynamics (microbiological analysis)

	Farm					Number of samples		Day of laboratory testing after sampling				
Type of product	1.	2.	3.	4.	5.	1	5	+ 1	+ 3	+ 6	+ 7	+ 9
Grilled chicken	1 SU*	1 SU	1 SU	1 SU	1 SU	1 x 5 SU	5 x (1 x 5 SU)	MBA**	MBA	MBA	MBA	MBA
Drumsticks	1 SU	1 SU	1 SU	1 SU	1 SU	1 x 5 SU	5 x (1 x 5 SU)	MBA	MBA	MBA	MBA	MBA
Wings	1 SU	1 SU	1 SU	1 SU	1 SU	1 x 5 SU	5 x (1 x 5 SU)	MBA	MBA	MBA	MBA	MBA
Liver and hearts	1 SU	1 SU	1 SU	1 SU	1 SU	1 x 5 SU	5 x (1 x 5 SU)	MBA	MBA	MBA	MBA	MBA
Gizzards	1 SU	1 SU	1 SU	1 SU	1 SU	1 x 5 SU	5 x (1 x 5 SU)	MBA	MBA	MBA	MBA	MBA

**SU – subunit*
***MBA – microbiological analysis*

Table 2 Sampling method and laboratory test dynamics (determination of pH value)

	Farm					Number of samples		Day of laboratory testing after sampling				
Type of product	1.	2.	3.	4.	5.	1	5	+ 1	+ 3	+ 6	+ 7	+ 9
Grilled chicken (pcs)	1	1	1	1	1	1 x 5	5 x (1 x 5)	pH	pH	pH	pH	pH
Drumsticks (pcs)	1	1	1	1	1	1 x 5	5 x (1 x 5)	pH	pH	pH	pH	pH
Wings (pcs)	1	1	1	1	1	1 x 5	5 x (1 x 5)	pH	pH	pH	pH	pH
Liver with heart (pcs)	1	1	1	1	1	1 x 5	5 x (1 x 5)	pH	pH	pH	pH	pH
Gizzards (pcs)	1	1	1	1	1	1 x 5	5 x (1 x 5)	pH	pH	pH	pH	pH

res involved in analysis. After the collection, samples were individually packed in PVC bags, coded and delivered to the laboratory in portable refrigerators, where they were stored at + 4 °C, until the analysis was performed. We paid particular attention to coding the samples in order to clearly establish their traceability.

Methods

Microbiological tests were carried out on microorganisms referred to in Regulation (EC) No 2073/2005 (Anon, 2005), using the referenced methods. This included: *Salmonella* spp. (EN ISO 6579), *Listeria monocytogenes* (EN ISO 11290), the total number of aerobic mesophilic bacteria (EN ISO 4833), *Escherichia coli* (EN ISO 16649) and *Enterobacteriaceae* (EN ISO 21528). The analyses were carried out in accredited laboratory.

Statistical analysis of results

We used the Pearson correlation coefficient to determine the dynamics between the presence of variable factors (product type, origin farm (lot), microbiological test type) and changes in pH values on the day of testing, as well as to establish possible correlations.

The farms were rated and classified based on obtained results. The lowest number of microorganisms found in examined product from each farm was given the grade 5. As the observed values increased, due grade was lowered, until the lowest grade 1. The rating relating to the number of aerobic mesophilic bacteria (all products), enterobacteria (grilled chicken, drumsticks and wings), and E. coli (liver, heart and gizzards) was performed on the seventh and ninth day of testing, for all five farms. Each product from each farm was assigned a rating on the seventh and ninth day. The rating was subsequently averaged. Based on average values for both days, we also calculated the mean. Furthermore, we calculated the standard deviation. The lowest standard deviation was given 1.00 point. Due points were later added to the mean for each farm. Since standard deviations increased inversely proportional, the points assigned to them decreased by 0.20. The farm with the highest standard deviation thus received 0.20 points. During the evaluation and classification, farms were given a letter grade (A, B, C, D and E), where the best grade (average value + standard deviation points) was assigned the letter A.

RESULTS AND DISCUSSION

a) The quality and safety of products with regard to microbiological criteria

The microbiological criteria in accordance with the Guideline on Microbial Food Criteria (Anon, 2013) used for grilled chicken, drumsticks, wings, and liver,

Table 3 Microbiological criteria for packaged fresh meat and poultry carcass process hygiene – grilled chicken, drumsticks and wings (a minimum of 0.5 cm below the surface)

Microorganism	Sampling plan		Limits	Stage of applying the criterion
	n	c		
Salmonella spp.	5	0	None in 25 g	End of manufacturing process
Listeria monocytogenes	5	0	None in 25 g	End of manufacturing process
Enterobacteriaceae	5	2	m=10^2 cfu/g M= 10^3 cfu/g	End of manufacturing process
Aerobic mesophilic bacteria	5	2	m=10^4 cfu/g M= 10^5 cfu/g	End of manufacturing process

n = number of units comprising the sample; c = number of sample units producing values between m and M, a sample is considered acceptable when maximum c/n values range between m and M and the rest of observed values amount to ≤ m; m= the limit below which all results are considered satisfactory; M= the limit value above which the results are not considered satisfactory, if only one result exceeds this value, the sample is considered unsatisfactory.

Table 4 Microbiological criteria for offal process hygiene – liver, hearts and gizzards

Microorganism	Sampling plan		Limits	Stage of applying the criterion
	n	c		
Salmonella spp.	5	0	None in 25 g	End of the manufacturing process
Listeria monocytogenes	5	0	M= 10^2 cfu/g	End of the manufacturing process
Escherichia coli	5	2	m=10^2cfu/g M=10^3 cfu/g	End of the manufacturing process
Aerobic mesophilic bacteria	5	2	m=10^5 cfu/g M= 10^6 cfu/g	End of the manufacturing process

n = number of units comprising the sample; c = number of sample units producing values between m and M, a sample is considered acceptable when maximum c/n values range between m and M and the rest of observed values amount to ≤ m; m= the limit below which all results are considered satisfactory; M= the limit value above which the results are not considered satisfactory, if only one result exceeds this value, the sample is considered unsatisfactory.

heart and gizzards are shown in Tables 3 and 4. During all nine days of experiment, *L. monocytogenes* and *Salmonella* spp. were not isolated in any 25 g sample.

The results of microbiological analysis for the presence of aerobic mesophilic bacteria, enterobacteria, E. coli and obtained pH values are presented in Tables 5, 6, 7, 8 and 9.

The results obtained for all tested bacteria were examined in accordance with Regulation (EC) No 2073/2005 (Anon, 2005) and are shown in Table 10.

Since bacteria *L. monocytogenes* and *Salmonella* spp. were not isolated in any 25 g sample, the results of testing are considered satisfactory for the entire experiment.

The results of all five test intervals obtained by the microbiological testing of grilled chicken produced satisfactory and/or acceptable microbiological parameters on the first, third, sixth, seventh and ninth day after production. Even though there was a higher increase of enterobacteria (two subunits produced a value between m and M) (2.7x10^2 and 5.2x10^2 CFU/g) and aerobic mesophilic bacteria (two subunits produced values between m and M) (5.1x10^4 and 9.9x10^4 CFU/g) on the ninth day, these values still fell within the prescribed criteria (Table 3) and were, thus, considered acceptable.

The results for first four test intervals obtained by the microbiological testing of drumsticks produced satisfactory and/or acceptable microbiological parameters on the first, third, sixth and seventh day after production. Even though we observed a higher increase of enterobacteria (one subunit produced a

Table 5 Microbiological analysis results and pH values for the product "grilled chicken"

Farm	Parametar	Day of testing				
		1	3	6	7	9
1	AMB, CFU/g*	8,5x10^2	9,5x10^2	2,5x10^3	7.5x10^3	9,5x10^3
	Enterobacteriacae, CFU/g	<10	<10	<10	52	77
	pH	5,99	5,86	6,6	6,2	6,37
2	AMB, CFU/g*	9,6x10^2	9,9x10^2	2,9x10^3	6,9x10^3	9,1x10^3
	Enterobacteriacae, CFU/g	<10	<10	<10	61	79
	pH	6,13	5,99	5,83	6,2	6,2
3	AMB, CFU/g*	7,8x10^2	8,7x10^2	1,7x10^3	5,7x10^3	8,2x10^3
	Enterobacteriacae, CFU/g	<10	<10	<10	58	88
	pH	5,93	6,26	6,22	6	6,01
4	AMB, CFU/g*	8,6x10^2	9,7x10^2	2,3x10^3	7,3x10^3	5,1x10^4
	Enterobacteriacae, CFU/g	<10	<10	<10	63	2,7 x 10^2
	pH	5,91	6,15	6,39	5,95	5,99
5	AMB, CFU/g*	9,8x10^2	1,3x10^3	4,9x10^3	9,9x10^3	9,9x10^4
	Enterobacteriacae, CFU/g	<10	<10	<10	72	5,2 x102
	pH	6,46	6,09	6,26	6,21	6,26

* *Aerobic mesophilic bacteria*

Table 6 Microbiological analysis results and pH values for the product "drumsticks"

Farm	Parametar	Day of testing				
		1	3	6	7	9
1	AMB, CFU/g*	6,7x10^2	9,3x10^2	8,9x10^3	9,1x10^3	5,1x10^4
	Enterobacteriacae, CFU/g	<10	50	78	98	5,2x10^2
	pH	6,22	6,5	6,25	7,03	6,68
2	AMB, CFU/g*	6,6x10^2	9,2x10^2	8,1x10^3	8,3x10^3	4,9x10^4
	Enterobacteriacae, CFU/g	<10	55	89	91	2,1x10^2
	pH	6,44	6,59	6,48	6,61	6,6
3	AMB, CFU/g*	6,5x10^2	9,1x10^2	5,7x10^3	5,9x10^3	9,6x10^3
	Enterobacteriacae, CFU/g	<10	65	75	87	98
	pH	6,05	6,5	6,12	6,45	6,63
4	AMB, CFU/g*	6,7x10^2	8,9x10^2	5,3x10^3	5,8x10^3	8,8x10^3
	Enterobacteriacae, CFU/g	<10	45	69	73	86
	pH	6,42	5,19	6,53	7,02	6,67
5	AMB, CFU/g*	6,2x10^2	8.9x10^2	4,9x10^3	5,9x10^3	9,7x10^3
	Enterobacteriacae, CFU/g	<10	85	99	1,5x10^2	3,7x10^2
	pH	6,63	6,26	6,53	6,42	6,34

* *Aerobic mesophilic bacteria*

value between m and M) (1.5x10^2 CFU/g) on the seventh day, the value remained within the prescribed criteria (Table 3) and the result was, thus, considered acceptable. We observed a greater increase of aerobic mesophilic bacteria on the ninth day (two subunits produced values between m and M) (4.9x10^4 and 5.1x10^4 CFU/g). This meant that chicken drumsticks maintained acceptable microbial growth on the ninth day after production. However, on the same day we also found a greater increase of enterobacteria (3 subunits produced values between m and M) (2.1x10^2, 3.7x10^2 and 5.2x10^2 CFU/g). Because such result is not considered satisfactory, drumsticks cannot be considered nutritionally useful.

The results of first four test intervals obtained by the microbiological testing of chicken wings produced satisfactory microbiological parameters on the first, third, sixth and seventh day after production. We observed a higher increase of enterobacteria and aerobic mesophilic bacteria on the ninth day. The growth of enterobacteria, nevertheless, remained within acceptable limits (two subunits produced values between m and M) (1.2x10^2 and 5.5x10^2 CFU/g). However, the growth of aerobic mesophilic bacteria was greater than acceptable (one subunit produced a value > M) (1.5x10^5 CFU/g), which means that chicken wings showed an unacceptable microbial growth on the ninth day; the results were unsatisfactory and thus cannot be considered nutritionally useful.

The results of first four test intervals obtained by the microbiological testing of chicken liver and

Table 7 Microbiological analysis results and pH values for the product "wings"

Farm	Parametar	Day of testing				
		1	3	6	7	9
1	AMB, CFU/g*	2,1x10^2	5,2x10^2	1,2x10^3	5,3x10^3	4,3x10^4
	Enterobacteriacae, CFU/g	<10	15	55	70	90
	pH	6	5,93	6,25	6,19	6,25
2	AMB, CFU/g*	3,6x10^2	6,6x10^2	2,6x10^3	7,6x10^3	3,6x10^4
	Enterobacteriacae, CFU/g	<10	25	75	80	90
	pH	6,21	6,21	6,12	6,23	6,24
3	AMB, CFU/g*	3,9x10^2	6,9x10^2	1,7x10^3	6,5x10^3	2,5x10^4
	Enterobacteriacae, CFU/g	<10	27	71	85	1,2 x10^2
	pH	5,98	5,85	6,45	6,29	6,3
4	AMB, CFU/g*	4,1x10^2	7,2x10^2	2,8x10^3	8,5x10^3	1,5x10^5
	Enterobacteriacae, CFU/g	<10	32	69	90	5,5 x10^2
	pH	6,11	5,98	6,14	6,21	6,27
5	AMB, CFU/g*	5,5x10^2	7,2x10^2	3,2x10^3	7,7x10^3	4,7x10^4
	Enterobacteriacae, CFU/g	<10	44	54	80	95
	pH	6,21	6,18	6,29	6,3	6,26

** Aerobic mesophilic bacteria*

Table 8 Microbiological analysis results and pH values for the product "liver and hearts"

Farm	Parametar	Day of testing				
		1	3	6	7	9
1	AMB, CFU/g*	7,9x10^2	9,9x10^2	3,9x10^3	1,9x10^4	7,9x10^5
	E. coli, CFU/g	<10	12	68	78	3,9x10^2
	pH	6,45	6,39	6,42	6,59	6,04
2	AMB, CFU/g*	7,6x10^2	9,6x10^2	3,6x10^3	5,6x10^4	1,6x10^5
	E. coli, CFU/g	<10	29	79	85	9,1x10^2
	pH	6,37	6,45	6,42	6,45	6,09
3	AMB, CFU/g*	6,9x10^2	8,5x10^2	2,5x10^3	5,5x10^4	3,5x10^5
	E. coli, CFU/g	<10	22	68	74	3,5x10^3
	pH	6,52	6,09	6,46	6,54	6,06
4	AMB, CFU/g*	6,3x10^2	8,5x10^2	2,8x10^3	3,8x10^4	8,8x10^5
	E. coli, CFU/g	<10	31	79	82	9,5x10^2
	pH	6,48	6,46	6,45	6,44	6,08
5	AMB, CFU/g*	6,1x10^2	8,8x10^2	2,3x10^3	5,3x10^4	2,9x10^5
	E. coli, CFU/g	<10	27	84	91	92
	pH	6,45	6,47	6,44	6,41	5,99

** Aerobic mesophilic bacteria*

hearts produced satisfactory microbiological parameters on the first, third, sixth and seventh day after production. On the ninth day we observed a greater growth of *E. coli* (one subunit produced a value > M) ($3.5x10^3$), as well as a greater growth of aerobic mesophilic bacteria (5 subunits produced values between m and M) ($7.9x10^5$; $1.6x10^5$; $3.5x10^5$, $8.8x10^5$ and $2.9x10^5$ CFU/g), which means that chicken liver and hearts showed an unacceptable microbial growth on the ninth day after production (Table 4); such result is unsatisfactory and thus cannot be considered nutritionally useful.

The results of first four test intervals obtained by the microbiological testing of chicken gizzards produced satisfactory microbiological parameters on the first, third, sixth and seventh day after production. On the ninth day, the count of *E. coli* greatly increased (three subunits produced values between m and M) ($1.9x10^2$; $2.7x10^2$ and $2.5x10^2$ CFU/g), as well as the count of aerobic mesophilic bacteria (5 subu-

Table 9 Microbiological analysis results and pH values for the product "gizzards"

Farm	Parametar	Day of testing				
		1	3	6	7	9
1	AMB, CFU/g*	$3,1x10^3$	$6,1x10^3$	$8,1x10^3$	$3,1x10^4$	$8,1x10^5$
	E. coli, CFU/g	10	20	30	50	80
	pH	6,85	6,76	6,91	7,18	6,96
2	AMB, CFU/g*	$3,5x10^3$	$7,5x10^3$	$9,5x10^3$	$4,5x10^4$	$3,5x10^5$
	E. coli, CFU/g	15	25	25	65	$1,9x10^2$
	pH	6,76	6,87	6,64	7,32	6,98
3	AMB, CFU/g*	$5,9x10^3$	$7,9x10^3$	$8,9x10^3$	$3,9x10^4$	$6,9x10^5$
	E. coli, CFU/g	20	40	45	65	$2,7 x10^2$
	pH	6,64	6,81	6,74	7,2	7,14
4	AMB, CFU/g*	$4,7x10^3$	$9,7x10^3$	$1,7x10^4$	$8,7x10^4$	$5,7x10^5$
	E. coli, CFU/g	20	30	53	83	$2,5x10^2$
	pH	7	6,49	7,25	7,59	6,96
5	AMB, CFU/g*	$4,6x10^3$	$6,6x10^3$	$9,6x10^3$	$4,6x10^4$	$4,9x10^5$
	E. coli, CFU/g	30	40	32	42	90
	pH	6,73	6,88	7	7,14	6,79

** Aerobic mesophilic bacteria*

Table 10 Hygienic safety of products according to microbiological criteria

Type of product	Day of analysis	Salmonella spp.	Listeria monocytogenes	Enterobacteriacae	AMB*
Grilled chicken	0+1	satisfactory	satisfactory	satisfactory	satisfactory
	0+3	satisfactory	satisfactory	satisfactory	satisfactory
	0+6	satisfactory	satisfactory	satisfactory	satisfactory
	0+7	satisfactory	satisfactory	satisfactory	satisfactory
	0+9	satisfactory	satisfactory	acceptable	acceptable
Drumsticks	0+1	satisfactory	satisfactory	satisfactory	satisfactory
	0+3	satisfactory	satisfactory	satisfactory	satisfactory
	0+6	satisfactory	satisfactory	satisfactory	satisfactory
	0+7	satisfactory	satisfactory	acceptable	satisfactory
	0+9	satisfactory	satisfactory	unsatisfactory	acceptable
Wings	0+1	satisfactory	satisfactory	satisfactory	satisfactory
	0+3	satisfactory	satisfactory	satisfactory	satisfactory
	0+6	satisfactory	satisfactory	satisfactory	satisfactory
	0+7	satisfactory	satisfactory	satisfactory	satisfactory
	0+9	satisfactory	satisfactory	acceptable	unsatisfactory
Liver and hearts	0+1	satisfactory	satisfactory	satisfactory	satisfactory
	0+3	satisfactory	satisfactory	satisfactory	satisfactory
	0+6	satisfactory	satisfactory	satisfactory	satisfactory
	0+7	satisfactory	satisfactory	satisfactory	satisfactory
	0+9	satisfactory	satisfactory	unsatisfactory	unsatisfactory
Gizzards	0+1	satisfactory	satisfactory	satisfactory	satisfactory
	0+3	satisfactory	satisfactory	satisfactory	satisfactory
	0+6	satisfactory	satisfactory	satisfactory	satisfactory
	0+7	satisfactory	satisfactory	satisfactory	satisfactory
	0+9	satisfactory	satisfactory	unsatisfactory	unsatisfactory

** Aerobic mesophilic bacteria*

nits produced values between m and M) ($8.1x10^5$, $3.5x10^5$, $6.9x10^5$, $5.7x10^5$ and $4.9x10^5$ CFU/g). This means that chicken gizzards showed an unacceptable microbial growth on the ninth day after production (Table 4); such result is unsatisfactory and thus cannot be considered nutritionally useful.

b)The microbiological growth of certain bacteria in individual products at test intervals

We calculated the Pearson correlation coefficient for determined bacteria (aerobic mesophilic bacteria, enterobacteria and *E. coli*) and established a positive correlation between the day of testing and all bacteria.

We discovered a strong correlation regarding the presence of aerobic mesophilic bacteria in grilled chicken for farms 1, 2 and 3 (0.90, 0.92, 0.88) and a moderately strong correlation for farms 4 and 5 (0.73 and 0.72). The correlation regarding the presence of enterobacteria for farms 1, 2 and 3 was similar. Namely, we established a strong correlation for all three farms (0.83) and a moderately-strong correlation for farms 4 and 5 (0.76 and 0.73).

When it comes to chicken drumsticks, we found a moderately strong correlation regarding aerobic mesophilic bacteria for farms 1 and 2 (0.79 and 0.78), and a strong correlation for farms 3, 4 and 5 (0.97, 0.98, 0.97). We also established a moderately strong correlation regarding the presence of enterobacteria for farms 1 and 5 (0.78; 0.88), as well as a strong correlation for farms 2, 3 and 4 (0.93; 0.91; 0.96).

A moderately strong correlation regarding aerobic mesophilic bacteria found on chicken wings was established for all five farms (0.73, 0.77, 0.79, 0.70, 0.75), while the presence of enterobacteria showed a perfect positive correlation for farms 1 and 3 (1.00), a strong correlation for farms 2 and 5 (0.98, 0.97), and a moderately strong correlation (0.77) for farm 4.

We established a moderately strong correlation regarding the presence of aerobic mesophilic bacteria found on chicken liver and hearts originating from all five farms (0.68, 0.80, 0.74, 0.69, 0.75), a moderately strong correlation regarding the presence of E. coli for farms 1, 2, 3 and 4 (0.80; 0.73; 0.68; 0.73), and a strong correlation for farm 5 (0.96).

Chicken gizzards from all five farms showed a moderately strong correlation with correlation factors of 0.68; 0.72; 0.69; 0.74 and 0.71 for aerobic mesophilic bacteria, a strong correlation regarding the presence of *E. coli* for farms 1 and 4 (0.94; 0.82), and a moderately strong correlation for farms 2, 3 and 5 (0.79; 0.77; 0.73).

During the research, the presence of aerobic mesophilic bacteria in all five products was consistent with the linear progression model. The number of enterobacteria found on grilled chicken on the first, third and sixth day amounted to < 10. The number of

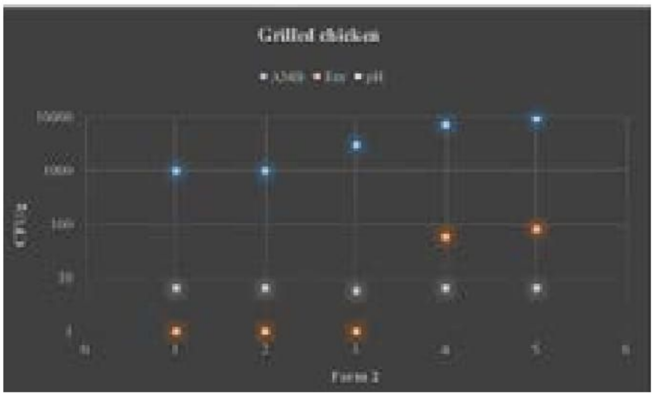

Figure 6 Example of moderately strong positive correlation for "grilled chicken" product

AMB – Aerobic mesophilic bacteria
Ent – Enterobacteriacae

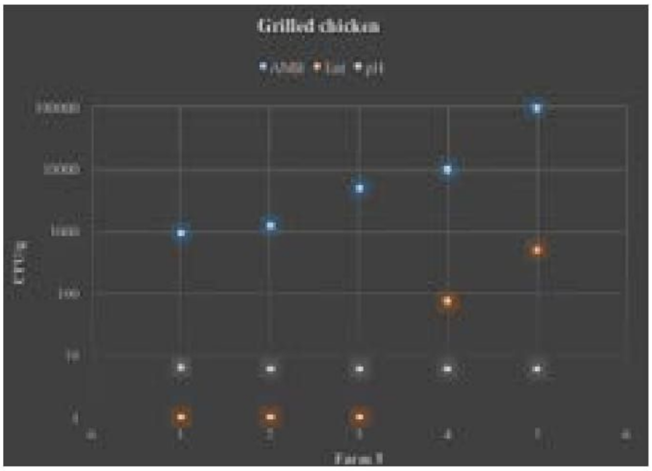

Figure 7 Example of complete absence of correlation

AMB – Aerobic mesophilic bacteria
Ent – Enterobacteriacae

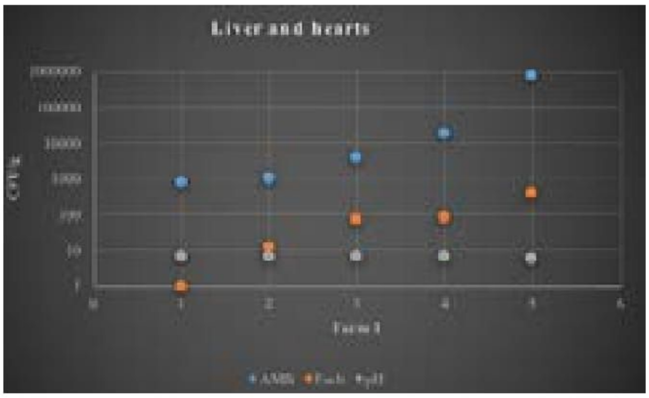

Figure 8 Example of strong negative correlation

AMB – Aerobic mesophilic bacteria
Esch – Escherichia coli

enterobacteria on chicken drumsticks and wings amounted to < 10 only on the first day and was consistent with the linear progression model on all other days. The number of *E. coli* on chicken liver and hearts amounted to < 10 on the first day and was, just like with chicken gizzards, consistent with the linear progression model on all other days throughout the study.

c) The relationship between the microbiological growth of certain bacteria in individual products and pH values obtained during the test interval

Unlike the correlation between the growth of microorganisms and the test interval for which we established a positive correlation of varying degree in all cases, we found both positive and negative correlation of varying degree between the growth of microorganisms and pH values.

For grilled chicken, the positive correlation regarding the growth of aerobic mesophilic bacteria was relatively weak for farm 1 (0.43) and moderately strong for farm 2 (0.58). We established a relatively weak negative correlation (-0.42; -0.28) for farms 3 and 4 and a complete absence of correlation (0) for farm 5. In addition, we found a positive correlation regarding the growth of enterobacteria for the same product on farms 1 and 2. It was relatively weak for farm 1 (0.29) and moderately strong for farm 2 (0.74). We established a relatively weak correlation (-0.47; -0.34; -0.01) for farms 3, 4 and 5. An example of moderately strong positive correlation for grilled chicken from farm 2 is demonstrated in Figure 6 and the complete absence of correlation in Figure 7.

For chicken drumsticks, the correlation regarding the growth of aerobic mesophilic bacteria was established for all farms, as follows: a relatively weak correlation for farms 1 and 2 (0.32, 0.43), a moderately high correlation for farms 3 and 4 (0.50, 0.65), and a relatively weak negative correlation for farm 5 (-0.27). We found a relatively weak positive correlation regarding the growth of enterobacteria for the same product for farms 1 and 4 (0.35), a moderately strong correlation for farms 2 and 3 (0.60, 0.74), and a moderately strong negative correlation for farm 5 (-0.53).

Chicken wings represented the only product for which we established positive correlation for all farms. Such correlation regarding the growth of aerobic mesophilic bacteria was moderately strong for farms 2 and 4 (0.56, 0.67) and relatively weak for farms 1, 3 and 5 (0.48, 0.34, 0.24). The growth of enterobacteria for the same product showed a moderately strong correlation for farms 1, 4 and 5 (0.63, 0.69, 0.86), and a relatively weak correlation for farms 2 and 3 (0.42, 0.30).

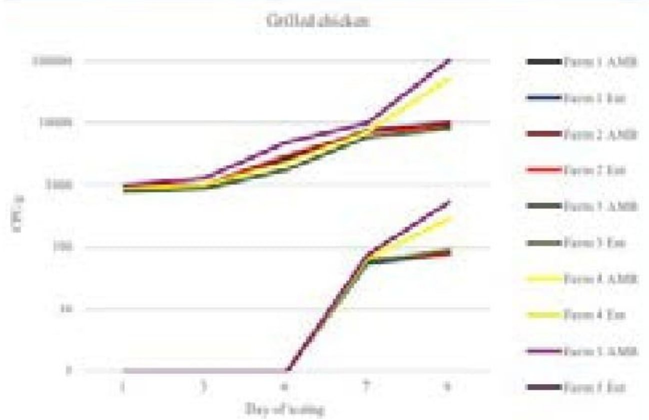

Figure 1 Microbiological status of grilled chicken in relation to farm of origin

AMB – Aerobic mesophilic bacteria
Ent – Enterobacteriacae

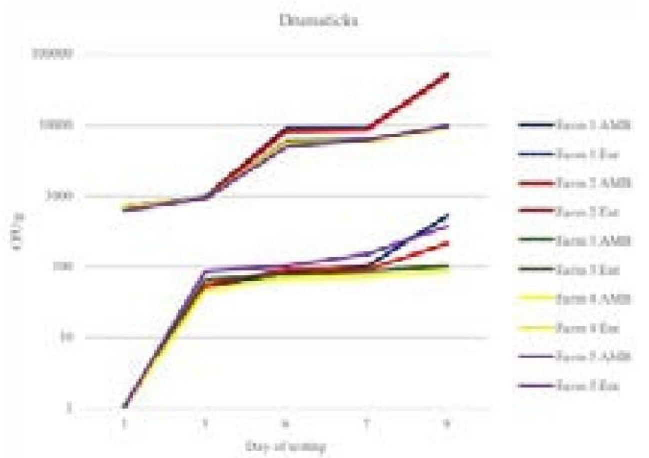

Figure 2 Microbiological status of drumsticks in relation to farm of origin

AMB – Aerobic mesophilic bacteria
Ent – Enterobacteriacae

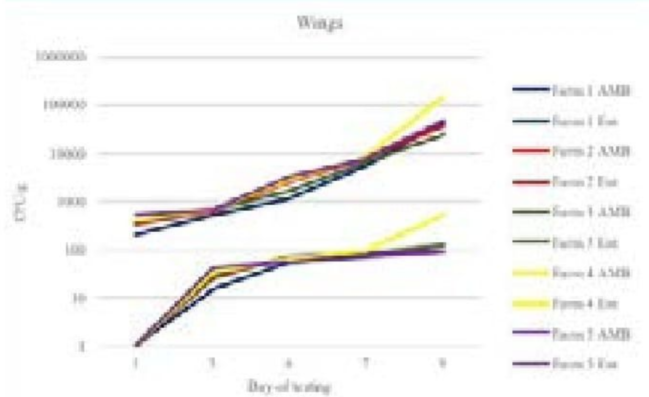

Figure 3 Microbiological status of wings in relation to farm of origin

AMB – Aerobic mesophilic bacteria
Ent – Enterobacteriacae

Unlike chicken wings, chicken liver and hearts represented the only product for which we established negative correlation for all farms. Such correlation regarding the growth of aerobic mesophilic bacteria was perfectly negative for farm 4 (-1), strong for farms 1, 2 and 5 (-0.92, -0.88, -0.99), and moderately strong for farm 3 (-0.58). The growth of E. coli for the same product showed a perfectly negative correlation for farm 4 (-1), strong correlation for farms 1 and 2 (-0.86, -0.96), and moderately strong correlation for farms 3 and 5 (-0.64, -0.50). An example of strong negative correlation for liver and hearts from farm 1 is shown in Figure 8.

A positive correlation regarding the growth of aerobic mesophilic bacteria for chicken gizzards was found on three farms. It was weak for farm 1 (0.13), relatively weak for farm 2 (0.24) and moderately strong for farm 3 (0.56). We established negative correlation for farms 4 and 5. It was very weak for farm 4 (-0.03) and relatively weak for farm 5 (-0.34). The growth of *E. coli* for the same product showed moderately strong positive correlation for farms 1 and 3 (0.56, 0.64), relatively weak correlation for farm 2 (0.38) and very weak correlation for farm 4 (0.08). The correlation was relatively weak negative only for farm 5 (-0.27).

d) The relationship between the microbiological status of product before expiry date and the farm of origin

We further analysed laboratory results for each product (grilled chicken, drumsticks, wings, and liver,

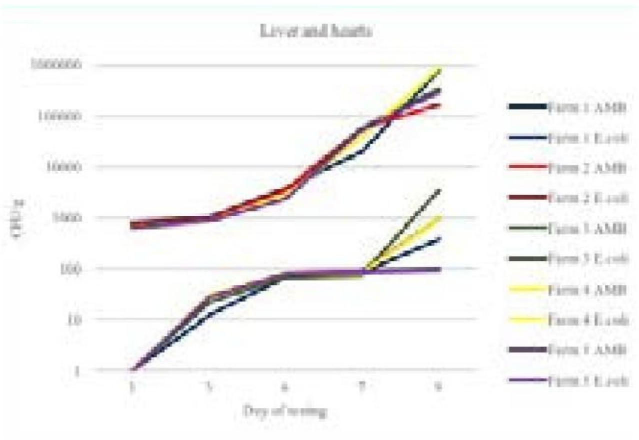

Figure 4 Microbiological status of liver and hearts in relation to farm of origin

AMB – Aerobic mesophilic bacteria
E.coli – Escherichia coli

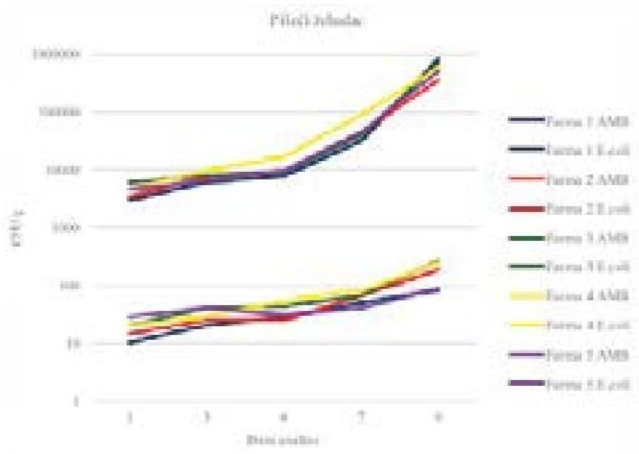

Figure 5 Microbiological status of gizzards in relation to farm of origin

AMB – Aerobic mesophilic bacteria
E.coli – Escherichia coli

Table 11 Rating and classification of farms based on isolated bacteria (1,2 and 3)

Type of product	Day 7	Day 9	Day 7	Day 9	Day 7	Day 9	Day 7	Day 9	Day 7	Day 9
	Farm 1		Farm 2		Farm 3		Farm 4		Farm 5	
Grilled chicken[1]	2	3	4	4	5	5	3	2	1	1
Drumsticks[1]	1	1	2	2	3,5	4	5	5	3,5	3
Wings[1]	5	3	3	4	4	5	1	1	2	2
Liver and hearts[1]	5	2	1	5	2	3	4	1	3	4
Gizzards[1]	5	1	3	5	4	2	1	3	2	4
Grilled chicken	5	5	3	4	4	3	2	2	1	1
Drumsticks[2]	2	1	3	3	4	4	5	5	1	2
Wings[2]	5	4,5	3,5	4,5	2	2	1	1	3,5	3
Liver and hearts[3]	4	4	2	3	5	1	3	2	1	5
Gizzards[3]	4	5	2,5	3	2,5	1	1	2	5	4
Sum	38	29,5	27	37,5	36	30	26	24	23	29
Mean value	3,80	2,95	2,70	3,75	3,60	3,00	2,60	2,40	2,30	2,90
SD[4]	1,47	1,56	0,81	0,93	1,04	1,41	1,56	1,43	1,33	1,30
SD[4] (day 7 + day 9)/2	1,51		0,87		1,23		1,50		1,31	
Average grade	3,38		3,23		3,30		2,50		2,60	
SD4 grade	0,20		1,00		0,80		0,40		0,60	
Total grade	3,58		4,23		4,10		2,90		3,20	
Classification	C		A		B		E		D	

1 Aerobic mesophilic bacteria; 2 Enterobacteriacae; 3 Escherichia coli; 4 Standard deviation hearts and gizzards) obtained during the study and examined its relationship with the farm of origin. The results in Figures 1, 2, 3, 4 and 5 are presented in logarithmic values for clearer presentation.

Even though the type of product (grilled chicken) was the same, we saw certain differences in the total number of found bacteria depending on the farm of origin. As shown in Figure 1, we found the greatest number of aerobic mesophilic bacteria (9.9x10^3 and 9.9x10^4 CFU/g) and enterobacteria (72 and 5.2x10^2 CFU/g) on grilled chicken from farm 5, on the seventh and ninth day. We obtained somewhat lower values for the same type of product from farm 4. The corresponding values relating to products from other farms were similar.

The highest values of aerobic mesophilic bacteria were determined for chicken drumsticks from farms 1 (9.1x10^3 and 5.1x10^4 CFU/g) and 2 (8.3x10^3 and 4.9x10^4 CFU/g), and the highest values of enterobacteria for chicken drumsticks from farm 1 (98 and 5.2x10^2 CFU/g), on the seventh and ninth day of the study. The corresponding values relating to the same type of product from other farms were similar (Figure 2).

The highest values of aerobic mesophilic bacteria (8.5x10^3 and 1.5x10^5 CFU/g) and enterobacteria (90 and 5.5x10^2 CFU/g) were determined on the seventh and ninth day of the study, for chicken wings originating from farm 4. The corresponding values relating to products from other farms were similar (Figure 3).

We determined that on the seventh day of the study chicken liver and hearts from all farms showed similar values of aerobic mesophilic bacteria. The exception was farm 1, where we found somewhat lower values (1.9x10^4 CFU/g). The highest values for the ninth day were obtained for farms 1 and 4 (7.9x10^5 and 8.8x10^5 CFU/g). The values of E. coli determined on the seventh day of the study were similar for all farms. However, the results were significantly different on the ninth day. Namely, we obtained the highest values for products from farm 3 (3.5x10^3 CFU/g) and the lowest values for products from farm 5 (92 CFU/g) (Figure 4).

We found the highest count of bacteria on the seventh day of the study, for chicken gizzards originating from farm 4. The values concerning the presence of aerobic mesophilic bacteria for products from all other farms were lower and more or less similar. The number of aerobic mesophilic bacteria determined for farms on the ninth day was as follows: farm 1>3>4>5>2 (8.1x10^5 > 6.9x10^5 > 5.7x10^5 > 4.9x10^5 > 3.5x105 CFU/g). The count of *E. coli* on the seventh day of the study was nearly the same. In comparison with farms 1 and 5 (80 and 90 CFU/g), we obtained somewhat higher values for farms 2, 3 and 4 (1.9x10^2, 2.7x10^2 and 2.5x10^2 CFU/g) on the ninth day (Figure 5).

e) Rating and classification of farms

The method we have used to rate and classify farms is presented in Table 11. Based on the analysis of results and taking into account the rating parameters listed in section "Materials and methods", we can classify farms in the following way: 2 (A), 3 (B), 1 (C), 5 (D) and 4 (E).

f) Comparison with other research

Unlike some other authors, we failed to isolate Salmonella spp. in any of our 125 25 g samples during this experiment. Samples contaminated with Salmonella spp. in other research accounted for 7.46% (Kozačinski, et al., 2012), 32.8% (Živković, 2001), 10.6% (Živković et al., 1997) and 36% (Bailey et al., 2001) of the total number of examined samples.

In addition, none of our 125 25 g samples used during the experiment contained *L. monocytogenes*. In studies carried out by other authors *L. monocytogenes* was isolated in 4.5% (Kozačinski et al., 2012) 3.03% (Kozačinski et al., 2006), 36.1% (Vitas et al., 2004) and 34% (Bohaychuk et al., 2006) of the total number of examined samples.

When we compared our results with the study focused on chicken meat (fillet without skin and drumsticks) carried out in the Republic of Croatia (Kozačinski et al., 2012), which established a linear progression of the total number of bacteria relating to the number of days, we found that its results coincide with the results of our study. Values obtained for fillet without skin were as follows: day 1 (4.22 $\log_{10}$ CFU/g) < day 3 (4.65 $\log_{10}$ CFU/g) < day 6 (5.14 $\log_{10}$ CFU/g), while values for chicken drumsticks in our study amounted to as follows: day 1 (3.60 $\log_{10}$ CFU/g) < day 3 (4.01 $\log_{10}$ CFU/g) < day 6 (4.56 $\log_{10}$ CFU/g).

CONCLUSION

All five products (grilled chicken, drumsticks, wings, and liver, hearts and gizzards) were still microbiologically safe for human consumption on the seventh day after their production.

On the ninth day after the day of production only values obtained for grilled chicken remained within the limits of prescribed microbiological criteria, while no other products (drumsticks, wings, and liver, hearts and gizzards) met the criteria of microbiological safety and were, thus, considered hygienically unsafe for human consumption.

We established a positive correlation with variable degree of correlation between the total number of bacteria detected in chicken meat from all farms and test interval.

We also observed both positive and negative correlation with variable degree of correlation and, in one case, a complete absence of correlation between the total number of bacteria and pH value.

Though we were comparing the same type of product, the highest values of bacteria on the seventh and ninth day of testing varied for different farms. The farms were, in turn, accordingly classified as follows: Class A – farm 2; Class B – Farm 3, Class C – Farm 1, Class D – Farm 5 and Class E – Farm 4.

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